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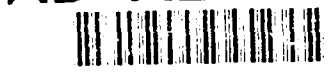
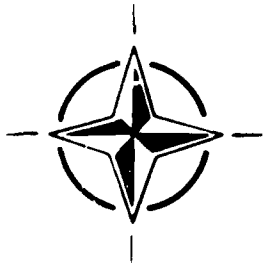


AGARD CONFERENCE PROCEEDINGS 518

Allergic, Immunological and Infectious Disease Problems in Aerospace Medicine

(Les Problèmes Causés par les Maladies Allergiques, Immunologiques et Contagieuses en Médecine Aérospatiale)

Papers presented at the Aerospace Medical Panel Symposium held in Rome, Italy from 21st to 25th October 1991.



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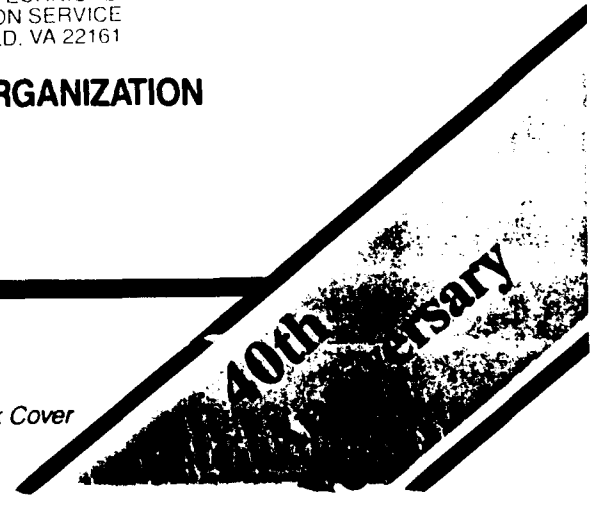

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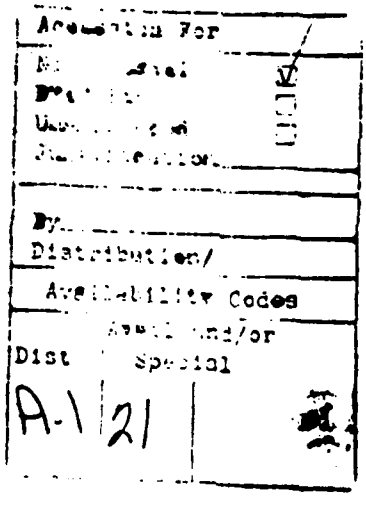

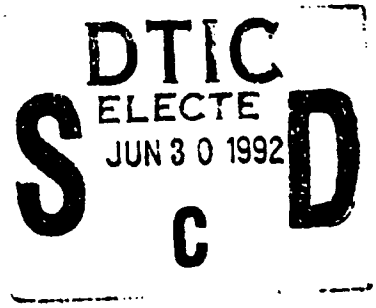

Preface

Allergic diseases are very frequent in the general population (15—17%) and are therefore regularly encountered in Aerospace Medicine. Respiratory diseases, especially those associated with bronchospasm, represent a threat to flying safety. Other allergic phenomena, such as hay fever, sinusitis, and dermatoses, occur because of the rather diverse antigenic exposure of aircrew who are deployed worldwide. How should such problems be dealt with in trained aircrew, and what is the current status of screening techniques for high risk subjects before they are trained? Which medications are acceptable in trained aircrew?

Preliminary evidence indicates that multiple commonly encountered conditions in aerospace operations (microgravity, hypergravity, normobaric oxygen and general stress) may alter the immune response. Further, some data regarding the AIDS virus have come from military populations, but no consistent recommendations for aircrew with HIV positivity have emerged. Infectious diseases, especially tropical diseases, continue to have great impact on operational readiness. Continual changes in the prophylaxis and treatment of infectious diseases, such as malaria, require the constant attention of Aerospace Medicine practitioners.

Because of their potentially adverse effects on aircrew readiness, flight safety and mission completion, the areas of allergy, immunology and infectious diseases must be continually reviewed.

Préface

Les maladies allergiques sont très fréquentes au niveau de la population générale (15—17%) et se retrouvent donc régulièrement en médecine aérospatiale. Les maladies respiratoires, en particulier celles associées au bronchospasme, représentent une menace pour la sécurité des équipages. D'autres phénomènes allergiques tels que le rhume des foins, la sinusite et la dermatose se produisent à cause l'exposition antigène diverse des équipages déployés à travers le monde. Comment faut-il aborder de tels problèmes au niveau des équipages qualifiés et quelle est la situation en ce qui concerne les techniques de filtrage des sujets à haut risque avant l'entrainement? Quels sont les médicaments acceptables pour les équipages qualifiés?

Les premiers résultats des travaux de recherche indiquent que les conditions multiples qui sont rencontrées typiquement dans les opérations aérospatiales (la micropesanteur, l'hyperpesanteur, l'oxygène à pression normale et le stress) risque de modifier la réaction immunitaire. En outre, certaines données concernant le virus du SIDA ont été fournies par les populations militaires mais aucune série de recommendations concernant la positivité VIH des équipages n'a été publiée. Les maladies contagieuses, et surtout les maladies tropicales, ont toujours un fort impact sur la disponibilité opérationnelle. L'évolution constante de la prophylaxie et du traitement des maladies contagieuses, telles que la malaria, exige l'attention permanente des médecins de l'air.

En raison de leurs effets néfastes possibles sur la disponibilité des équipages, la sécurité en vol et l'accomplissement de la mission, les domaines de l'allergie, de l'immunologie et des maladies contagieuses doivent être revus en permanence.

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OPENING ADDRESS

by

Major General **Guido Olivero**

Mr Chairman, Ladies and Gentlemen, in the capacity of AGARD Italian National Delegate I have the pleasure of warmly welcoming you here in Rome for the 72nd AMP/AGARD Fall Symposium.

The theme of this Symposium "Allergic, Immunological and Infectious Disease Problems in Aerospace Medicine" is of great importance for military and civilian aviation. Today aircraft have reduced even long distances, so it is possible for people to reach, in a considerably short time, countries that are completely different from those of origin, in many different ways, such as climate, environmental hygienic conditions, the presence of insects and, consequently, infectious and parasitic diseases. Aircraft, therefore, may also become a very effective means of spreading infectious diseases, if we consider the high number of travellers worldwide today. The same applies to military aviation, because, in the last few years, operating in regions very far from its own has also become quite frequent for some NATO countries traditionally less accustomed to that. This created the need to establish a continuous surveillance, to limit the spread of infectious diseases and to improve the resistance of aircrew and, more in general, of military personnel, to different infectious diseases. The topics treated in this Symposium will contribute to clarify the ideas in this important area and to adopt up-to-date hygienic measures.

Also, respiratory allergies, whose frequency is today growing, mainly in industrialized countries, such as the NATO ones, may be highly invalidating for pilots, considering the solicitations of the new generation of high performance fighter aircraft. A better knowledge of these diseases and the ways to prevent or to handle them safely in pilots are crucial topics for Aerospace Medicine. I wish therefore that your work be useful and fruitful this week in Rome, so the basis for further improvement of the physical fitness of pilots and, more in general, of military personnel and travellers may be set in our city.

I wish to thank the President of the National Research Council, Prof. Luigi Rossi Bernardi, for the opportunity that he has given us to use this beautiful and well equipped building and all the chairmen speakers, a few of whom have also undertaken long trips to contribute culturally to the success of this Meeting. I wish all the participants a pleasant stay in Rome.

TECHNICAL EVALUATION REPORT

by

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1. INTRODUCTION

The Aerospace Medical Panel symposium on "Allergic, Immunological and Infectious Disease Problems in Aerospace Medicine" was held at the National Research Council, Rome, Italy, from October 21 to 25, 1991. Thirty-six papers, instead of the 38 originally scheduled, were collected from seven NATO countries and one non-NATO.

2. THEME

The theme of the symposium was the fight against the infectious diseases, mainly the vaccine-preventable ones, and the study of inhalant allergic diseases. The Special Clinical and Physiological Problems in Military Aviation Committee of the Aerospace Medical Panel first recognised the importance of the proposed theme for Aerospace Medicine in 1986 and definitively in 1989. This event is a new one (in the almost 40 years of life of AMP/AGARD this is the first time that a symposium is dedicated to this theme), as until now the traditional field of interest of Aerospace Medicine has been the study of the respiratory, cardiovascular and central nervous systems as well as the sense organs during environmental stimuli, such as hypoxia, accelerations, microgravity, vibrations, etc... The enormous development in the field of Allergo-Immunology in the last few years has allowed us to understand and prevent many pathological conditions, whereas the setting up of laboratory tests has allowed us to monitor with great precision the immune functions, which may be depressed by the environmental stimuli linked to space or military flight. The exposure of pilots to the increasing demands of high performance modern aircraft require complete fitness in relation to mild symptomatology, such as allergic oculorinhitis. Lastly, the development of military and civilian air travel, which favours the spreading of infectious and parasitic diseases, requires the setting up of correct prophylactic measures. The Committee recognised the actual value and the importance for Aerospace Medicine of the above reported considerations; consequently recommended to the 68th Business Meeting of the Aerospace Medical Panel, which was held in November 1989, that the Panel should hold a symposium on allergic, immunological and infectious disease problems in Aerospace Medicine. This recommendation was accepted by the Panel and Prof. H. Andersen (NO), Col. J.R. Hickman (USA), Col. P. Vandenbosch (BE) and Col. R. D'Amelio (IT) were appointed Session Organisers of the Symposium. The call for papers was issued by the AMP Executive in January 1991. A total of 40 abstracts were received, of which 38 were accepted and 36 actually presented. The programme for the symposium was finalised by the Session Organisers early in May 1991.

3. PURPOSE AND SCOPE

The purpose of the symposium was to sensitize the AGARD community and the National Delegates from the individual NATO countries to the Allergo-Immunological problems in Aerospace Medicine. This was accomplished by bringing together military and civilian researchers, from universities, industry, defence and other governmental research laboratories or representatives of international and authoritative institutions, such as WHO. Awareness was necessary, considering that it was the first time that these topics were faced in an AMP/AGARD Symposium.

The scope was mainly practical, the attempt was to propose some standardization agreements on the treated topics, i.e. the screening of inhalant allergic diseases and of HBV and HIV, and the improvement of the vaccination schedule for military personnel.

4. SYMPOSIUM PROGRAMME

The symposium was organised into 2 Lectures and 4 Sessions as follows:

The 2 Lectures, the first one on "Space flight and immune system" and the other on "Mechanisms of the immune failure in burn injury", were set aside because of the relevance of the topics and for the lack of other contributions in the field. Consequently it was impossible to include them in a session.

The first session, Viral Hepatitis, which consisted of 4 papers, mainly treated hepatitis B, regarding epidemiology, clinical history and specific vaccination.

The second session, HIV infection, which consisted of 10 papers, treated the present epidemiology as well as the projections to the end of the century, the impact on the military, the problem of the silent infection and of the virus variability, which makes the creation of a vaccine difficult. In the second half of the session the early prognostic markers, the analysis of disease progression and the central nervous system involvement were treated.

The third session, Infectious Diseases and Vaccinations, consisted of 11 papers (12 originally). After a general introduction to the theme, topic addressed were the diphtheria toxoid vaccination, the meningococcal meningitis vaccination, two types of thyphoid vaccination, the anti-malarial chemoprophylaxis and the studies for new human vaccine adjuvants.

The fourth session, Inhalant Allergic Diseases, consisted of 9 papers (10 originally). Topics addressed were the epidemiology in North America and in Europe of the respiratory allergies, the methods of screening and clinical viewpoints on the study of respiratory allergies in pilots.

5. TECHNICAL EVALUATION

5.1. The author of the first paper (Cogoli) reported the more recent results of his studies on the relationship between space flight and immune system. A. Cogoli started his studies more than 10 years ago and the results show a consistent and constant depression of lymphocyte activation, most probably due more to stress (physical and psychological) than to weightlessness per se or to cosmic radiation (this last consideration suggests that the same immune abnormality may be present in other stressing conditions, such as military flight). Only recently have these results been adequately taken into account in the planning of long (3 months - 1 year) space missions.

5.2. The author of the second paper (B. Sparkes) reported very interesting results on the pathogenesis and the prognostic monitoring of immune abnormalities during burn injury, which are unfortunately not rare in our environment. The immune abnormality is characterized by a strong reduction of T lymphocytes positive for Interleukin-2 receptor (IL-2R); this reduction is due to the massive shedding from the cell surface of IL-2R. The quantity of soluble IL-2R is also a prognostic marker, as the higher levels correspond to non survivors. A lipid protein complex (LPC) produced in skin by burning has been shown to inhibit the immune response. LPC may probably be fixed in the burn by cerium nitrate, topically applied. So, cerium nitrate seems to be a concrete hope to improve chances of survival in burn injury, by reducing the immunodepression after burn, as observed in a study of 10 patients.

5.3. In the first session, Viral Hepatitis, moderated by prof. M. Rizzetto, (the discoverer of virus delta), and by prof. R. Steffen, (from Zurich), prof. Rizzetto introduced the theme, discussing the epidemiology and the clinical history of Hepatitis B. The epidemiology of hepatitis B, which is today clearly considered a sexually transmitted disease, is very variable among the different NATO countries. The prevalence of HBsAg positive subjects, in the general population, is in fact 0.1% in North America and Northern Europe (UK and Scandinavia), 0.5-0.6% in Western Europe and 1.5-2% in the Mediterranean Regions. The number of HBsAg carriers worldwide is approximately 300 million. The clinical history shows that the disease is normally benign; less than 1% of hospitalized cases run a fulminant course and less than 3% of total cases progress to chronicity. In its typical form, the disease has 2 phases, the first one characterized by florid replicative activity of the virus and strong cell-mediated reaction of the host directed against nucleocapsid antigens, such as HBcAg and HBeAg. Subsequently, replication of HBV declines, while part of the viral genome integrates in the host genome. In this phase

the virus is capable of coding only HBsAg. The carrier of HBsAg loses the markers of virion replication and seroconverts to anti-HBe.

Recently some cases of progressive or fulminant hepatitis have been reported, serologically characterized by HBsAg, absence of HBeAg and presence of anti-HBe. This serological anomaly is due to a point mutation in the precore region of the genome which creates a "stop" codon preventing transcription of the HBeAg polypeptide. The knowledge of this anomaly is not only important from scientific and clinical points of view, but also to correctly plan serological screening, that should not be based on the only identification of both, HBsAg and HBeAg, to decide on the presence or not of viral replication.

The fourth paper (Parkinson) showed epidemiologic data, in relation to demographic variables, of hepatitis B in the USAF in the last 10 years, underlining the risk of infection due to the problem of worldwide deployment for possible operational scenarios, whereas the fifth paper (Steffen) stressed the same problem of worldwide deployment and subsequent risk of hepatitis B and A, for civilian travellers. Prof. Steffen, therefore, recommended hepatitis B and A (this vaccine will be marketed in a very short time) vaccinations for pilots serving in developing countries.

The sixth paper (Zanetti) treated the problem of active immunization by anti-HBV vaccination. This is a very effective and well tolerated vaccine, but the high cost limits its wide use, mainly in developing countries, where HBsAg prevalence is higher. The risk of contracting HBV infection for deployable troops or civilian travellers must be taken into account well in advance, considering the time (2-3 months at least) necessary to reach anti-HBs protective levels. Prof. Zanetti, in addition, illustrated different strategies for control of hepatitis B by immunization and particularly the strategy followed in Italy, where vaccination is compulsory for all newborns and adolescents under 12 years of age, starting from May 1991. Within 12 years, all Italian young people under 24 years of age will be immunized against hepatitis B.

This brief, but very stimulating session, has clearly illustrated the danger of hepatitis A and B both of which luckily can now be prevented by effective, specific vaccines.

5.4. In the second session, HIV infection, moderated by Col. D.S. Burke, responsible for the AIDS Project of the US Armed Forces, and by Prof. F. Aiuti, one of the Italian researchers most engaged in the fight against AIDS, the seventh paper (Kallings) reported epidemiological data of HIV infection in the world, as estimated by WHO, Global Programme on AIDS. Presently AIDS cases have been reported from 162 countries. By 1991, WHO estimates the accumulated number of AIDS cases to be about 1.4 million and the number of HIV-infected adults 9 to 10 million (approximately 3.5 million in women). Approximately 60% of all HIV infections have occurred in sub-Saharan Africa, about 25% in North and South America and Australia, about 6% in Europe and about 10% in Asia. By the year 2000, according to a conservative estimate, there may be up to 10 million adult AIDS cases and, in addition, as many as 20 million HIV infected adult persons worldwide, plus at least 10 million HIV infected children. The rapid increase in Asia may force an upward revision of these projections. These numbers represent the challenge that will have to be faced by the Military and Civilian Health Services both in industrialized and developing countries. Another worrying observation reported by prof. Kallings is the increasing way of transmission by heterosexual route, that is now estimated to be about 70%, and that identifies HIV infection as a typical sexually transmitted disease, spread in the same way as syphilis and gonorrhoea.

The eighth paper (Burke) stressed the impact of HIV infection on the military. Noteworthy is the parallel established by Col. Burke between HIV infection and war regarding the impact on a nation. In both situations, in fact, young, productive, infected adults become disabled and dysfunctional, and die, leaving behind the very young and the old. HIV infection potentially impacts the complete spectrum of military activities, especially the activities requiring psycho-physical fitness, such as pilot activity. The comprehensive approach developed by the USA Department of Defence in the fight against HIV infection is: surveillance of infection rates (intelligence) around the world and in the military; behavioral research to develop more effective means of education to change behavior; and biological research to develop a quick and easy field test, and a vaccine or drug to prevent the disease from occurring despite exposure. In the USA the compulsory screening of military personnel for HIV antibodies

represented a crucial help to the civilian Health Service in relation to the knowledge of the epidemiology of the infection in the general population.

The ninth paper (Warner) reported the epidemiological and medicolegal experience of USAF in the last 5 years. During this period over 700,000 USAF active duty personnel had been screened for HIV antibodies and a total of 942 HIV-positives were detected, of which only 29 were female. The infection was concentrated in the young (20-35 year old), unmarried, enlisted males. Aviators and crews are not considered at increased risk.

The tenth paper (Aiuti) discussed a very crucial problem, the silent HIV infection, that refers to seronegative infected individuals, who are able to potentially transmit the virus. Twelve out of 65 high risk seronegative individuals were found to have HIV proviral sequences in peripheral blood lymphocytes, by polymerase chain reaction (PCR). Only 1 out of these 12 patients seroconverted during the follow up (after 4 months). These results must be taken into account in the planning of serological screening, where PCR should be performed in seronegative risk individuals.

The eleventh paper (Verani) discussed the problem of the preparation of a specific vaccine, in the presence of HIV variability. The gp120 portion most suitable for the planning of a HIV vaccine is the so-called V3 loop, able to recruit neutralizing antibodies, but strongly variable in the different isolates, except an apical region of 6 aminoacids (GPGRAF). A peptide formed by these aminoacids should be a suitable vaccine candidate. Many clinical trials are now in progress in the USA with different types of vaccine.

The twelfth paper (Wolfe) is very interesting because it represents a reference point for the correct interpretation of the immunological data obtained in patients with HIV infection. The authors, in fact, reported a study of some immunological parameters (lymphocyte subpopulations and functional stimulation assays) in more than 800 normal subjects and the influence of some factors, like age, race, percent body fat, tobacco use and alcohol consumption.

In paper No 13 (Dolan) a careful immunological study of the more than 900 HIV-infected cases identified in the USAF in the last 5 years is reported. In these cases the incidence rate was of 15.6/100,000 person-years. Noteworthy was the rate of initial occurrence of opportunistic infections, that was 1 and 4% at 1 and 2 years, respectively, in patients with >400 CD4 cells/mmc, and 21 and 36% in patients with <400 CD4 cells/mmc. Beta-2 microglobulin and neopterin showed an inverse relation with CD4 levels and the decline of CD4+ T cells was quicker in the p24 antigen-positive group than in antigen-negative. Lastly an increase of CD4/CD29 memory T-cell subset was observed in the AZT-treated group.

In paper No 14 (Warner) the authors discussed a crucial problem, the time spent in the different WR stages, mainly in stages 5 and 6. In a sample of 92 USAF infected individuals at WR stage 6, the median survival time has been calculated in approximately 23 months. Finally papers No 15 (Rundell) and 16 (Rundell) treated the central nervous system involvement in HIV infection, the first one investigating the types of cognitive changes observed in early HIV infection (slowing of information processing speed in a significant minority of HIV positive subjects) and the other reporting the neuropsychiatric sequelae, among whom a high rate (17%) of suicidal thoughts and behaviors in the male population was observed.

The final message from this session is the large diffusion of the infection, the great impact on military activities, the early involvement of cognitive function, with subsequent decrease of pilot performance. Particularly this last topic has been widely debated during the discussion.

5.5. In the third session, Infectious Diseases and Vaccinations, moderated by dr. G. Torrigiani, Chief of the Division of Communicable Diseases, WHO, and by prof. R.E. Spier, Editor of the Scientific Journal "Vaccine", dr. Torrigiani (paper No 17) made an introduction to the problem of infectious diseases, their impact in the world and the ways of preventing them. It is estimated that 5 million deaths occur per year from diseases which can be prevented by vaccines available today, and that another 5 million people are being crippled, blinded or mentally retarded as a result of the same diseases. Malaria affects 150 million people annually and tuberculosis and leprosy still remain major public health problems in developing countries. Sexually transmitted diseases are on the increase everywhere with a general shift towards the teenage group. Environmental management, such as the provision of a safe water supply and the disposal of refuse, control of vectors

transmitting disease, new drugs and antibiotics and vaccines are the most effective weapons in the fight against infectious diseases which Health Services and research may develop.

Paper No 18 (Niemann) was not presented.

Col. Clardy (paper No 19) reported a study on the susceptibility rates for mumps, measles and rubella in 276 USAF recruits. Based upon the susceptibility rates, serological screening and selective immunization for all three diseases were determined to be cost effective when compared to mass immunization of all recruits.

In paper No 20 dr Rappuoli reported a study on the levels of anti-diphtheria toxoid antibodies in 334 Italian recruits, showing that 22.9% of them were negative for antibodies. A recommendation to include the anti-diphtheria vaccine in the compulsory vaccination schedule for Italian recruits was advanced. This recommendation was made possible and more feasible because a highly purified non toxic mutant of diphtheria toxin was developed by dr. Rappuoli in the laboratories of the Sclavo Research Center. In this mutant, only one (position 52) out of the 535 aminoacids composing the diphtheria toxin has been modified; the result is the complete lack of toxicity, without any quantitative and/or qualitative modification of antigenic power.

Dr. Biselli (paper No 21) reported an immunological study on meningococcal meningitis vaccine (A + C) in Italian recruits, documenting a good antibody response. A good antibody response was also recorded in unfavourable environmental conditions, such as hypoxia, as demonstrated during permanence at 16.000 feet for more than 20 days. Since the introduction of this vaccine in the compulsory vaccination schedule of Italian recruits, a dramatic reduction of meningococcal meningitis has been observed in the military personnel. The clinical response has been excellent because, before the introduction of the vaccine among the compulsory vaccinations for military recruits, the majority of cases of meningococcal meningitis in Italy were caused by *N meningitidis* serogroup C, whose polysaccharide is present in the vaccine composition.

In paper No 22 dr Didelot illustrated the French vaccination schedule for the aircrew travelling to tropical areas This schedule includes compulsory measures (anti-yellow fever vaccination), recommended measures (anti-tetanus and diphtheria toxoids, anti-polio, BCG and anti-thyphoid fever), and suggested measures (anti-meningococcal meningitis, anti-hepatitis B, anti-rabies and anti-influenza).

Dr. Nisini (paper No23) and Prof. Bellanti (paper No24) presented two new oral live thyphoid vaccines, the first of which (the well known mutant strain Ty21a) has been included for the last 5 years in the compulsory vaccination schedule of Italian recruits, whose efficacy has been discussed under the epidemiological, clinical and immunological points of view. Whereas for the other, a phase I study has been recently completed. The advantage of this second type of thyphoid vaccine (ts 51-1), in comparison to the strain Ty21a, seems to be a greater stability.

Prof. Steffen (paper No 25) reported a careful epidemiological study on the safety and efficacy of malaria chemoprophylaxis, from which mefloquine appears to be relatively safe and highly efficacious in areas with widely distributed chloroquine- resistant P. falciparum.

Paper No 26 (Gordon) discussed a study targeted to improve the immunogenicity of malaria vaccine candidate antigens by the use of novel adjuvants and delivery systems, such as chemical conjugation to bacterial carrier proteins, emulsification in "Freund's-like preparations, and incorporation into liposomes. The improvement of immunogenicity refers mainly to cellular immunity, because the only licensed human vaccine adjuvant, alum, may improve antibody production, but it is a poor stimulator of cellular effector mechanisms. This intervention is closely linked to paper No 27 (Tagliabue) on the animal trials with a new biological adjuvant, the nonapeptide 163-171 (VQGEESNDK) of Interleukin (IL)-1, which is devoid of all the proinflammatory activities, but maintains the immunostimulating activity of the whole IL-1, and to No 28 (Aguado) on the future approach to vaccine development. This approach consists of avoiding multiple injections in order to reach protective antibody levels. In fact multiple injections bring, as a consequence, a high percentage of drop outs. The possibility of obtaining protective antibody levels with only a single injection may consist in the use of controlled-release systems. The copolymers of polylactic acid/polyglycolic acid in form of microspheres seem to be the most suitable controlled-release system for vaccines. It is hoped that in the near future phase I human

trials will be conducted with a mixture of different vaccines (tetanus toxoid, hepatitis B, inactivated polio vaccine)-containing biodegradable microspheres.

All the presentations in this session, with the expertise of the chairmen and speakers, gave us an up-to-date survey, further amplified by a very stimulating discussion, of the basic and applicative research in the continuously evolving field of vaccine development.

5.6. In the fourth session, Inhalant Allergic Diseases, moderated by prof. J.A. Bellanti, former Editor of the Scientific Journal "Annals of Allergy", and by Col. P. Vandenbosch, prof. Bellanti (paper No 29) introduced the theme from a general point of view, by classifying the allergic diseases, giving epidemiological, pathogenetic and clinical data and underlining the impact on the pilot. Such an impact may be direct, as a consequence of the symptomatology, or indirect, as a consequence of adverse effects of medications used in the treatment of these disorders. Regarding this last point, new medications with minimal side effects, i.e. non-sedating antihistaminic drugs, are now available.

Prof. Bonini (paper No 30), in the role of General Secretary of European Academy of Allergology and Clinical Immunology, showed the activity of the Academy, which is mainly involved in the congress and meeting organization.

In paper No 31 (Matricardi) the experience of the Italian Air Force in the serological screening of inhalant allergic diseases was reported. Inhalant allergic diseases are clinically characterized by long periods of wellbeing between crises. In the universally accepted diagnostic algorithm of these pathologic conditions the first, most important step is clinical history. The same diagnostic algorithm is not valid for the screening of military pilots, because the clinical history could be unreliable, consequently we can risk enrolling allergic pilots (the contrary is true for the applicants to military service in the countries where military service is compulsory; in this case malingering phenomena may occur). There is therefore the need to invert the normal diagnostic algorithm, starting from a serological test and not by clinical history. This test should be sensitive and specific, quick, easy to perform and not very expensive. In the Italian experience a multi-RAST with these characteristics gave a good performance, showing a nearly absolute sensitivity and an acceptable specificity. On the basis of this experience this assay has now been included among the compulsory tests for the screening of applicants who wish to become part of the Italian Air Force. Probably this experience could be useful also for other NATO countries, where a serological screening is not performed.

In paper No 32 (Wever) the same diagnostic test was validated on two populations of different mean age (one of which with a high prevalence of allergic asthma) from the Netherlands, giving consistently excellent results.

In paper No 33 dr Anzalone reported the experience of the Italian Navy in the screening of bronchial asthma, by the methacholine (or allergen) airway challenge test, and the validation, on a sample of 200 subjects, of a new panel multi-RAST assay.

Dr Gray (paper No 34) reported the Canadian experience in the screening of bronchial asthma in aircrews by the methacholine (or histamine) airway challenge test, giving also the limits for exclusion: PC20 <4 mg/ml for pilots, <2 mg/ml for the other members of the aircrew. Dr Gray also showed a diagnostic algorithm, based on clinical history and physical examination, followed by a chest X-ray and pulmonary function testing before and after bronchodilator and lastly by a standardized methacholine airway challenge test.

Paper No 35 was not presented.

In paper No 36 (Mortier) the experience of the Belgian Air Force in the screening of allergy on 135 student pilots is reported. The conclusions of the authors are that the serological screening with total IgE is not helpful in comparison to clinical history, physical examination and possibly pulmonary function testing. This is also the experience of dr Matricardi (paper No 31) and of dr Wever (paper No 32). In adults in fact the diagnostic or predictive value of total IgE is very poor, whereas that of multi-RAST assays is very efficient.

In paper No 37 (Pappas) a study aimed to identify allergic and non allergic rhinitis in 144 male Greek pilots was reported. The majority of them presented allergic (80.5%) rhinitis, mainly seasonal from pollens. IgE levels were higher in allergic than in non allergic rhinitis patients.

Lastly in paper No 38 (Palermos) a correlation among serum alphal-antitrypsin, cigarette smoking and

pulmonary function status in 113 male Greek pilots over a 10 year period is reported. The levels of alphal anti-trypsin were significantly higher in smokers than in non smokers, whereas the lowering of forced vital capacity and 1second forced expiratory volume after 10 years was significanly greater for smokers than non smokers.
In conclusion this session documented the marked interest, also underlined by a very interesting discussion, for inhalant allergic diseases in the Air Force of some NATO countries. The multi-RAST assays and the methacholine airway challenge test seem to be the most reliable means to screen respiratory allergies and asthma.

6. CONCLUSIONS AND RECOMMENDATIONS

In the last few years Immunology has grown up enormously both, from theoretical and practical points of view. Many laboratory tests have been set up, which are able to monitor the different immune functions, during environmental, pharmacologic and infectious stimuli. In spite of this, little attention has been paid until now to Immunology in Aerospace Medicine. Consequently the Aerospace Medical Panel of AGARD showed a marked scientific sensitivity to dedicate a Symposium to allergo-immunological problems in Aerospace Medicine. Before discussing recommendations linked to the topics treated in the meeting, I would like to stress a general consideration, reported by the AMP chairman, prof. Guy Santucci, during the opening ceremonies of our meeting. Aerospace and Military Medicine, which are medicines of extreme environmental conditions, such as the heat, the cold, hypoxia, hypogravity, etc..., represent a major help for the World Health Organization, through mandatory behaviors, such as vaccinations, screening for infectious diseases, general hygienic measures. Military personnel are continuously monitored from the medical point of view and, being part of the general population, significantly contribute to limit the spreading of infectious agents in the general population too.
The results of dr Cogoli's observations on the immune abnormalities deriving from space flight, even if constantly observed and consistent, were at first underestimated, because of more immediate abnormalities in other systems, such as cardiovascular and vestibular ones, traditionally treated by Aerospace Medicine. Now immune problems are considered with much more interest in the planning of long space missions, because there are concerns about the possible development of infectious and neoplastic diseases. In addition, the last results of Cogoli seem to attribute more responsibility to physico-psychological stress than to environmental stimuli. Also our results (1) agree with such an interpretation. Student pilots were in fact found to present constant and consistent modifications of lymphocyte subpopulations in comparison to their sex and age-matched instructors.
Moreover there are data in the literature (2-5) on the phagocyte abnormalities during hypoxia.
In conclusion there are now clear data demonstrating a close correlation between environmental stimuli linked to military or space flight, stress and immune abnormalities. These abnormalities must be carefully considered, consequently a recommendation is made to monitor cellular (lymphocyte and phagocyte) immune functions in spacemen, in student pilots and in pilots of high performance aircraft.
Also in a field, such as burn injury, in which the infectious complications until a while ago were only attributed to the loss of the cutaneous barrier, it is now known that a *major* responsibility is linked to the immune abnormalities, so clearly spelled out by dr Sparkes.
Regarding hepatitis B, the epidemiological and clinical aspects were very clearly discussed and the dimensions of the phenomenon are very well known. Nonetheless, an analysis of the policies of the individual NATO countries regarding the screening and vaccination of military recruits or, at least, of the pilots, for the hepatitis B, shows that some of them, including some Mediterranean NATO countries, where the prevalence of the hepatitis B markers in the general population is higher compared to Northern Europe and North America, do not perform such tests, not even for deployable personnel (Tab.I). Three orders of considerations should induce us to revise the medicolegal policies regarding this easily diagnosable and vaccine-preventable disease: 1) The prevalence of HBV infection in the general population in the country. In the presence of a high prevalence, compulsory screening and vaccination not only reduce the circulation of HBV in the military environment, but, considering the nature of

hepatitis B as a sexually transmitted disease (6), also contributes to a reduction of the prevalence in the general population. 2) The possibility of operational scenarios in countries with endemic HBV infection (the recent Persian Gulf War is very instructive to this respect). The same applies for international travellers, as prof. Steffen has demonstrated (paper No5). 3) The consideration of the high cost of pilot training necessitates a higher need to protect them in comparison to other categories of personnel.

Regarding HIV infection, whose increasing prevalence in subjects infected through heterosexual transmission is reported in many NATO countries, as reported by prof. Kallings (paper No 7), and whose tremendous impact on the military has been stressed by Col. Burke (paper No 8), the compulsory screening of personnel is only performed by USA, followed by Belgium, Denmark and Greece (these last three countries only for selected categories of personnel, including pilots) (Tab.II). Also periodical screenings are only performed by USA, because the other NATO countries perform HIV testing only on entry. The policy for medical dispositions is generally oriented to the grounding of pilots and minor, if any, restrictions for other categories of personnel, including the medical one. The lack of a clear knowledge of the phenomenon among the Armed Forces of NATO countries is reflected by the very few precise answers to the last two questions in Table II, that is the number of seropositive military personnel until now discovered and the therapeutical behavior with AZT. My personal opinion is that also in this area a revision of behaviors would be desirable and could allow us to reach the ideal condition where military personnel would be considered a "walking blood bank" (7). The problem is even more important for pilots, considering that HIV infection may clinically start as a dementia. I would also like to underline the danger and the responsibility of performing compulsory vaccinations with live agents, such as BCG, smallpox, yellow fever, oral polio virus, in subjects whose serological situation is unknown and who can consequently be seropositive for HIV. Lastly, the social relevance and interest for civilian health services of the knowledge of epidemiological data for HIV among military personnel can not be forgotten. The frequent discussion of the unreliability and the high cost of serological tests for HIV are easily overcome by what has been reported by Col. DS Burke during the HIV session. In the USA experience serological HIV assays have a nearly absolute specificity, an acceptable sensitivity, a very low cost (3 US dollars for each determination) all attainable in a reasonable short time (72 hours).

Of equal importance is the fight against the other vaccine-preventable infectious diseases. The development of international travels (in 1985, close to 100 million travellers crossed international borders legally [World Tourism Association, unpublished data]) and the possible modern operational scenarios make the spreading of infectious and parasitic diseases very easy. Paradigmatic to this respect is the recent experience of the Persian Gulf War, during which the American Military Health Services were alerted on the problem of infectious diseases, mainly of the gastrointestinal tract, in the operational scenario and in Americans returning to USA from the operation "Desert Storm". The analysis of this problem has been published in "The New England Journal of Medicine" (8-9). In spite of these considerations, the last standardization agreement among the NATO countries in this field, which dates back to 1988, contains only general rules and recommendations. The actual analysis of the vaccination schedule for military personnel in the NATO countries (Tab.III) shows in fact a high variability of behavior. Such a variability should reflect the epidemiology of the infectious diseases as well as the vaccination schedule for children in the country. But, in addition to that, other factors are probably operating, such as the high cost of some vaccines (this could be the case for the hepatitis B vaccine) and lack of knowledge/experience with some new vaccines, the frequent consideration "This way everything is fine, why change?". A scientific approach to the problem should take into account the epidemiology of the infectious diseases in the country, the organization of a vaccination schedule for military personnel rationally linked to that for children, and possibly a good level of scientific research to continuously improve the quality of the single vaccines, in such a way to induce a higher percentage of seroconverters, higher antibody levels, in a shorter time. The reports of dr. Rappuoli (paper No 20), prof. Bellanti (paper No 24), dr Gordon (paper No 26), dr. Tagliabue (paper No 27) and dr Aguado (paper No 28) are clear and evident expressions that science today

can do that , and that each delay in adopting modern measures and technology to limit the spread of infectious diseases might be dangerous and guilty. Another consideration to be taken into account in the organization of the vaccination schedule is the possible operational scenarios of deployable troops, from a double point of view, the local epidemiology of infectious diseases and the possibility of the use of biological weapons, as recently dramatically underlined during the Persian Gulf War (a direct consequence of this last consideration seems to be the maintainance of smallpox vaccination in the US Army [10], in spite of the WHO statement on the disappearance of smallpox in the world, with subsequent unnecessity of vaccination). Not all the NATO countries presently comply with these considerations, and the vaccination of deployable troops is frequently an emergency operation. An up-to-date version of a standardization agreement on vaccinations for military personnel, with more stringent guide lines, would be highly desirable.

Lastly the identification of pilots with inhalant allergic diseases during screening procedures was another declared goal of our Meeting. The increasing pilot exposure during military flight with high performance aircraft imposes a fitness requirement also in relation to mild symptomatologies, such as allergic oculorhinitis. Consequently the Air Forces from all the NATO countries agree on the necessity not to enroll pilots with respiratory allergies. The real problem is the correct screening procedure. Allergic diseases, in fact, are pathologic conditions characterized by long periods of well being between crises. Consequently a diagnostic screening based only on the clinical history and physical examination could fail to identify all of the allergic military pilots. A serological screening based on an objective and efficient test is therefore necessary. A diagnostic test with good performance is now available and validated (paper No 31-32) and is included among the compulsory tests for the screening of applicants who wish to become part of the Italian Air Force. This experience should be useful for other NATO countries, where a serological screening is not performed (Tab.IV).

In conclusion this Meeting, with the expertise of the selected chairmen/speakers, clearly demonstrated the present importance of the immunoallergological and infectious problems, and the actual wide array of potential solutions. The most relevant practical result should be to generate the interest on the topics and to study the possibility of a gradual introduction, in the Armed Forces of the individual NATO countries, of the recommendations arising from the Meeting in a coordinate way. The coordination among AMP/AGARD, the biomedical group of NATO and WHO should be ensured by a permanent international Scientific Military Committee, whose operating arm should be an international laboratory of military Allergo-Immunology and Infectious Diseases "ex novo" set up in the seat of the Committee. Alternatively, the Committee could coordinate the activity of the individual national research laboratories of Immunology. Such a proposal should be the most relevant signal which AMP/AGARD may give to the National Authorities, NATO and WHO. The enthusiastic, qualified and highly expert attendees to our Meeting are the proof that the indispensable premise to such a realization, i.e. a sufficient body of researchers, even military, in this field, is already present.

7. ACKNOWLEDGEMENTS

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8. APPENDIX

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TAB. I

NATO POLICY FOR HBV SCREENING AND VACCINATION

	HBV SEROLOGICAL SCREENING		*HBV VACCINATION*	
	GENERALIZED	SELECTED CATEGORIES	GENERALIZED	SELECTED CATEGORIES
Belgium	NO	YES	NO	YES
Canada	NO	NO	NO	YES
Denmark	NO	NO	NO	NO
France	NO	NO	NO	YES
Germany	NO	YES	NO	YES
Greece	NO	YES	NO	NO
Italy	NO	YES	NO	YES
Netherlands	NO	YES	NO	YES
Norway	NO	NO	NO	YES
Portugal	NO	NO	NO	NO
Spain	NO	NO	NO	YES
Turkey	NO	YES	NO	YES
UK	NO	NO	NO	NO
U.S.A.	NO	YES	NO	YES

TAB. II

HIV INFECTION: BEHAVIOR OF NATO COUNTRIES REGARDING SCREENING, PERIODICAL CHECKS, MEDICO-LEGAL MEASURES, THERAPEUTIC APPROACH. PREVALENCE IN MILITARY ENVIROMENT.

	HIV TESTING						MEDICO-LEGAL BEHAVIOR			ESTIMATED # OF HIV (+)			THERAPY
NATION	AIRCREW	NON AIRCREW	MEDICAL PERSONNEL	ON ENTRY	PERIODICAL CHECKS	SPECIAL CIRCUMSTANCES	AIRCREW	NON AIRCREW	MEDICAL PERSONNEL	AIRCREW	NON AIRCREW	MEDICAL PERSONNEL	AZT STARTED AT
BELGIUM	YES	ON REQUEST	ON REQUEST	YES	NO	AFTER MISSIONS TO AFRICA	CASE BY CASE	CASE BY CASE	CASE BY CASE	2	U	0	CD4<500
CANADA	NO	NO	NO	NO	NO	NO	INITIAL GROUNDING (1)	RESTRICTIONS (2)	RESTRICTIONS (2)	2	17	1	CD4<400
DENMARK	YES	NO	NO	YES	NO	NO	NO	NO RESTRICTIONS	NO RESTRICTIONS	0	U	U	CD4<400
FRANCE	NO	NO	NO	NO	NO	BEFORE AND AFTER MISSIONS TO TROPICAL AREAS	GROUNDING FOR FIGHTERS, RESTRICTIONS (2)	RESTRICTIONS (2)	NO	6	U	U	CD4<400
GERMANY	NO	NO	NO	NO	NO	NO	NO	NO	NO	0/850	120/180 000	0	CD4<400
GREECE	YES	NO	NO	YES	NO	AFTER TRIPS TO CENTRAL AFRICA	GROUNDING	NO	NO	0	3	0	U
ITALY	NO	NO	NO	NO	NO	BEFORE MISSIONS TO USA	CASE BY CASE	CASE BY CASE	CASE BY CASE	U	U	U	U
NETHERLANDS	NO	NO	NO	NO	NO	BEFORE MISSIONS TO USA	GROUNDING	NO	NO	U	U	U	U
NORWAY	NO	NO	NO	NO	NO	BEFORE MISSIONS TO USA	GROUNDING	MEDICAL SUPERVISION	NON PATIENT DUTY	0	0	0	U
PORTUGAL	NO	NO	NO	NO	NO	BEFORE MISSIONS TO USA	NO	NO	NO	0	FEW RECRUITS	0	U
SPAIN	NO	NO	NO	NO	NO	NO	GROUNDING	MEDICAL SUPERVISION	NON PATIENT DUTY	0	U	U	U
TURKEY	NO	NO	NO	YES (3)	NO	BEFORE AND AFTER MISSIONS ABROAD	PUT OUT OF THE MILITARY SITE	PUT OUT OF THE MILITARY SITE	PUT OUT OF THE MILITARY SITE	0	0	0	U
UK	NO	NO	NO	NO	NO	NO	GROUNDING	CASE BY CASE	NO	U	U	U	U
USA (AF)	YES	YES	YES	YES	2 YEARS	NO	GROUNDING (CASE BY CASE)	NO OVERSEAS ASSIGNMENTS	NO INVASIVE PROCEDURES (CASE BY CASE)	0	18	118	CD4<500
USA (ARMY)	YES	YES	YES	YES	2 YEARS	(4)	CASE BY CASE	CASE BY CASE (5)	CASE BY CASE	27	2666	46	CD4<500
USA (NAVY)	YES	YES	YES	YES	1-3 YEARS	NO	GROUNDING	RESTRICTIONS DEPENDING ON WR STAGE (6)	NO INVASIVE PROCEDURES	11	1500		CD4<400

U= Unknown data (1) Returns to restricted duties if all investigations are normal; (2) If clinical and immunological examinations are abnormal; (3) Only for Turkish people who have worked in Europe. (4) Pending overseas assignments, pending selection to military shools, pending assignment to combat arms units deployable overseas, all routine hospital admissions, emergency care of trauma, rape, gunshot, and wound victims, pregnant females, prenatally, child and spouse abuse cases, patients admitted to drug and alcohol treatment programs, all contacts of HIV (+) individuals, all patients with sexually transmitted diseases, patients scheduled for outpatient surgery, all DOA's, personnel scheduled for OCONUS trips; (5) If asymptomatic, returns to duties inside the USA. (6)WR I-II remain on duty within 300 miles of a Navy Hospital.

TAB. III

VACCINATION SCHEDULE OF MILITARY PERSONNEL IN NATO COUNTRIES

NATION — TYPE OF VACCINATION

	Adeno 4-7	BCG #	Cholera	Diptheria	Influenza	Yellow F.	Measles	Meningo (tetra)	Mumps	Polio	Rabies	Rubella	Smallpox	Tetanus	Typhoid
BELGIUM			S		V	S				C		V		C	S
CANADA	C		S	C		C	C	C	C	C	S	C			C
DENMARK		S	S	C		S				S				C	S
FRANCE		C		C	S	S		S		C				C	C
GERMANY *			S			S							C	C	
GREECE		C	S	S		S				S				C	S
ITALY		C	S		V	S		C						C	C
NETHERLANDS *		S	S	C	V	S				C				C	C
NORWAY *		C	S	S		S		S		S	S			S	S
PORTUGAL (Air Force)			S	S		S				S			S	C	C
PORTUGAL (Army) *		C											C	C	C
PORTUGAL (Navy) *		C	S	S		S							C	S	S
SPAIN						S								C	C
TURKEY *		S	S	S						S			S	C	C
UK		S	V	S	S	V				V	V	S		V	V
U.S.A. (Air Force)	S			C	C	C	S	C	S	C		S		C	C
U.S.A. (Army)	C		S	C	C	S	C	C		C	S	C	C	C	S
U.S.A. (Navy)	C			C	C	S	C	S	C	C		C		C	S

C Compulsory, S=Selected categories, V Voluntary — * Data obtained from STANAG 2037 MED (Ed. 6 - 1988) — # On Mantoux negative subjects

TAB. IV

SEROLOGICAL SCREENING FOR INHALANT ALLERGIC DISEASES

	GENERALIZED	PILOTS
Belgium	NO	NO
Canada	NO	NO
Denmark	NO	NO
France	NO	NO
Germany	NO	NO
Greece	NO	NO
Italy	NO	YES
Netherlands	NO	NO
Norway	NO	NO
Portugal	NO	NO
Spain	NO	NO
Turkey	NO	NO
UK	NO	NO
U.S.A.	NO	NO

SPACE FLIGHT AND IMMUNE SYSTEM

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SUMMARY

Depression of lymphocyte response to mitogens in cosmonauts after spaceflight was reported for the first time in the early seventies by Soviet immunologists. Today we know that depression of lymphocyte function affects at least 50% of space crew members. Investigations on the ground on subjects undergoing physical and psychological stress indicate that stress is a major factor in immune depression of astronauts. This despite the fact that weightlessness per-se has a strong inhibitory effect of lymphocyte activation in-vitro. Although the changes observed never harmed the health of astronauts, immunological changes must be seriously investigated and understood in view of long-duration flights on space stations in an Earth orbit and to other planets like Mars and the Moon.

LIST OF SYMBOLS

Con A	concanavalin A
D-1	Spacelab Mission D-1
ELISA	enzyme-linked immunosorbent assay
HDT	head down tilt bedrest
IF	interferon
IL	interleukin
IML-1	International Microgravity Laboratory Spacelab Mission 1
IML-2	International Microgravity Laboratory Spacelab Mission 2
NK	natural killer
OAF	osteoclast activating factor
PHA	phytohemagglutinin
SL-1	Spacelab Mission 1
SLS-1	Space Life Sciences Mission 1
TNF	tumor necrosis factor
WBC	white blood cells

1 INTRODUCTION

The purpose of this paper is, first, to review and discuss the results of the immunological studies performed in space on humans, on laboratory animals and with isolated cells, second, to present parallel investigations carried out on the ground and, third, to analyze the impact of the changes on the health of astronauts during and after long-duration space flights. The work done so far was dedicated to the study of the specific immune system, while very little or nothing is known on the effect of space flight on innate immunity.

More than thirty years of manned space flight have clearly demonstrated that man can work and live for at least one year in space without immediate effects on his health. Very little is known, however, on possible "hidden" effects which may appear later after flight due to muscle and bone degradation in 0g or to the damages at the cellular level caused by cosmic radiation or to depression of the immune system. In fact, in the past the attention was focused on more evident symptoms of the exposure to weightlessness like the space adaptation syndrome (space motion sickness) and cardiovascular effects.

Concerning the immune system, it is difficult to develop diagnostic tests to provide immediate information on its efficiency in humans. In laboratory animals this is much easier since the specific immune response can be measured experimentally after immunization with an antigen.

The first report on changes in lymphocyte function was published by Konstantinova et al. in 1973 (1). Later, immunological tests were carried out by Kimzey et al. on US astronauts on the Apollo missions (2) and on Skylab (3). While the Soviets continued their immunological studies throughout the last 20 years, it is with the advent of the Space Shuttle and Spacelab that US and European investigators were able to study immunological changes systematically.
Part of the work presented here was carried out in my laboratory.

The immunological parameters measured were (i) lymphocyte activation after exposure to mitogens, (ii) cellular immunity by skin-test, (iii) immunoglobulin, α-interferon and interleukins levels in peripheral blood. Especially with the development of analytical kits, based on monoclonal antibodies, the analysis of lymphocyte subpopulations as well as of interleukins (interferon-gamma, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, TNF etc.) in the blood is becoming a common procedure. However, it is difficult to correlate the changes of these parameters with a real pathological situation. More investigations are required to assess the risks of immunodeficiency during and after spaceflight.

The main change observed in astronauts consists of the depression of the level of lymphocyte activation after flight. No health consequences were reported and the activation values returned to the preflight baseline within one week. Nevertheless, the fact that lymphocyte

function is impaired cannot be ignored. A comprehensive review on the subject will be published elsewhere (Gmünder and Cogoli (4)).

2 WHAT DO WE KNOW TODAY?

This section contains a short overview on the relevant results of immunological investigations in space. Related ground-based studies are also described.
In order to avoid misunderstandings, it is necessary to make an important distinction: One set of investigations consisted mainly of triggering lymphocyte activation in blood samples drawn from space crew members prior to, during and after flight. I.e. lymphocyte probes were incubated in a culture flask in the presence of a mitogen (see below). I call this approach "ex-vivo experiments".
In another set of studies, lymphocytes were purified prior to launch from the peripheral blood of test subjects who were not astronauts. The cultures were exposed to the mitogen during flight. I refer to this second approach as "in-vitro experiments". To try to correlate the results of the two approaches may lead to erroneous interpretations of the effects of space flight on the immune system.
Due to its importance as widely used lymphocyte test, it is useful to describe here how mitogenic activation and its measurement are performed in the laboratory.

2.1 The Lymphocyte System

Lymphocytes are easily purified from peripheral blood and can be activated chemically or by exposure to mitogens in culture. After activation cells start to proliferate, and T-lymphocytes secrete lymphokines (IL-2,IL-3, IL-4, Il-5, IL-6 as well as interferon). The mechanism of activation in-vitro is analogous to that occurring in in-vivo when the immune system is challenged by an antigen. The main difference is that activation by antigens is based on clonal selection of a restricted population of cells, whereas mitogenic activation is polyclonal, i.e. involves most or all T- and/or B-lymphocytes. Therefore, lymphocytes are a good model with which pursue three major goals of investigations in gravitational biology: (i) basic science, i.e how gravity affects differentiation processes, (ii) biomedicine, i.e. how the immune system of astronauts adapts to space flight conditions, and (iii) biotechnology, i.e. how the production of important substances like interferon and interleukins may be qualitatively and quantitatively evaluated and/or improved in space.

There are several substances having mitotic action on lymphocytes. Those used in space so far were Con A, a substance extracted from Canavalia ensiformis and PHA from Phaseolus vulgaris. Both substance are T-lymphocyte mitogens.

Lymphocytes are prepared in two ways: (i) cultures of purified cells are obtained by centrifugation of fresh blood on density gradients of Ficoll; (ii) "whole-blood" cultures were made by diluting blood with culture medium 1:10. The last method was developed in my laboratory to work with samples drawn in flight from crew members (5).

The extent of activation is measured (usually 72 h after the beginning of mitogenic stimulation) with two methods: (i) first, cells are incubated either with pulses of tritium labeled thymidine or uridine, second, the radioactivity incorporated either into DNA or RNA respectively is measured. (ii) the number of radioactively labeled cells is determined by autoradiography on microscope slides.
In addition, the extent of the activation can be judged qualitatively by comparing thin-section electron micrographs of active and resting cells. This method gives a reliable insight into the status of a lymphocyte culture.

While incubation with the mitogen, labeling of DNA or RNA followed by chemical fixation or criopreservation are carried out in space, measurement of radioactive label, autoradiography and microscopy are performed on the ground after the mission.

2.2 Human Subjects - "Ex-vivo" Experiments

Two kinds of measurements were used to establish the fitness the immune system of space crew members prior, during and after flight: one consisted of the determination of the blood levels of a number of parameters like differential leukocyte counts, immunoglobulin, complement and lysozyme levels, cytotoxicity index of natural killer cells; the other kind of measurement was based on the assessment of lymphocyte function upon activation with inducers and mitogens in cell cultures prepared from blood samples of the astronauts. Thereby the rate of DNA and RNA synthesis and the production of interferon and interleukin-2 were determined.

2.2.1 Immunoglobulins

No significant changes of IgG and IgM levels were found after the Apollo flights (2); the same was reported for IgG, IgA, IgM, IgD and IgE after the three Skylab missions (3). A large increase of IgA, IgG and IgM serum concentrations was observed after the 49-days flight on the Soviet space station Salyut 5 (6). This effect has been put in relationship with the secretion of autoantibodies against degradation products from the atrophy of skeletal muscles occurring during space flight. Less important immunoglobulin changes are also reported (1-4, 6-8). Immunoglobulins G, M, A, D and E concentrations in the serum of four crew men of Spacelab-1

in 1983 did also not show significant changes pre-, in- and post-flight (9). It is important to notice that these results do not necessarily exclude an impairment of B-lymphocyte function in space. In fact only the measurement of the specific response to an antigenic challenge in-vivo may give information on the efficiency of B-lymphocytes. For obvious reasons, such experiments may be carried out with laboratory animals (see below) but not on humans.

2.2.2 Lysozyme

Lysozyme, a protein with bactericidal activity, is localized in the saliva, and, in small quantity, in the blood plasma. Lysozyme levels were significantly lower than preflight in the saliva of cosmonauts after 49 days on Salyut 5 (8) and 96 days on Salyut 6 (7). Slightly higher levels were found in the serum of the Salyut 5 cosmonauts (7) and in two astronauts of Skylab II (3). No changes were observed in the serum of all other Skylab crew members.

2.2.3 Complement

C_3 was found higher after the Apollo flights (2), slightly decreased after Skylab II, unchanged after the other Skylab III and IV missions (3), unchanged after a 2-day Soyuz flight, significantly higher after 16, 18 and 49-days Salyut flights (6,8).
C_4 was unchanged after the three Skylab missions (3) and after 2, 16 and 18-days Soviet flights, whereas it was significantly higher after the 49-days Salyut 5 flight (6,8).

2.2.4 White blood cells counts

A dramatic increase in total WBC counts was always observed after spaceflight (3,10-12). Differential counts indicated that the number of circulating neutrophils was increased (the ratio between segmented and nonsegmented cells remained unchanged), while lymphocyte counts did not change significantly or decreased. For instance, Taylor et al. (12) reported an increase of 102% (mean of 41 subjects, range +348% to -21%) in leukocyte counts and a decrease in lymphocyte counts of -13% in 41 subjects (range -56% to +87%) immediately after several shuttle missions.

2.2.5 Lymphocyte subpopulations

Changes in numbers of lymphocyte subpopulations appear less dramatic and occurred within normal physiological limits. Taylor et al. (12) found no significant changes in subpopulations from 11 crew members after two Space shuttle flights. No changes were noted in the numbers of pan T-cells and size of suppressor subset, only a weak increase in size of the helper subset and a weak decrease in the numbers of B-lymphocytes. By contrast, after long-term flights (75-185 days), Vorobyev at al. (13) found a decrease in numbers of T-cells in most of the cosmonauts after landing. This finding was confirmed by Konstantinova (7,14) who noted a decrease in numbers of T-cells in 8 of 10 subjects and a smaller size of the B-cell subset in 2 of 4 cosmonauts.

2.2.6 Natural Killer Cell Activity

NK cell activity was measured in blood samples from several soviet cosmonauts by Konstantinova and her co-investigators in Hungary and in the USA (15-17). The NK cytotoxicity test consists of exposing ^{3}H-uridine labeled target cells (human myoleukemia K-562 cells) to NK test cells and to determine the radioactivity released after treatment with pancreatic RNase (17).
Cytotoxic activity of NK cells was measured prior to (30 days) and after flight (1, 7, and 16-76 days) in 35 cosmonauts from short-duration flights (7-10 days) and in 22 subjects from long-duration flights (112-366 days) respectively. The response varied considerably from one subject to another.
In 15 of 35 subjects from short flights there was a slight decrease of cytotoxic activity which lasted only few days and was back to baseline levels 7 days after landing.
In 11 of 13 subjects from flights lasting 112-175 days NK activity was decreased. The same was in 6 of 9 cosmonauts from 211-366 days missions. Despite the individual differences, Konstantinova et al. were able to identify three types of response: (i) 3 of 12 cosmonauts who spent between 112 and 175 days in space showed a remarkable decrease of the cytotoxic index (60-90% lower than preflight) on the first day after landing. Recovery to preflight level, however, occurred within 7 days; (ii) 5 of the 22 cosmonauts who spent at least 112 days in space showed no alteration of cytotoxic activity immediately after flight but a significant decrease in the following week. Recovery to baseline level was slow; (iii) 4 subjects displayed low activity immediately after landing followed by recovery time of 1-2 months.

2.2.7 Lymphocyte function

A. Lymphokines

Lymphokines are secreted mainly by T-lymphocytes upon antigenic or mitogenic activation. An important class of lymphokines are the interleukins. While IL-1 and IL-6 can be produced by activated macrophages as well as by activated B-lymphocytes, IL-2, IL-3, IL-4, IL-5, Tumor necrosis factor are specific T-cell lymphokines. Another important T-lymphocyte product is gamma-interferon. All these substances play an important role in the immune response. However their biological mechanism of action is not yet fully understood. Also most of the analytical tools to determine routinely their level in the blood were developed in the last two or three years. Therefore not little is known on how space-

flight may affect their production in the blood. The few data available concern IL-2, interferon-alpha and -gamma.

Interleukin-2: IL-2 biosynthesis by lymphocytes of cosmonauts was analyzed after induction in-vitro with PHA (17,18). Two aspects were considered: (i) Biological activity of IL-2 was evaluated by measuring its ability to induce proliferation of IL-2-dependent CTLL cells; (ii) the amount of IL-2 secreted was determined with monoclonal antibodies to a human recombinant IL-2 using the ELISA test. Interestingly enough, while biological activity in 12 of 13 cosmonauts (staying in space between 65 and 366 days) dropped significantly after flight (five subjects showed a drop of 50%), the amount of IL-2 produced was higher after flight in 8 cosmonauts. It appears that an inactive form of IL-2 was secreted.

Interferon: Lymphocytes from cosmonauts from two 7 and 9 days flights respectively were induced to produce alpha interferon in-vitro. In two subjects the level dropped by 75% one day after landing. In one of them it came back to preflight baseline within 6 days, in the other remained low. In other two subjects no change was observed, however both had an extremely low baseline (15,16). When gamma interferon biosynthesis was tested after short flights, a tendency to lower post flight level was noticed (17).

B. Cell Proliferation

DNA synthesis is confined to the S phase of the cell cycle and in lymphocytes it reaches its maximum between 60 and 72 h after exposure to the mitogen. The rate of DNA synthesis, measured as incorporation of ^{3}H-thymidine (usually a 2 h pulse after 72 h of incubation) correlates fairly well with the proliferation rate of the cells.
The increase of RNA synthesis begins shortly (within hours) after contact with the mitogen and can be measured with pulses of ^{3}H-uridine (usually 1 h) already after 20 h of culture. The rate of RNA synthesis gives an overall view of the activation of the lymphocytes. Both methods were used when assessing the capacity of lymphocytes from astronauts to be activated after space flight. The lymphocyte activation/proliferation test, based on the in-vitro activation either with PHA or Con A, has been widely used to check the fitness of T-lymphocytes after space flight. I have reviewed the data of the "pre-Shuttle" era in an earlier paper (19), Konstantinova et al. are giving an updated overview of the tests on soviet cosmonauts in Ref. (17).

Apollo (2): 21 subjects participating to seven missions (three of which to the Moon) lasting between 6 and 12 days were tested. No effect on lymphocyte activation by PHA was observed.

Skylab (3): The three missions lasted 28, 59 and 84 days respectively. The lymphocytes of 9 astronauts were exposed to PHA. RNA and DNA synthesis were measured. A remarkable depression of RNA synthesis (up to 90%) was detected after flight in 8 subjects. The effect was less evident after the longest mission, indicating a possible adaptation to the space flight conditions. Depression of DNA synthesis was also observed in 6 subjects, however the effect was much less evident on DNA than on RNA. All parameters recovered to baseline values within 7-21 days after landing.

Apollo-Soyuz (20): DNA synthesis was reduced in the three astronauts tested after a 9 d flight.

Space Shuttle (11,12): T-Lymphocyte activation by PHA was determined as DNA synthesis rate in 41 astronauts flying on 11 missions lasting between 2 and 8 days. Depression of activation was detected in 36 subjects. The average depression calculated for 41 subjects was 25.7%.

Spacelab D-1 (10,21): In an experiment carried out in my laboratory we tested lymphocyte activation (DNA-synthesis) by Con A on 4 Spacelab astronauts on whole-blood cultures. For the first time a 1 g reference centrifuge on board permitted to conduct the measurement in orbit on samples drawn in-flight on the third day in flight. Three subject showed depression (20-50%) in flight as well as immediately after recovery. Baseline value were reached within 7 days after landing. The experiment has been repeated on the recent flight (June 1991) of the SLS-1 mission. The data are not yet available.

Soviet Cosmonauts (1,17): The results from soviet cosmonauts are particularly important due to the duration (up to 366 days) of the space flight. Konstantinova et al. summarized the data obtained from 50 subjects by activating T-lymphocytes with PHA and measuring RNA and DNA labeling respectively. The mean responsiveness to PHA in terms of RNA synthesis was decreased by 20% in 16 subjects who spent between 122 and 175 days in space and by 14% in a group of 9 who were in orbit between 211 and 366 days. In contrast, the mean response was not lowered in 25 crew men who flew in space for 7 to 10 days. Less evident changes were detected, as an average, in all groups when DNA labeling was measured. For those cosmonauts showing depression of activation, recovery occurred within 3-7 days after the 7-10 days flights, whereas it took 2-4 weeks in the subjects from longer flights.

C. Skin Tests

Cellular immunity can be tested by skin tests in which an antigen is applied to the skin of the subject. The extent of the reaction can be determined by measuring the diameter of the induration on

the skin 48 hrs after the application. G. Taylor (paper presented at the 1990 Annual meeting of the American Society for Space and Gravitational Biology) carried out skin tests on several Shuttle astronauts. My Laboratory, in collaboration with the Institute of Biomedical Problems in Moscow is presently involved in a program of skin tests with soviet cosmonauts spending approximately 6 months on the MIR station.

2.3 Experiments with Animals

Rats were used for immunological experiments on a number of space missions. The two main advantages are that rats can be used for in-vivo experiments, i.e. their immune system can be challenged by injection of a selected antigen and that they can be flown on automated biosatellites.
Mandel and Balish (22) studied the specific cell-mediated response to Lysteria monocytogenes in rats flown during 19.5 days on the soviet satellite Kosmos 782. The rats were immunized before flight with the antigen, the lymphocytes isolated from their spleens after recovery were challenged with the same antigen. No difference in the response was found between flight and control animals. However, the extent of the response was low. A possible cause might have been the degradation of the biological samples due to the long time between sacrifice of the animals at the landing site in the USSR and the processing of the spleens in the USA.
Immunological studies were were conducted also on two recent Biokosmos flights (Kosmos 1667, 7 days and 1887, 13 days respectively) (17). Increases of splenic T-cells (identified as W3/13+), T-helper (W3/25+), T-suppressor (0x-8+) and cells displaying IL-2 receptors (0X-39+) were detected.
In addition, a higher amount of T-lymphocytes was found in the bone marrow after flight thus supporting the hypothesis of a stress reaction causing migration of blood cells to the bone marrow. Conversely, IL-2 production by the splenocytes of flown rats was increased.
Rats were also flown for seven days on the Spacelab 3 mission in 1985. Spleens were processed 12 hr after landing (23,24) and lymphocytes were exposed to Con A. IL-3 and interferon-gamma production was tested in the supernatants. While IL-3 levels remained normal, there was a clear reduction of interferon-gamma.
More immunological tests were conducted by US and soviet Scientists on rats flown on Spacelab SLS-1 in 1991. The data are not available yet.
Finally, several experiments with rats are planned on the automatic biosatellite LIFESAT presently under development by NASA.

2.4 Experiments in-Vitro

In this section I have briefly reviewed experiments with cultures of lymphocytes conducted in space. These are basic biological rather than immunological experiments. In fact, lymphocyte activation with mitogens in-vitro is a widely used model for the study of the mechanisms of mammalian cell differentiation. Therefore, as pointed out earlier in this paper, the results of such investigations shall not be misused to describe the effect of space flight on the human immune system.

My laboratory was deeply involved in this type of experiments since the flight of Spacelab 1 in 1983 (25). A review on the subject has been published recently (26).
Extensive studies in Space, stratospheric balloons, sounding rockets, in clinostats and centrifuges have revealed that lymphocytes in culture are sensitive to changes of the gravitational environment. Briefly, activation by Con A is reduced by more than 90% in real microgravity in space and by 70-50% in simulated low-g in the fast rotating clinostat. Conversely, activation is enhanced by 20-40% in the centrifuge at 10 g. Several hypotheses have been formulated to explain these effects as discussed in ref. (26). Signal transduction leading to activation of the T-lymphocyte system is an extremely complex and still poorly understood mechanism. Therefore, further studies in space are needed.

The most important question to be asked today is whether direct or indirect effects of gravity are playing a role in lymphocyte activation. Gravity may interfere directly with cellular structures and organelles (e.g. the nucleus, nucleolus and the cytoskeleton) or indirectly by altering the environment of the cell (e.g. cell-cell contacts, cell-substratum adhesion, fluid convection). The data available are in favor of direct gravitational effects on the cell. The analysis of an experiment on this subject carried out on SLS-1 is presently in progress in my laboratory.

Talas, Batkai et al. (15,16) reported that lymphocytes cultured on the soviet space station Salyut-6 in the presence of inducers were able to produce an amount of interferon-alpha that was five times higher than that of the corresponding ground controls.
In summary, there is no doubt the microgravity is causing profound metabolic alteration at the level of single cells in lymphocytes. Microgravity might be used as a tool to better understand the complex intracellular events leading to their activation.

3 THE CONTROL OF THE IMMUNE RESPONSE AND THE SPACE ENVIRONMENT

The data presented in the previous section clearly indicate that certain important aspects of the immune response are altered during and after space

flight. The main change is concerning the reduction of the mitogenic response in T-lymphocytes. The clinical significance of the effect is still unclear. Concerning the origin of the effect, the data from ground simulation strongly support a link between the physical and psychological stress of space flight and immunodepression. The neuroendocrine control of lymphocyte function in relationship with space flight has been reviewed recently by Gmünder and Cogoli (3).

The principal environmental factors and physiological systems interacting with the immune system are summarized in Fig. 1. Nearly all elements sketched in Fig.1 are related to manned space flight as shown in Fig. 2. In this section, these relationships are analyzed and ground-based key investigations are reviewed.

3.1. The Space Environment

Stress: It is well known that stress, both psychological and physical, plays an important role in the efficiency of the human immune system, (27,28). Stress provokes a response of the neuroendocrine system, resulting in a change of the blood levels of a variety of stress-associated hormones and neuropeptides. These included ACTH, cortisol, catecholamines, enkephalins, and endorphins.

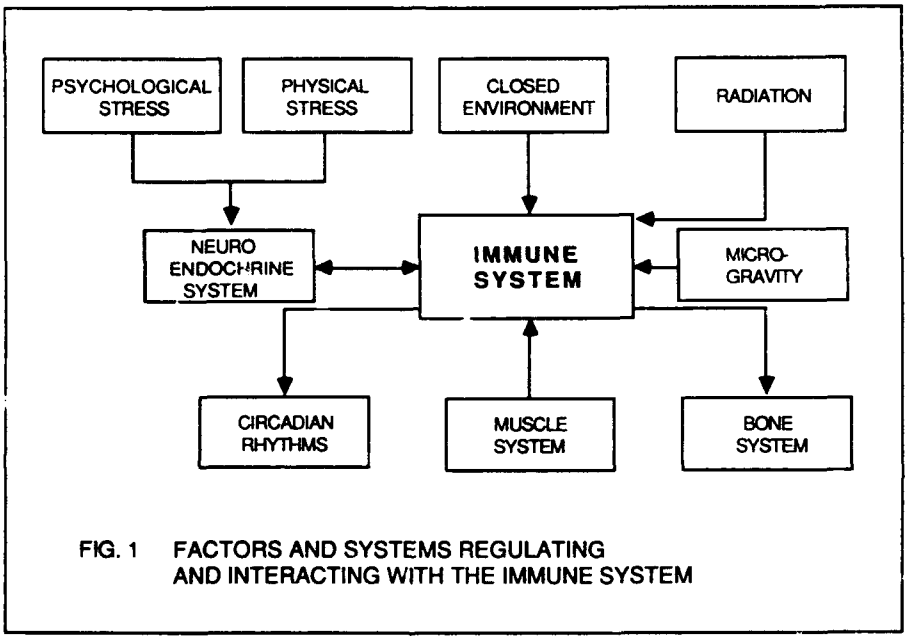

FIG. 1 FACTORS AND SYSTEMS REGULATING AND INTERACTING WITH THE IMMUNE SYSTEM

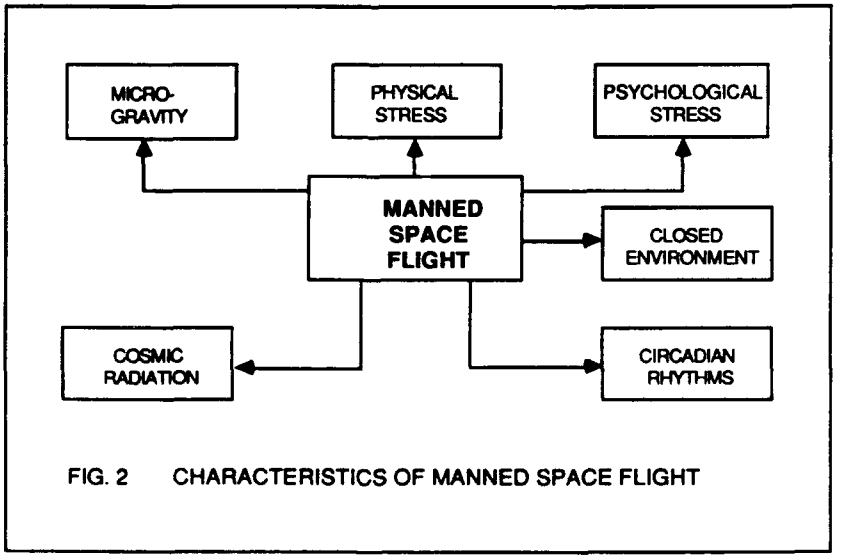

FIG. 2 CHARACTERISTICS OF MANNED SPACE FLIGHT

Immunocompetent cells express specific receptors to these hormones and neuropeptides and in turn secrete ACTH, endorphins, enkephalins and lymphokines that may affect the nervous system and other immunocompetent cells. Thus the neuroendocrine and immune systems seem to form a complete regulatory circuit (27).
The type and amount of each stress-associated hormone and neuropeptide release may alter depending on the sort of stress. This flexible response may result in different types and degrees of immunodepression.
The mitogenic response of lymphocytes to con A is significantly reduced following in-vivo administration of corticosteroids (29). Therefore, it is conceivable that spaceflight which is regarded as a psychological, psycho-social and physical stress may depress immunity.

Circadian Rhythms: PHA-responsiveness is modulated by the circadian changes in plasma cortisol levels (30). It is obvious that living and working in a space station, without the usual Earth-bound day-night cycle, will cause significant changes in several physiological functions.

Radiation: Cosmic irradiation is, together with microgravity, the most important environmental parameter affecting living systems in space. Astronauts on the Space Shuttle and in the Spacelab module are exposed to radiation doses of approximately 10 mrad/day (31). Doses of 127mrad/day were measured during the Apollo 14 lunar orbital flight. Damage by cosmic rays, e.g. by heavy ions, on the cellular level car be expected during manned space missions. An analysis of the effects is given in Ref. (32). In addition, according to the "Theory of hormesis" low radiation doses may have a positive stimulative effect on biological systems (33).

Microgravity: Reduced gravity in space leads to profound changes in a number of physiological functions (34). For instance, within the first minutes of weightlessness fluid shifts from the lower limbs to the thorax and the head. This shift (2-3 liters in volume) persists during space flight although a slight normalization is noted after 2-4 days in space.
Another effect is the loss of water (approx. 2 liters) inflight, known as the Gauer-Henry effect (35). It has been shown that under the conditions of altered fluids distribution in the body, the control systems of fluids, electrolytes, blood pressure and metabolism are affected as well (36). It is conceivable that these changes are reflected also in the environment of the lymphocytes distributed in the blood, in the lymph and in their storage organs like the spleen and the lymph nodes. Consequently, lymphocyte function may also be affected.

Closed environment: Immunological fitness against infection is maintained through the continuous challenge by the infectious agents of the environment. This is not the case of people confined in a close and controlled environment for prolonged periods of time like in a spaceship. Therefore, astronauts may be exposed to higher risks either by visiting crews, as it is the case on a space station like MIR, or after return to Earth. A parallel between people in antarctic and space station is discussed by Cosman and Brandt-Ruf (37). Although earth-bound studies of this kind are delivering important information, the data available are not yet sufficient to draw conclusions and more investigations on the subject are required.

Bones: A new hypothesis linking bone metabolism and immune system in astronauts has been formulated by Konstantinova (7). It is known since a long time that prolonged exposure to weightlessness causes loss of calcium and bone structure in a way very similar to osteoporosis. Whereas bone formation depends on osteoblasts, bone resorption is initiated by osteoclasts. Osteoclasts originate from hemopoietic stem cells like macrophages and are released from the bone marrow. They collect at sites of bone resorption. Bone formation and resorption are regulated by a variety of growth factors. In the early seventies it was discovered that lymphocytes produce the lymphokine osteoclast activating factor, OAF. OAF, in the presence of prostaglandin PGE_2 produced by monocytes, leads to the activation of osteoclasts at sites of bone resorption (38,39). It appears that there are lymphokines with OAF-like activity like IL-1, TNF-α and -β, but their roles in bone metabolism are poorly understood. Recent investigations on the relation between immune and bone systems during and following HDT bedrest revealed interesting results (Ref. 7,40, see section 3.2.2).

Muscles: According to another new hypothesis formulated in 1988 by Newsholme et al. (41), there is a close relation between muscles and the immune system: Lymphocytes and macrophage display a very high rate of glutamine utilization. The main source of glutamine is muscle and liver and only a small proportion of the total dietary glutamine enters the body as such. Glutamine is essential for the synthesis of DNA and RNA during lymphocyte activation. According to the Authors, skeletal muscle metabolism can be viewed as part of the immune system. The fact that muscle degradation is occurring during space flight might be related to some of the effects observed on immunological parameters.

3.2 Ground-Based Simulations

The implication of the stress of space flight and of the fluid shift in microgravity in the depression of the immune system has been investigated by Gmünder et al. in my laboratory. Two ground-

based simulations have been used: long-distance running and HDT bedrest.

3.2.1 Physical Stress

The response of critical immunological parameters was measured in 24 subjects undergoing the sustained physical stress of long-distance running (8 subjects run the marathon distance of 42 km, 16 subjects run 21 km) (42,43). Immediately after the run lymphocyte responsiveness to con A was severely depressed to 1-70% of the values at rest. Lymphocyte counts did not change. Leukocyte counts were increased 2.8-fold. No dramatic changes were found within the lymphocyte subsets although an increase in pan T-cells and in the helper/inducer subset was significant 2 days after the run. In addition, the numbers of B-cells decreased significantly. No change was observed within the suppressor/cytotoxic subset. Cortisol increased 2.1-fold, epinephrine 3.2-fold and norepinephrine 2.7-fold. All these parameters returned to baseline values within 2 days. These data were compared with data obtained during and after space flight. It was concluded that the prolonged physical stress of marathon and ½-marathon running induces changes in immunological responsiveness that are strikingly similar to those arising from the stress of space flight (42,43).

3.2.2 Head Down Tilt Bedrest

A protocol analogous to that used above was applied to a study on six males undergoing bedrest and head down tilt test for 10 days by Gmünder et al. (44). HDT test is normally used to study fluid redistribution and adaptation of the cardiovascular-pulmonary and renal system to weightlessness. Again, lymphocyte activation by con A was severely reduced immediately before, during and after HDT bedrest. In contrast, delayed-type hypersensitivity (skin test) was not affected. Counts of WBC, total lymphocytes, T-lymphocytes and T-cell subsets did not change with the exception of NK cells. NK cells decreased markedly in all test subjects immediately after HDT test and returned to baseline levels within 6 days. Plasma cortisol levels were above normal before and during HDT test, indicating that the subjects were under stress. At difference from the runners test (see above), epinephrine and norepinephrine levels remained within the normal range thus giving a closer analogy to what is seen in astronauts.
The data suggest that the mitogenic response of lymphocytes was affected by psychological stress before the HDT test and probably by a combination of psychological- and fluid shift-stress during the test. It was concluded that the HDT-test gives even a better simulation of the effect of space flight on immunological parameters than long-distance run (44).

In another HDT bedrest study lasting 370 days (40), an increase in the production of OAF was noted in the supernatants of peripheral mononuclear blood cells from the test subjects. The increase in OAF, however, coincided with a steady increase in lymphocyte responsiveness to PHA during the HDT test. This is in contrast with what Gmünder et al. (44) observed during the 10 days test and with the loss of lymphocyte responsiveness after space flight.

4 WHAT SHALL BE DONE?

A group of scientists from the USA, Europe and the Soviet Union (R. Ballard, W. Berry, S.K. Shapes, A.Cogoli, G. Coulter, R. Janney, B.Masterton, D. Morrison, R. Meehan, A. Rakhmilevich, L. Schaffar, D. Schmitt, G. Sonnenfeld, G. Taylor and P. Todd.), involved in investigations on the behavior of lymphocytes in microgravity, met in November 1990 to discuss the state of the art of the study of the immune system of man in space. It was recognized that too little attention was paid to the issue so far and that more work is required to fully understand the potential risks of an impaired immune function in space. The issue is particularly important in view of the long duration flights on the International Space Station Freedom, the establishment of colonies on the Moon and the three-years travel to and from Mars. In fact, despite individual differences, several immunological parameters are changed in a large number of astronauts during and after flight.
A program of immediate research needs involving astronauts as test subjects has been suggested. The main points are summarized here:
* Determine the immune response of space crew members to common antigens in terms of antibody production and delayed type hypersensitivity reactions.
* Further investigate the T-lymphocyte response of space crew members to non-specific mitogens like Con A and PHA in terms of IL-2, IL-3 and gamma-interferon production, of expression of IL-2 receptors and of blastogenic response.
* Examine the function of neutrophils, mononuclear cells and macrophages.

Based on the findings, further steps can be planned in order to gain a full picture of the impact of space flight on the immune response.

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MECHANISMS OF IMMUNE FAILURE IN BURN INJURY

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Summary

The burden on military medical services in handling burn casualties is daunting as all physiological systems will become affected. Severe burns in a battlefield setting have a very low salvage rate, to a great degree because of the immune failure which invariably develops. Evaluations of responses of lymphocytes taken from burn patients over several weeks following the burn (> 30% TBSA), have revealed that the immune failure which follows thermal injury involves T cell activation events. Interleukin 2, which is normally produced by activated T lymphocytes, is very poorly produced by cells cultivated in vitro taken from non-surviving patients, whereas some production continues, although at below normal levels, in patients who ultimately survive their injury.

IL2 exogenously added to lymphocyte cultures enhances the proliferation of cells from surviving patients but gives no such help to cells from non-survivors. The TAC portion of the IL2 receptor (IL2Rα), expressed on the T cell surface, appears to be responsible for this difference, as the number of lymphocytes able to express IL2Rα falls post-burn. A lipid protein complex (LPC) produced in skin by burning has been shown to inhibit the immune response in vivo and the growth of IL2-dependent lymphocytes in culture. Cerium nitrate, applied topically to the burn patient, is thought to fix the LPC in the burn eschar and prevent its entry into the circulation. In a study of 10 patients, bathed in cerium nitrate, some T lymphocyte activities were found to be in the normal range rather than suppressed. Such a treatment promises to be useful in improving chances of survival in severe burn injury.

Introduction

For military medical services the burn casualty poses enormous logistic problems, since the burn, as a form of trauma, evokes to an exaggerated degree, all of the systemic responses seen in other injured patients. An extensive burn makes the patient with such an injury the universal trauma model. Not only is there extensive skin damage but potentially every organ system can become affected. It follows that all treatment procedures applied to patients, and information gathered from studies of burned patients, have application also to all other trauma patients (24). Hence, research into burn injury is actively supported in many world centres and any understanding which leads to novel approaches to treatment may reduce the burden of care universally.

Unlike mechanical injury the burn induces quantitative changes in relation to the magnitude of the injury, and all systems may become affected in time, after a severe burn injury (19). Reduction of early death due to shock and acute renal failure achieved by fluid resuscitation, has only revealed later, other previously obscured problems. Once out of the critical stage severely burned patients typically fluctuate from serious to critical condition several times as respiratory, cardiovascular and infectious complications develop. In a military setting stabilization of the patient may be the initial challenge, but appropriate longer term care of the patient has to be modified according to the service in the available time frame. On the assumption that problems of casualty sorting, of evaluation, and first treatment techniques are optimized, the major problem for the burn casualty then is the ultimately higher than normal risk of late death simply due to delay in achieving high level critical care.

Very early fluid resuscitation may appear to be critical for survival, but only in the short term. This was demonstrated by the disaster at the *Los Alfaques* campground in Spain where a tanker truck, carrying a volatile liquid fuel, crashed on the roadside at the camp. The fuel's expanding vapour engulfed 242 campers in a flaming explosion. The truck blocked the road such that of the 140 immediate survivors 58 were evacuated north to Barcelona and 82 south to Valencia. Only the northbound group received fluids en route, from small local hospitals. Thus, as the *Los Alfaques* disaster has revealed (3), by having two comparable groups of burn victims of 85.5 ± 19.5 % total burn surface area (TBSA) and 81.2 ± 24.0 %TBSA, with ages of 26.3 ± 17.0 and 28.0 ± 16.2 respectively, early fluid resuscitation in only the first group, permitted greater survival for the first few weeks but no significant difference between groups after two months (21% and 28% surviving) (Figure 1). It is for these reasons that heroic first attempts to treat the severely burned, with existing procedures, under wartime conditions, will have doubtful long term consequences. This fact underlies policies for treatment on the battlefield.

With increasing numbers of burn casualties in warfare (Table 1) the burden on field medical services becomes daunting. In anticipation of these events some NATO countries have defined their policy for choosing which burn cases will be treated. Whereas a young healthy adult burn patient, in a civilian setting, will have a risk of survival of 0.5, if his lesions represent 60% TBSA (5), there have been policies stating that casualties with burns

over 40% are "beyond the scope of therapeutic capabilities in times of crisis" (8). These thresholds have been supported by the indication that with burns over 60% TBSA the salvage rate of military patients has been low and survival could not be assured (32).

The main cause of death after severe burn injury has been listed as sepsis (25), which most often is accompanied by multiple organ failure (MOF). However, the apparent lack of resistance of the burn patient indicates a "host problem" rather than a problem of increased virulence of the invading microorganism. Furthermore, in the late stages of burns circulating bacteria cannot be detected in up to half the patients who die from what appears to be sepsis (9). Hence the MOF is thought to be due to devitalized tissue activating phagocytic and other cells to secrete nefarious mediators which dysregulate homeostasis.

A significant effect of these burn-induced mediators is on host defences, and this in particular involves first the destruction of the skin barrier, the impairment of tissue blood flow, the depression of the immune response, and then the secondary effects of therapy and complications. Implications of cellular immune response failure (T lymphocyte failure) had been suggested by observations in burn injury, noted over 4 decades ago, of prolonged allograft survival in skin grafting procedures (4). Graft rejection represents activity of the T lymphocyte compartment of the immune response, and other T cell functions were also noticed to have failed following burn injury, such as the delayed type hypersensitivity (DTH) response (23), the proliferative response of lymphocytes to a T cell mitogen (7), the cytolytic response (16), and the mixed lymphocyte response (20) (Table 2).

Immune Failure in Burn Injury.

In 1984 the Defence & Civil Institute of Environmental Medicine initiated an investigation into the mechanisms of immune failure in burn injury, through the Ross Tilley Burn Centre at the Wellesley Hospital, Toronto.

To study T cell activity of burn patients, peripheral blood mononuclear cells (PBMC), which represent 27% of the leukocytes (Table 3), were taken every week or ten days over the course of hospitalization, and *in vitro* lymphocyte IL2 production was assessed by culturing them with mitogen and assaying the culture supernatants for IL2 by the proliferative response of an IL2-dependent cell line (36). IL2 production was below normal, and both non-survivors and survivors could not be differentiated on the day of the burn. However, over the hospitalization period, *in vitro* IL2 secretion levels increased closer to normal levels in survivors, but decreased to undetectable levels in non-survivors (Figure 2).

An assay of the PBMC proliferative response in the presence of exogenous IL2 is shown in Figure 3. Low responses to IL2 on day 1, and a week later, were common to all patients. However, survivors' responses recovered by the third week in contrast to those of the others. Once again a marked difference was observed between survivors and non-survivors with regards to IL2 activity in the burn (37).

Survivors' PBMC cultivated *in vitro* 3 days with mitogen, displayed a high level of the surface α receptor for IL2 (IL2Rα or TAC) when cells were taken on the day of the burn. Figure 4 shows that at some point in the second or third week the IL2Rα levels would drop, but later return, in survivors, to day 1 levels. The lowest point may be Day 10 or 20 or both, as shown for 3 selected patients (Figure 4). In non-survivors the cell surface IL2Rα would steadily decrease from Day 1 to the time of death (36) (Figure 4). Overall results from a large group of patients showed that during the course of hospitalization, IL2Rα would be expressed on cells at the time of the lowest levels observed, at a mean level of about 50% of the surviving individuals' first day levels, but at virtually undetectable levels for non-survivors (Figure 5). Low levels of *in vitro* lymphocyte IL2 production and low levels of the α IL2 receptor, inducible *in vitro* on the surface of the lymphocytes, appeared therefore to be critical indices for non-survival, and suggested problems with the T cell activation mechanism.

Burn-Induced Eschar Toxic Products.

For over a century (39) researchers have believed that burn injury causes the production and/or release of mediators which have toxic or suppressive effects on normal physiological functions. Indeed, many T cell functions are inhibited by factors circulating in the serum (Table 2). Present knowledge of the cytokine cascade and its relationship to prostaglandin activity may provide ready explanations for the inhibition of any particular function, as the cytokines (e.g the interleukins) serve to mediate a regulatory system within the immune response, consisting of specific ligands and cell membrane receptors. As we have seen, it is this cytokine system which has clearly become dysregulated in burn injury, more so in non-survivors than in survivors.

Quantitative differences in burn immunosuppression were found to bear a clear relationship with quantity and type of burn tissue. Mice implanted with increasing amounts of burned skin (eschar) (but not burned liver, for example), were increasingly immunosuppressed (10). The quantitative toxicity of burned skin has also been shown to be related to the burn temperature (1). Consistent with these two findings, in the burned mouse, immediate excision of the eschar avoided immunosuppression (11). Beneficial effects of prompt eschar excision have also been demonstrated in humans, particularly in burned children (6,38) and young adults (12). Conclusions to the contrary (15) depend on how early the excision is made, for

burned skin has been shown to contain a toxic product (1,2) which can appear in the circulation within a day of the burn injury (33). This particular eschar product is not a tissue breakdown product but a heat-induced cross-linking of a complex of six skin cell membrane lipid-associated proteins (30). They are combined to form a lipid protein complex (LPC), of about 3 million kDa, which was shown to damage cell ultrastructure in the same fashion as does burn injury (13). LPC also disturbed cell metabolic function by its effect on mitochondrial membranes (29). Altogether many effects of the LPC have been found to mimic the various consequences of burn injury (14), and these even include immunosuppression. In vivo, LPC enhanced the mouse susceptibility to pseudomonas infection (31), and the mouse immune response to sheep erythrocytes (Figure 6), being 1000 times more immunosuppressive than bacterial endotoxins on a molar basis (34). It was also inhibitory of the T lymphocyte proliferative response to PHA *in vitro* (21), and inhibited PWM-induced IgG production (35) as well as the growth of IL2-dependent cells in culture (34) (Figure 7). Thus, it is implicated in the very mechanism, T cell activation, which appears to be critical to surviving major burns.

Use of topical cerium nitrate in burn treatment.

Serving as a topical antiseptic for burn wounds silver nitrate had been used extensively since 1945. However, it blackened the bed clothes and affected electrolyte balance of the patients adversely. Sulfamylon was also introduced as a topical agent but it also had drawbacks. Alternatives were sought and cerium nitrate and silver sulfadiazine (SSD) were identified. When assays for antibacterial activity were performed with these two agents SSD demonstrated superior antibacterial activity in *in vitro* tests on bacterial plate cultures, and thus rose to prominence. Its use is nearly universal today. However, in clinical trials on burn patients, Monafo, using topical cerium nitrate solution on gauze dressings, observed an unusually high survival rate among patients whose burns warranted a prediction of a lower survival rate (17,18) (Tables 4, 5). Cerium particularly minimized the incidences of late death in burn victims with larger %TBSA, yet clinically it appeared not to be a superior topical antiseptic, confirming the *in vitro* comparisons. However, its weaker antiseptic value is nevertheless useful in synergism with SSD (27).

The use of cerium in treating burns has not yet been widely accepted and is still at the experimental stage. However it became appreciated that the reports of improved clinical outcome from use of topical cerium might be explained by the local destruction of the toxic lipid protein complex in the burn eschar. Studies on the binding capability of cerium with the complex revealed a high affinity of the two with consequent denaturation of the LPC (13). Therefore it was reasoned that one bathing, long enough to allow for good contact of eschar toxic complex with cerium nitrate, should inhibit LPC resorption from the burned tissue into the circulation, effectively inducing a "chemical excision" of the eschar's dangerous properties. Indeed, experiments had confirmed that topical application of cerium nitrate to scalded skin of mice prevented the T lymphocyte failure common to the conventionally treated burns (22).

Based on the binding affinity of cerium for the eschar toxic complex, the intensive care unit of the Kantonsspital, Basel, Switzerland, has, for a number of years, used a treatment routine for burn patients which involves one tubbing only at the time of admission, in 0.04M cerium nitrate. The incidence of survival in large burns has been found to be exceptionally high (28) with 9 out of 10 patients surviving risks of mortality (calculated according to *Roi et al. (26)*) greater than 0.8 (Figure 8). A risk of 0.8 implies that 8 out of 10 patients with such complications should die, yet several survived with risks greater than 3 patients who did die, of particular complications not strictly burn related. With lower risk patients, who might all be expected to survive, the value of cerium is not revealed by checking survival or death. However if cerium binds to the LPC in the eschar and LPC is a key agent initiating immune dysregulation, the parameters of the immune response should be improved in patients who receive cerium treatment.

A group of patients was therefore bathed once in 0.04M cerium nitrate and their PBMC were taken at ten day intervals for an assessment of lymphocyte surface IL2Rα induction and of IL2 secretion, in response to mitogens *in vitro*. Compared to former burn survivors whose lymphocytes displayed, at the time of lowest immune responses, a surface IL2Rα level around 50% of the first day level (Figure 5), the cerium treated group was improved in this assessment. Preliminary results showed that the mean IL2Rα level had dropped only to 73% of the first day levels (Table 6; *manuscript in preparation*). The *in vitro* IL2 production by burn survivor's lymphocytes, which had been consistently below normal levels (Figure 2) was found to be in the normal range (Figure 9; *manuscript in preparation*) in comparable patients bathed once in cerium nitrate (Table 7; *manuscript in preparation*). Thus, preliminary results suggested that this treatment attenuated the immunosuppressive activity. This was demonstrable even in burn patients whose burns may not necessarily have led them to mortality, and who thus may not have been distinguished by a survival/non-survival classification.

Further examination of such results is continuing but it is noteworthy that cerium treatment promises hope for severely burned patients, to survive against previously estimated enormous odds. Casualties of burns on the battlefield, if treated one way or another with cerium, may therefore look forward to a more enthusiastic triage, the optimism coming equally from the military medical services who bear this responsibility.

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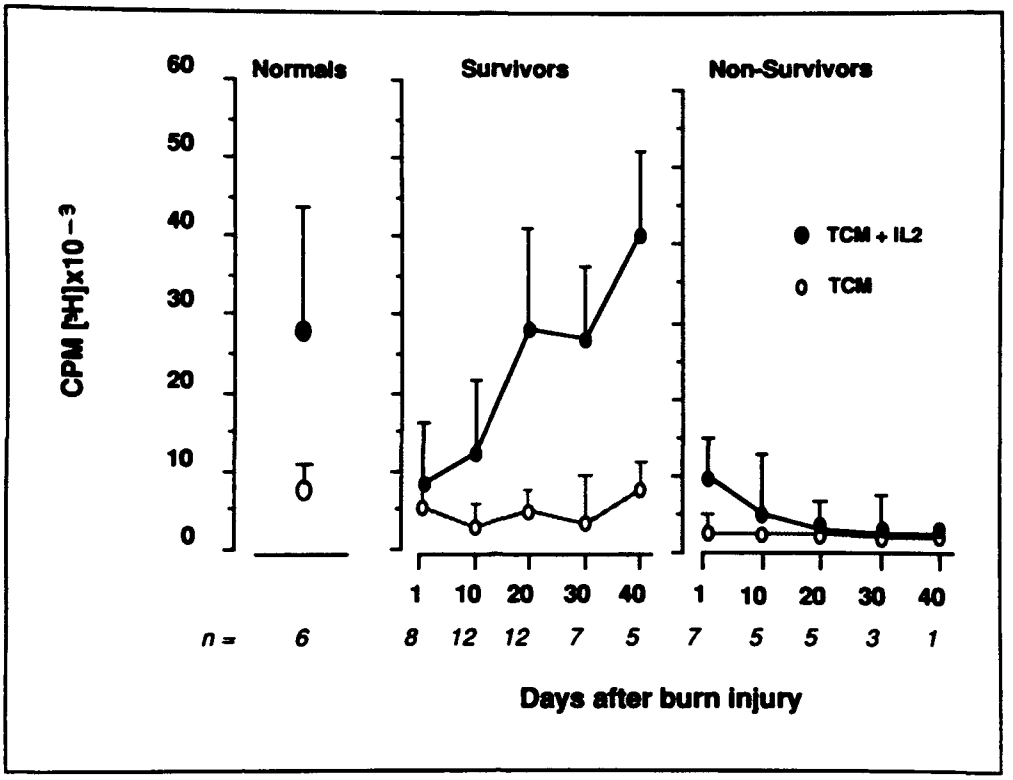

Figure 3. Mitogenic effect of exogenous IL2 added to the tissue culture medium (TCM) of lymphocytes from normal control subjects and burn patients distinguished as survivors or non-survivors (Ref. 37).

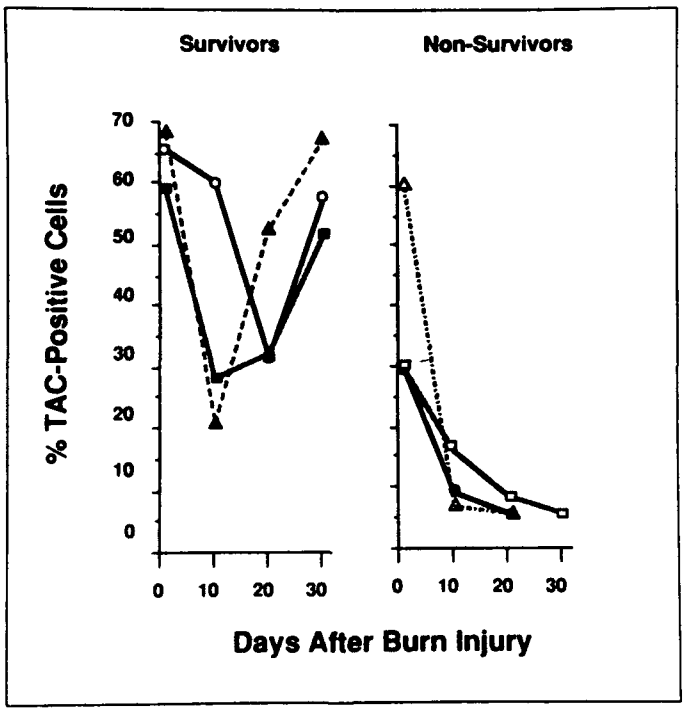

Figure 4. Percentage of the PBMC population displaying the surface IL2 receptor (IL2Rα, or TAC), after *in vitro* cultivation with mitogen (Ref. 36).

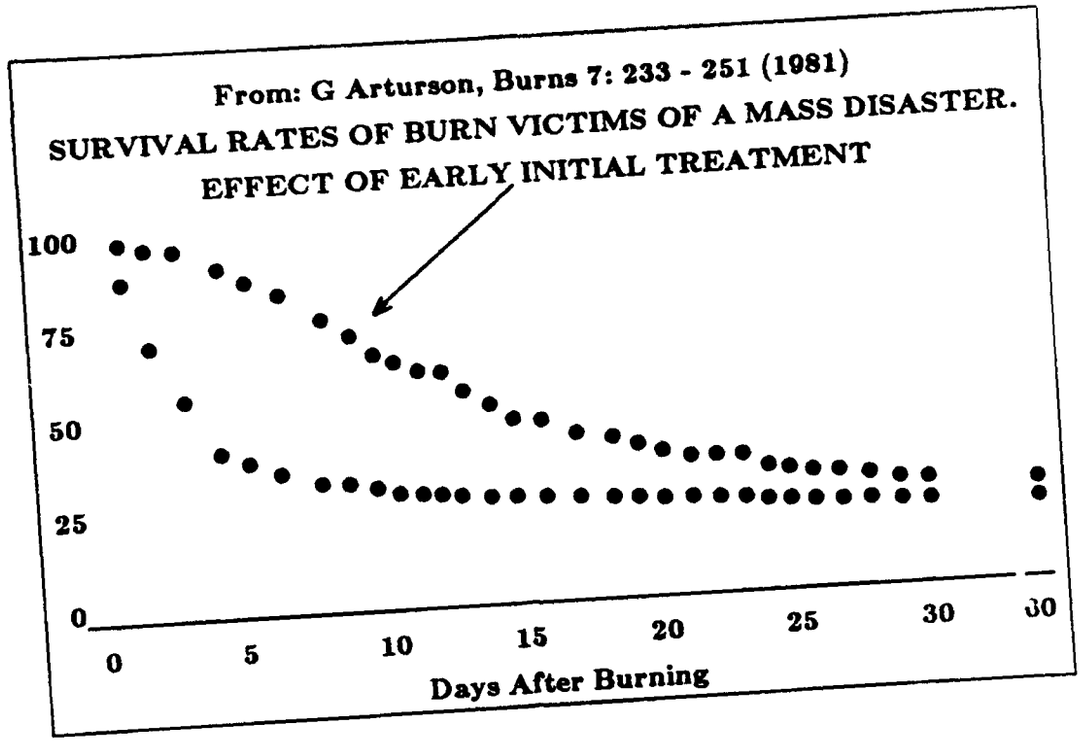

Figure 1. Percent survivors in each of two groups of burn victims, one (upper curve) which had received early fluid resuscitation (Ref. 3).

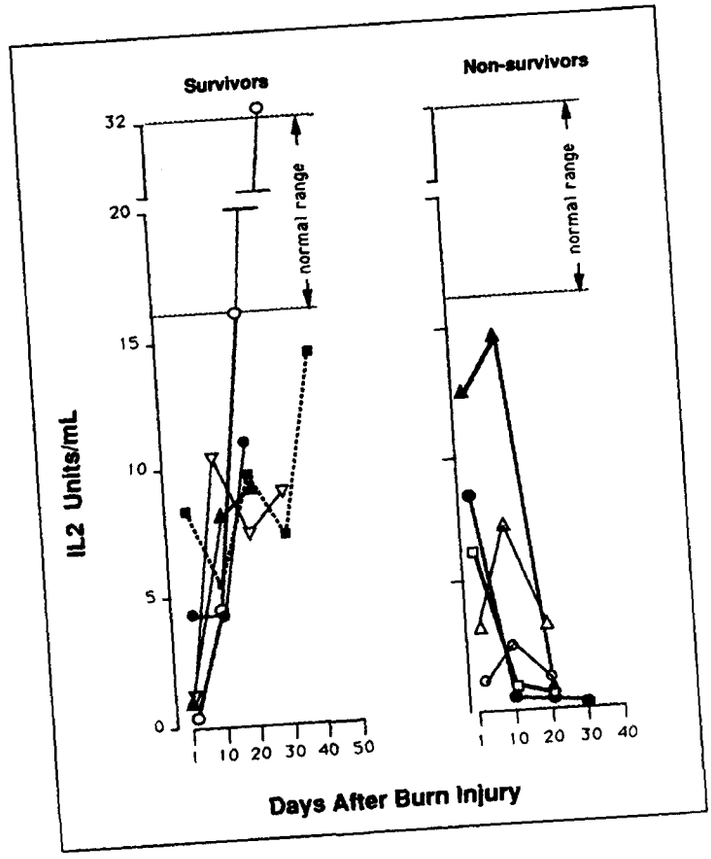

Figure 2. IL2 production by burn patients' PBMC stimulated by mitogenic lectin *in vitro* (Ref. 36).

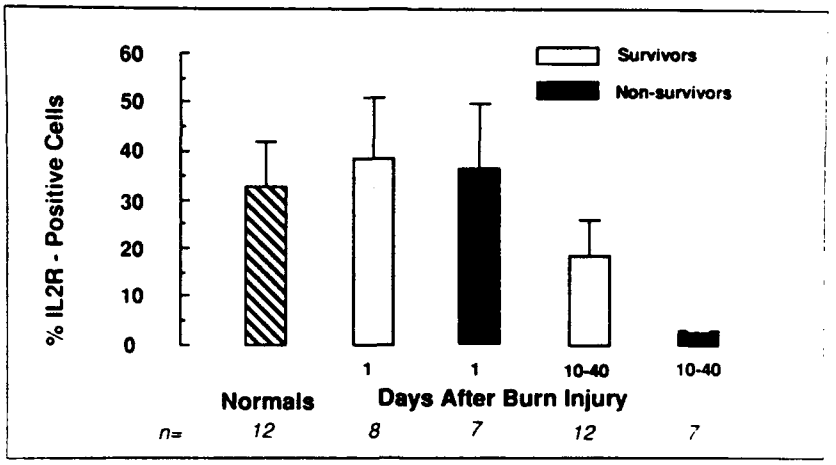

Figure 5. Percentage of the PBMC population displaying the IL2 receptor (IL2Rα or TAC) in normal subjects and in burn patients on Day 1 and at the time of the patients' lowest level of surface receptor (Ref. 37).

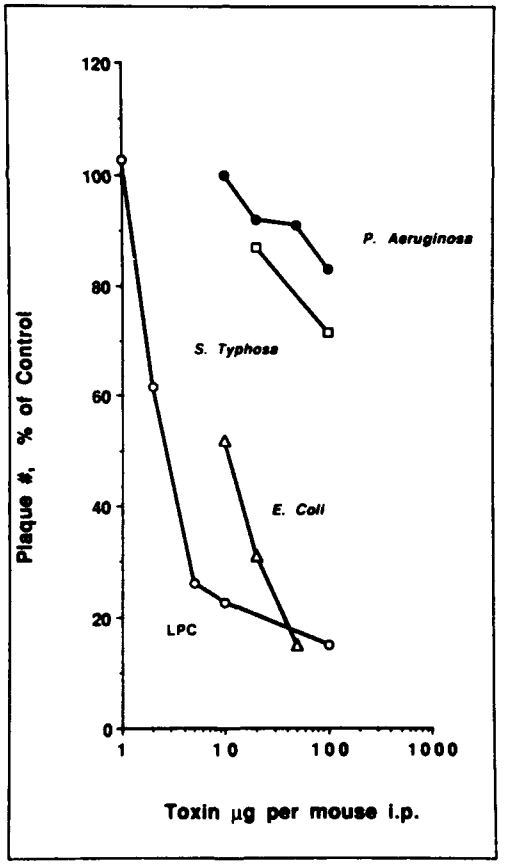

Figure 6. Effect of toxins on the plaque forming cell response *in vivo* in mice responding to antigen. Endotoxins from three bacteria, and the lipid protein complex (LPC) from burned skin were given two days before the antigen sheep erythrocytes (Ref. 34). For comparison on a molar basis weight of toxin should be divided by 3 million for LPC and by 20,000 for endotoxins.

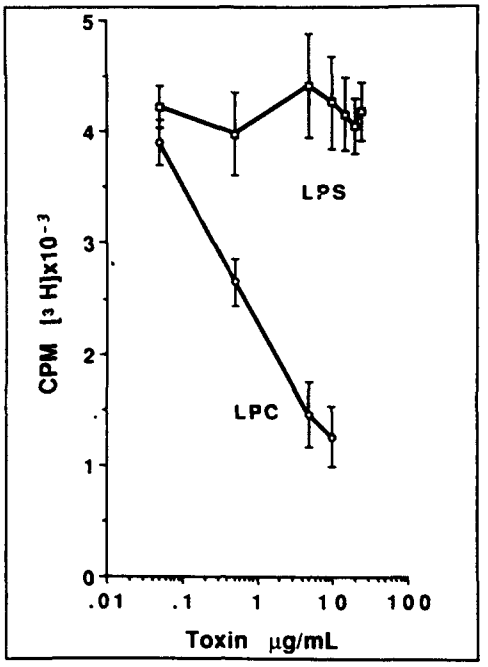

Figure 7. Effect of lipid protein complex (LPC) on growth of IL2-dependent cells in culture, in the presence of IL2, in comparison with the effect of endotoxin (lipopolysaccharide; LPS).

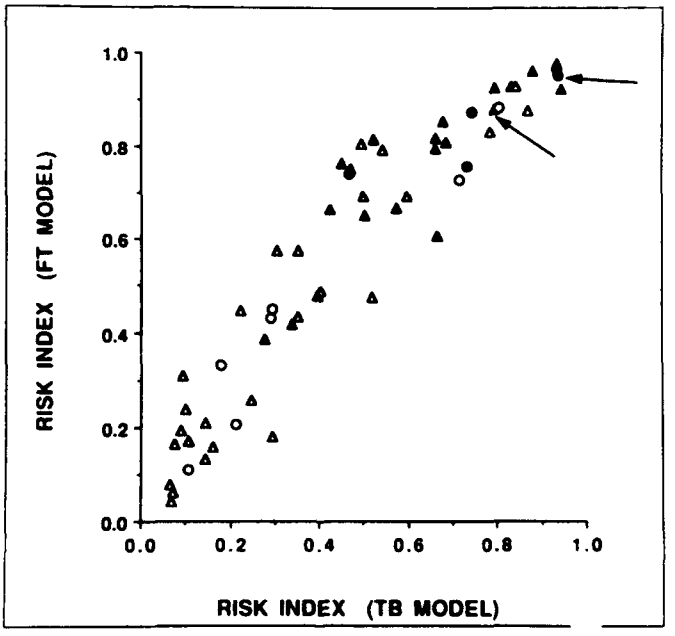

Figure 8. Relationship of two risk scores (Ref. 26) calculated on a population of burn patients given one bath of cerium nitrate. Risks were based on Full Thickness burns area (FT model) and on % TBSA (TB model) of burn patients. All patients survived except three. Two deaths are indicated by arrows (for one, a suicide, 90% TBSA, further resuscitation attempts were cancelled; the other suffered a pulmonary embolism. The third, with a fractured femur and internal injuries, had a TB Risk of 0.66 but no FT data was available.

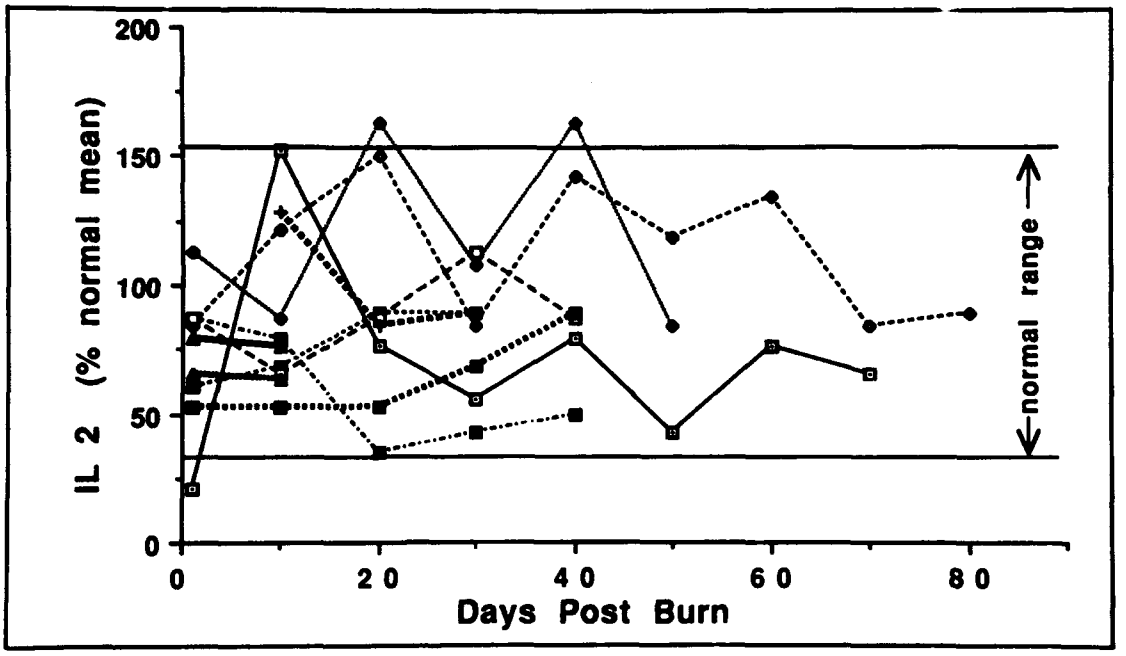

Figure 9. IL2 production by PBMC *in vitro*, taken from cerium treated (one bathing only) burn patients. Individual patient values are depicted as a percentage of the mean value of IL2 production by lymphocytes from normal subjects, assayed at the same time. The range of values found among normals is given as upper and lower limits.

Table 1. Percent of casualties of warfare suffering burns.

WARFARE BURN CASUALTY STATISTICS

Burn Victims as Percent of Casualties

WW II	**(Brit)**	**1.5%**
KOREA	**(US)**	**2.8%**
VIET NAM	**(US)**	**4.6%**
YOM KIPPUR	**(Israeli)**	**10%**
FALKLANDS	**(Brit)**	**14%**
LEBANON 82	**(Israeli)**	**8.6%**

Table 2. Observations that indicate that particularly T cell immune functions are impeded by burn injury.

THERMAL INJURY PRODUCES IMPAIRMENT OF CELLULAR IMMUNITY (T CELL IMMUNITY)

CIRCULATING SUPPRESSIVE FACTORS INHIBIT

1) T CELL CYTOLYTIC ACTIVITY
2) PROLIFERATIVE RESPONSE TO T CELL MITOGENS
3) PROLIFERATIVE RESPONSE TO ALLOGENIC CELLS
4) DELAYED HYPERSENSITIVITY
5) ALLOGRAFT REJECTION

Table 3. Peripheral blood components indicating that peripheral blood mononuclear cells (PBMC), the cells isolated for the *in vitro* immunology studies, make up only 27% of the leukocytes.

BLOOD COMPONENTS

PLASMA	**55% VOLUME**	**91% Water**
	8% PROTEIN	**0.9% SALTS**
FORMED ELEMENTS		**45% VOLUME**
	PLATELETS	**300,000 per μL**
	RED CELLS	**5 x 10^6 per μL**
	WBC (LEUKOCYTES)	**5 - 10 x 10^3 per μL**

GRANULOCYTES (POLYMORPHONUCLEAR CELLS)
73% of WBC
AGRANULOCYTES (MONONUCLEAR CELLS)
22% of WBC = LYMPHOCYTES
5% of WBC = MONOCYTES/MACROPHAGES

Tables 4 and 5. Mortality rate of patients treated over their course of hospitalization with topical cerium nitrate 0.04M solution applied to gauze dressings (From Refs. 17 and 18).

From Monafo et al. SURGERY **80:** *465 (1976)*

MORTALITY RATE OF PATIENTS TREATED WITH TOPICAL CERIUM NITRATE

SURFACE AREA BURNED (%)	N°. PATIENTS	N°. DEATHS PREDICTED	N°. DEATHS OBSERVED
1 - 19	32	1.1	0
20 - 39	16	3.3	1
40 - 96	12	8.7	6
Total	60	13.1	7

From Monafo et al. ARCH. SURGERY **113:** *397 (1978)*

MORTALITY RATE OF PATIENTS TREATED WITH TOPICAL CERIUM NITRATE

SURFACE AREA BURNED (%)	N°. PATIENTS	N°. DEATHS PREDICTED	N°. DEATHS OBSERVED
70 - 79	4	3	0
80 - 89	5	5	2
90 - 100	7	7	6
Total	16	15	8

Table 6. Percent of PBMC expressing surface IL2 receptor in vitro, comparing cells from burn patients treated in the standard fashion and those treated by one cerium bath. Lowest mean IL2R level is higher in cerium patients although due to large variance is not significantly increased.

PERCENTAGE OF CELL POPULATION EXPRESSING IL2R

n	*AGE*	*TBSA*	*3°*	*% IL2R+ DAY 1*	*LOWEST % IL2R+ PB PERIOD*
STANDARD TREAMENT					
SURVIVORS					
8	31.0 ± 8.2	42.0 ± 12.7	30.8 ± 13.9	38.3 ± 11.7 (26 - 51)	18.4 ± 7.3 (48%) (7 - 30)
NON-SURVIVORS					
7	54.6 ± 16.4	60.7 ± 22.0	46.7 ± 14.3	36.6 ± 16.4 (17 - 62)	<5 (0 - 6)
CERIUM BATHED					
10	37.2 ± 12.4	35.0 ± 9.3	27.8 ± 13.3	32.3 ± 21.8 (4 - 63)	23.7 ± 15.2 (73%) (7 - 58)
NORMALS					
10	36.8 ± 8.2				36.2 ± 9.7 (17 - 51)

Table 7. IL2 production in vitro by lymphocytes taken from patients given standard treatment and those given one cerium bath. Values for each patient sampling over his hospitalization period were averaged and the mean of these averages is given. There is no significant difference between normals and cerium treated patients.

IL2 PRODUCTION BY LYMPHOCYTES IN VITRO

n	*AGE*	*TBSA*	*3°*	*IL2 U/ml*	
STANDARD TREATMENT					
SURVIVORS					
6	38.0 ± 7.6	35.3 ± 8.7	27.0 ± 11.7	9.1 ± 2.9 (0 - 32)	
NON-SURVIVORS					
7	53.7 ± 12.1	57.0 ± 13.6	49.0 ± 20.4.	5.0 ± 3.7 (0 - 22)	p < 0.001
CERIUM BATHED					
10	37.2 ± 12.4	35.0 ± 9.3	27.8 ± 13.3	31.6 ± 7.6 (8 - 62)	
NORMALS					n.s.
10	36.8 ± 8.2			37.5 ± 17.2 (16 - 60)	

CLINICAL TYPES OF HEPATITIS B

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SUMMARY

Hepatitis B is a parenterally and sexually transmitted disease of global importance. The disease runs more frequently a subclinical and anicteric course, with a significative rate of cases that become chronic. Chronic hepatitis may progress to cirrhosis or cancer. The strategies by which Hepatitis B can be diminished and eventually eliminated are: immunization, measures to prevent exposure to infective blood or blood derivatives and education (in particular awareness that hepatitis B is a sexually transmitted disease).

INTRODUCTION

The clinical expression of Hepatitis B Virus (HBV) infection is heterogeneous, ranging from subclinical infections to fulminant hepatitis, cirrhosis and hepatocellular carcinoma (tab.I). HBV is possibly the major cause of medical liver disease world-wide and is carried by an estimated 300 million persons globally (1). Hepatitis delta virus (HDV) is a defective virus requiring helper functions of HBV to replicate. It develops only in patients with HBsAg and its presence aggravates the course of the underlying HBV infection. Current estimates indicate that the proportion of HDV-infected HBsAg carriers may exceed 5% world-wide (2).
Disease associated with HBV infection not only gives rise to much human suffering but places an intolerable burden on public health services, particularly in those countries least able to provide adequate sanitation.

THE VIRUSES

The causative hepatitis B virion consists of an envelope and a nucleocapsid. The Hepatitis B surface Antigen (HBsAg), formerly called Australia antigen, is located in the viral envelope. Immunogenic but not infectious, it is the basis of hepatitis B vaccines. The nucleocapsid contains a DNA polymerase and a circular, double-stranded DNA molecule. It also contains two important antigenic determinants: the Hepatitis B core antigen (HBcAg) and the Hepatitis B e Antigen (HBeAg), a protein subunit of the core. The double stranded DNA genome of HBV has been cloned and sequenced. The S gene was found to code for the protein of the viral envelope (HBsAg). In addition, a pre-S region (pre-S1 and pre-S2 regions) precedes the gene coding for HBsAg: it may be involved in interactions of the virus with the host hepatocytes. The C gene encodes the core protein (HBcAg and HBeAg), while the putative DNA polymerase-encoding P gene overlaps the S gene. A fourth reading frame, less well understood, is designated X (3) (Fig.1).
Active viral replication is indicated by the presence of any serological markers of the virion core. Hence, serum HBeAg correlates with ongoing viral synthesis and infectivity. Antibodies to HBeAg (α-HBe) appear in the integrated stage of infection.
The presence of IgM antibodies to HBcAg (α-HBc) implies the existence of ongoing HBV-related chronic liver disease. Low titres of IgG α-HBc with antibodies to HBsAg (α-HBs) are indicative of hepatitis B infection in the past, whereas higher titres of IgG α-HBc without α-HBs indicate the persistence of viral infection. Serum hepatitis B DNA (HBV-DNA) is the most specific and sensitive index of viral replication. It can be present in α-HBe-positive serum (4).
Hepatitis delta virus (HDV) is a defective virus requiring helper functions of HBV to replicate.
Commercial tests currently in general use detect HDV antigens and antibody to HDV (α-HDV) in serum by radioimmunoassay or enzyme-linked immunosorbent assay, and HD antigen in liver by immunohisto-chemistry. Patients co-infected by HBV and HDV develop the full battery of HBV markers and a variable response to HDV, ranging from hepatitis D antigenemia followed by the homologous antibody, to isolated α-HDV responses. In general the response to acute co-infections is weak and evanescent so the appearance of the antibody may be delayed several months after the onset of hepatitis. Recognition of past-HDV infection is impossible on serological ground: α-HDV usually does not persist and no protective antibodies similar to the antibodies to HBsAg have been identified for hepatitis D. By contrast, superinfected carriers with progressive

hepatitis D mount brisk antibody responses that give rise to high titres, which persist throughout the chronic course of HDV infection. Thus, upon cross-sectional analysis, the serum specimens which provide most information about the prevalence of HDV infection are those from HBsAg carriers, as exposure to the defective virus in these individuals results in persistent α-HDV that can be measured in any random sample (6).

EPIDEMIOLOGY

Hepatitis B is transmitted parenterally and by sexual intercourse.

The carrier rate of HBsAg varies world-wide from 0.1 to 0.5% in North and West Europe and US to ~2 % in Greece and Southern Italy and to as much as 10 to 15% in Africa and Asia. In some isolated communities, such as Alaskan Eskimos and Australian Aborigens, the rates of exposure to HBV are even higher (1).

A number of studies have documented the importance of sexual practices in trasmitting HBV. In prevalence studies, groups such as homosexual men, prostitutes (both male and female) and sexual contacts of HBV-positive patients were shown to be at increased risk of infection. In studies in developed countries, heterosexual attenders of Sexually Transmitted Disease (STD) clinics and persons with multiple sex partners or with a history of previous STD were also shown to be at risk (7).

In developed countries, the importance of sexual HBV transmission is variable. In the United States, sexual contact is the most important mode of disease transmission, accounting for over 35% of all infections and 50% of cases with identified risk factors. In recent years, the relative importance of heterosexual transmission has increased, while homosexual transmission has decreased. In developed countries in Northern Europe, disease incidence is lower and the importance of heterosexual transmission is less well defined. In Southern European countries, such as Greece and Italy, HBV prevalence is decreasing due to diminished horizontal transmission among children; in these areas, the relative importance of adult infection is increasing and heterosexual contacts account for a substantial proportion of acute hepatitis B cases.

Sexual transmission is important also in developing countries, sexual transmission also has variable importance. In Southern Asia, 40-60% of adults are susceptible to HBV infection and a consistent proportion acquire infection with sexual contact. Prostitutes are at high risk of infection: where paid sex is frequent, it may account for a high proportion of adult HBV infections. In developing countries, fewer adults have escaped childhood infection, but susceptible are far more likely to be exposed to the HBV. In the Middle East, sexual transmission of HBV contributes little to the overall disease burden. In contrast, travellers and military personnel from developed countries are at high risk of HBV infection if they share sex with the local population while on visit countries where HBV is endemic (7).

The epidemiology of HDV is characterized by great variations and intriguing contrasts. Despite dependance on HBV, its epidemiology is not simply a replica of the epidemiology of HBV. Mode of transmission are by necessity similar, but differs in the relative efficiency by which they can transmit the two viruses; for example, vertical transmission from mother to offsprings, which is important in maintaining the endemicity of HBV in underdeveloped countries, represents a very incospicuous mode of transmitting HDV (8). Likewise, the geographical prevalence of HDV overlaps but does not coincide with that of HBV. While in areas of tropical Africa and of the Amazon Basin the ratio of HDV infection in HBV-infected individuals is very high, often exceding 60%, the ratio is instead low in the entire South East of Asia (9) and among Alaskan Eskimos (10), where HBV infections are nevertheless hyperendemic. Remarkable differences also occur in areas with intermediate HBV rates, such as the Mediterranean Basin and Japan; infection is found in 20% to 30% of the patients with chronic hepatitis B in Greece(11) and Italy (12), but less than 3% of Japanese carriers of the HBsAg with similar clinical characteristics. In general, HDV can cause infection by one of two modes, either coinfecting together with HBV or superinfecting person already infected with HBV. Coinfections run most often a self limited course followed by clearance of HDV-HBV, while superinfections most often result in chronic HDV infection superimposed on a chronic HBV infection. In low endemicity areas, such as U.S. and Northren European countries, HDV infection is confined to group at risk, such as the drug addicts. Infection is acquired predominantly by coinfection, through the continuous recruitment of new drug abusers and disease occurs often in clustered outbreaks of severe hepatitis. In intermediate endemicity areas, such as the Mediterranean Basin, the

epidemiology of HDV is composite; it results from an epidemic pattern, similar to that seen in Northern Europe within groups at risk for the acquisition of HBV infection and from an endemic pattern in the general HBsAg-positive population. However, because the network of carriers is more dispersed and transmission mechanisms less efficient, dissemination of the infection is much slower than in hyperendemic areas, with the virus circulating costantly in a minorities of carriers rather than occurring in epidemic waves.

The importance of a core group of highly sexually active persons in maintaining transmission of HBV and HDV, as occurs with some other STDs, has not yet been defined. Prostitutes may play such a role in HBV-HDV transmission in some countries. Mathematical modelling may serve to enhance our understanding of the impact and cost effectiveness of various strategies of HBV control. Programmes which successfully reach susceptible individuals with a high risk of sexual behavior such as prostitutes or heterosexuals attending STD clinics, may have a large impact on disease transmission.

HBV infection is both an occupational and social risk to the soldier. Apart from sexual transmission, as in civilian life, hepatitis B is an occupational threat to the military surgeon, dentist, nurse and laboratory worker exposed to human blood. This risk is increased when health care workers from low-prevalence areas provide care to residents of high-prevalence regions. At particular risk is the soldier who provides emergency care to combat casualties. Any soldier who administers first aid may become infected while treating bleeding wounds or providing other life-support measure to injured soldiers or civilians. The demands and urgency of mass casualty care do not permit the health operator to use what would otherwise be routine protective measures for limiting exposure. In addition, during wartime conflicts or peace-time disasters, mass casualties require large numbers of blood transfusions quickly. Frequently, the only immediate sources of blood donations are other nearby soldiers (13).

CLINICAL COURSE

The features of acute HBV infection in adults are well defined, and include clinical hepatitis (25-33%), subclinical, anicteric disease (65-70%), fulminant hepatitis (1 to 3%o) (tab. I). The anicteric case is more likely to become chronic than the icteric one. Although the usual clinical attack of hepatitis B tends to be more severe than for virus A or C infections, the overall clinical picture is similar. Cholestatic hepatitis is unusual. A serum-sickness-like syndrome can complicate the prodromal period. A fulminant course in the first 4 weeks may be related to an enhanced immune response with more rapid clearing of the virus; titres of HBsAg may be low or undetectable. The diagnosis may be made only by finding serum IgM α-HBc. Another superimposed infection may precipitate a fulminant course: this may either be hepatitis A, delta virus or hepatitis C. Recently precore-mutants HBV have been shown to be responsible for fulminant cases of hepatitis B (14).

Approximately 10% of patients contracting hepatitis B as adults and 98% of those infected as neonates will not clear HBsAg from the serum. These patients become carriers of HBV. Classification of chronic hepatitis has been based on hepatic histology as determined by percutaneous liver biopsy. Chronic persistent hepatitis is a milder form of the disease formerly thought to have a good prognosis; chronic active hepatitis has a worse prognosis and severe chronic active hepatitis with bridging necrosis is particularly bad. A new classification of chronic hepatitis is based on the presence of viral replication (serum HBeAg and HBV DNA-positive with HBcAg detectable in hepatocellular nuclei) and hepatocellular necrosis (altered serum transaminases): A= HBeAg positive, viraemic (HBV-DNA positive) usually with chronic liver disease; B= HBeAg negative and α-HBe positive, viraemic and invariably with more severe and rapidly progressive disease; C= α-HBe positive without viraemia or liver disease; most of these patients being the so called healty carriers of HBsAg (15) (tab. II). It has become clear that the major factor influencing prognosis is not the original histologic picture , but whether or not viremia persist; thus chronic persistent hepatitis can also progress to chirrosis if the underlying HBV infection remains active.

In a consistent number of patients belonging to group B, particularly in those of Mediterranean descent, the presence of α-HBe is not synonymous of absence of HBV replication and of healthy liver state; such patients, in fact, have usually a severe and progressive hepatitis accompanied by the expression of HBV DNA in serum and HBcAg in liver. In many of these cases it has been shown that a

HBV variant is involved, which is not able to release HBeAg in the blood, due to a point mutation in the precore region of HBV genome that creates a stop codon (TAG) preventing transcription of the HBeAg polypeptide (16-18). It is not clear whether the variant is a defective mutant selected under immunological pressure of the host or is an autonomous infectious entity capable of inducing ex-novo α-HBe positive hepatitis.

The strong association between liver disease and HDV emerged from clinical studies in the last decade (19) led to the conclusion that HDV is highly pathogenic and invariably creates or aggravates disease in infected persons. Recent findings, however, that in the Greek island of Rhodes over half of the HBV carriers with anti-HDV had no biochemical or histologic signs of liver damage, have challenged the postulate. The lack of liver damage in a consistent number of carriers with anti-HDV raises the possibility that the natural history of HDV infection may differ from its medical history, the former remaining largely unexplored because attention has been drawn so far only by the clinical tip of the HDV iceberg. While it is clear that an unknown proportion of HDV infections run a subclinical course, patients who develop hepatitis D appear nevertheless to suffer from a disease that is more progressive than either ordinary hepatitis B or other forms of liver disorders. Worldwide HDV infection represents the true or the major cause of 30 to 80% of fulminant cases formerly thought to be caused by HBV(20). Characteristic features of HDV patients are the prevalence of anti-HBe in serum and frequent history of acute hepatitis, by contrast, an episode of hepatitis is reported in fewer than 5% of the HDV-negative carriers with anti-HBe in Mediterranean countries. Clinically, hepatitis D is more inclined than other forms of viral hepatitis to rapidly advance in cirrhosis; however, when a cirrhotic stage is reached, this is often a stable condition with a prolonged survival. The evolution of hepatitis D in 83 patients is shown in tab.III.

STRATEGIES FOR CONTROL

The strategies for global control and prevention of hepatitis B may be summarized in: i, mass immunization; ii, general measures to prevent exposure to infective blood or blood derivatives and sharp instruments and iii, education of the public, physicians and STD specialists that hepatitis is a sexually communicable disease.

The current list of six immunizable disease officially included in the Expanded Programme on Immunization (EPI) diphtheria, measles, pertussis, polio, tetanus and tuberculosis- has now been expanded under more limited conditions to include hepatitis B for countries that possess the economy capacities to purchase the vaccine and where the chronic HBV carrier rate exceeds 2.5%. Currently only 30% of the world's children are adequately immunized with the six official immunogens. The majority of countries where HBV mass immunization is needed are in the developing world, but such countries cannot afford to purchase hepatitis B vaccine. HBV vaccine became available in 1982 with the purification of HBsAg from plasma of human carrier. More recently, recombinant yeast-derived (R-HBs) vaccines were developed, which are of efficacy and long-term persistence comparable to that of plasma-derived vaccines but cost less and may thus render possible mass immunization programs. Active prophylaxis with HBV vaccines is likely to be also protective against HDV and HBV mutants infection.

General measures to prevent exposure to infective blood or blood derivatives and sharp instruments were developed in the early 1970s and remain the basis of the universal recommendations; these were more vigorously promulgated to health care workers when AIDS was also recognized to be a blood-borne disease. These measures are to be extended, whenever is possible, to the screening for infectivity of blood and blood derivatives for therapeutic use.

Finally the public, physicians and STD specialists must be aware that hepatitis is a STD comparable in mportance to syphilis in developing countries. Programmes to educate these groups about the epidemiology and consequences of HBV infection are of the highest priority and HBV-specific prevention and control measures should be incorporated into all aspects of standard STD prevention guidelines. The strategies for global control and prevention of hepatitis B summarized above should be gradually introduced according to HBV prevalence, economical income and health service organization of single countries.

Among the military, the routine immunization of all recruits should be adopted also in those countries with low incidence of HBV. The vaccination of military personnel will result in several

beneficial effects: it will reduce the burden of an important disease and assure that a large segment of adults are no longer suceptible to HBV after their military service has ended. It will be of strategical importance, like other vaccinations, in war scenarios in endemic areas to reduce the rate of unemployable personnel and, finally, in all those situation such as wartime conflicts or peace-time disasters, where large numbers of blood transfusions are quickly required and the only immediate source of blood donations is often the nearby soldier, HBV vaccination will contribute to render every soldier a *safe* walking blood bank.

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Table I: Outcome of HBV Infection.

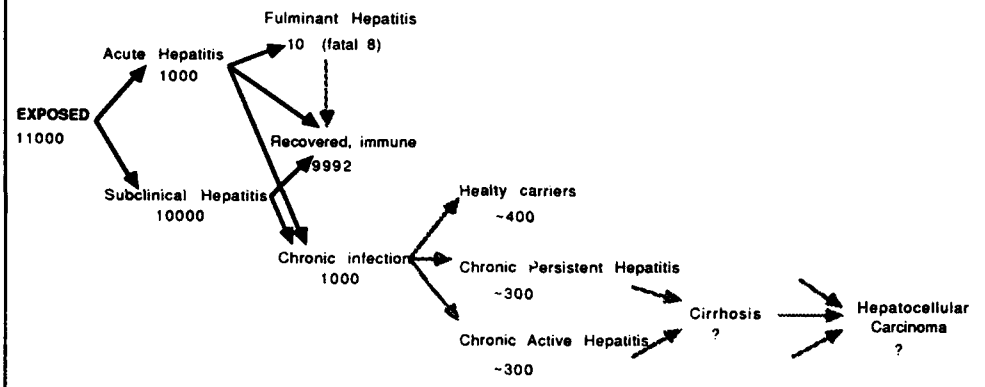


Table II : Classification of Chronic Hepatitis

	TYPE		
	A	B	C
Parameter			
Viral replication (a)	+	+	-
Inflammation (b)	+	+	-
Management	Antivirals	Antivirals?	Nil
5-Years mortality	<10%	20-30%	very low

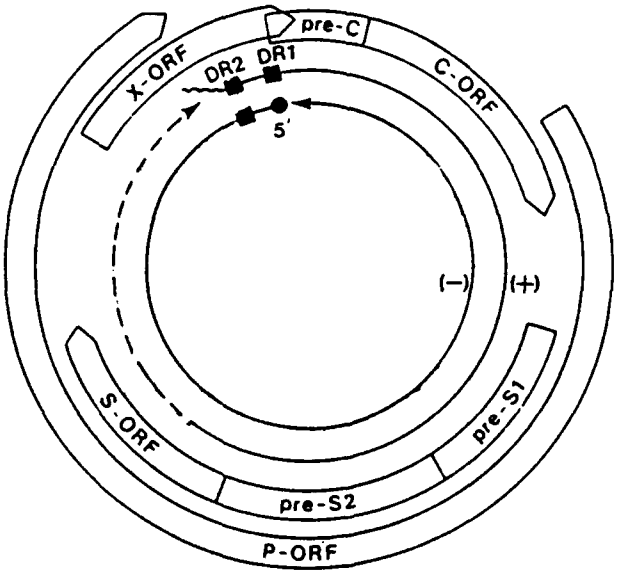
(a) HBeAg, HBV-DNA, HBcAg in nuclei
(b) transaminases increased

Table III: Evolution of hepatitis D in 83 Italian patients during a follow-up of years (mean: 4 years)

INITIAL DIAGNOSIS	LAST DIAGNOSIS
AH 23	3 CLH
	3 CPH
	7 CAH
	10 CIR
CLH 3	1 CLH
	2 CAH
CPH 19	1 CPH
	7 CAH
	11 CIR
CAH 33	14 CAH
	19 CIR

AH= Acute Hepatitis, CLH= Chronic Lobular Hepatitis CPH= Chronic Persistent Hepatitis, CAH= Chronic Active Hepatitis, CIR= Cirrhosis.

Fig. 1: Structure and genetic organization of the HBV genome. The positions of the direct repeats are represented by DR1 and DR2, and filled circle represents the protein covalently bound to the 5' end of the minus strand. The wavy line represents the short RNA oligomer at the 5' end of the plus strand. The positions of the four reading frames (ORFs) in relation to the genome are shown in the open boxes. The arrowheads indicate the direction of translation.



VIRAL HEPATITIS IN THE U.S. AIR FORCE, 1980 - 1989

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1. SUMMARY

Viral hepatitis and its acute and chronic complications continue to pose significant threats to the readiness of military personnel. Knowledge about the specific viral agents and their routes of transmission are important in developing prevention strategies. A recent analysis of hepatitis in the U.S. Navy for the period 1975-1984 is reviewed. In order to better characterize the risk of viral hepatitis among Air Force personnel, a comprehensive review of inpatient and quarters data for hepatitis A, B and "non-A, non-B" were reviewed from Air Force medical treatment facilities worldwide for the period 1980-1989. Following a discussion of the study methodology, preliminary data and hepatitis type-specific demographic risk variables are discussed. Preliminary results from a hepatitis serosurvey (A, B, and C antibody with use of a supplemental validating assay) of the subset of the study cohort who are currently on active duty are briefly reviewed.

2. INTRODUCTION

In a study of viral hepatitis in U.S. Navy members in 1975-1984 (1), Hyams et al concluded that 1) the highest incidence of acute viral hepatitis occurred in the youngest age groups, age 24 or less, 2) that a previous hospitalization for drug abuse or a concurrent discharge diagnosis of a sexually transmitted disease were strongly associated with the risk acute hepatitis, and 3) that the observed decline in the incidence of viral hepatitis during the 10 year period may have been due to decreasing drug abuse. The researchers concluded that based on these findings, immunization of high-risk groups in the U.S. Navy with hepatitis B vaccine could be an effective policy for the prevention of acute viral hepatitis.

The newly described hepatitis C agent is the leading cause of transfusion-related non-A, non-B hepatitis. Epidemiological studies have demonstrated that hepatitis C can be transmitted by transfusions, transplantations and intravenous drug abuse. Less is known about the transmission of the virus through sexual or other routes. Recent studies have demonstrated that hepatitis C may not be as readily transmitted through the sexual route as hepatitis B. Clinically, hepatitis C tends more frequently to present either asymptomatically or only as a brief nonspecific illness. However, it is estimated that as many as 50% of those infected with hepatitis C may eventually develop chronic hepatitis. With the discovery of hepatitis C and with the need to review prevention strategies for all forms of viral hepatitis, the Epidemiology Division designed a study to evaluate known or suspected risk factors for viral hepatitis in the Air Force.

3. METHODS

Hospitalization and quarters records for Air Force medical treatment facilities worldwide for the period 1980-1989 were abstracted for hepatitis A (ICD9CM codes 070.0, 070.1), hepatitis B (070.2, 070.3) and non-A, non-B hepatitis (070.4, 070.5, 070.6, 070.9). Age-, race- and sex-specific rates were calculated for the ten year time period. Concurrent discharge diagnoses in the case patients for a sexually transmitted disease (ICD9CM codes for gonorrhea, pelvic inflammatory disease, syphilis, chancroid, and lymphogranuloma venereum), for a previous history of a transfusion or for intravenous drug use were also abstracted. Patient records were matched with Air Force personnel records to obtain information regarding the presence/absence and number of overseas assignments, flying status and occupation.

In order to study the prevalence and risk factors for acquisition of the newly described hepatitis C a serosurvey was conducted. Members of the study cohort who remained on active duty were asked to donate serum for a complete hepatitis profile (hepatitis A IGM, IGG; Hepatitis B surface antigen/antibody, core antibody/antigen, "E" antigen; hepatitis C antibody). Specimens which were positive for the hepatitis C ELISA antibody test were validated with a Supplemental HCV neutralization assay, (Abbott Diagnostics). Hepatitis C-specific demographic and other factors were then determined. Those Air Force members who were identified as having viral hepatitis from the record review, but who were no longer in the military, were studied further by examining their cause for separation. Of specific interest was the proportion of

individuals who were separated for medical complications related to hepatitis (chronic hepatitis, hepatocellular carcinoma, non-alcoholic cirrhosis, etc.)

This presentation will summarize some of the preliminary analyses of the study which is still in progress.

4. RESULTS AND DISCUSSION

For the 10 year period, 1,911 records with viral hepatitis codes were analyzed. Incidence rates of viral hepatitis per 100,000 active duty person-years were calculated and revealed that sex-specific incidence rates were approximately equal for men and women for hepatitis A and non-A, non-B hepatitis. Hepatitis B however, was nearly twice as common among men as women (14.8 vs. 8.0 per 100,000 personnel, respectively). Blacks were at higher risk of hepatitis B acquisition (27.4) than whites (11.2) or members of "other" racial groups (13.0). Whites were slightly more likely to have non-A, non-B hepatitis than other racial groups.

Age-specific incidence rates, preliminary analyses of overseas assignment history and presence/absence of flying status, as well as initial analysis of serological data will be presented.

Comparison with U.S. Navy data and possible implications for U.S. Air Force preventive strategies will be discussed.

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HEPATITIS A AND HEPATITIS B: RISKS COMPARED TO OTHER VACCINE PREVENTABLE DISEASES AND IMMUNIZATION RECOMMENDATIONS.

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Summary

The incidence rate of hepatitis A is 3(-6)/1000 per month of stay in a developing country in unprotected travelers. Trampers and other persons feeding themselves under bad hygienic conditions have a rate of 20/1000. In many industrialized countries, persons below the age of 50 years have a seroprevalence rate of anti-HAV <20%. Hepatitis A morbidity and mortality in travelers is far greater than the one of any other vaccine preventable infection in travelers, with the exception that hepatitis B shows a slightly greater mortality in expatriates. Future studies will determine the role of hepatitis C and E. Typhoid fever shows an incidence rate of 0.3/1000 in foreigners on the Indian subcontinent, and in many parts of North and West Africa, excluding Tunisia, in other parts of the third world it is tenfold lower. In poliomyelitis, tetanus, diphtheria, cholera, rabies, Japanese encephalitis the incidence rate is ≤0.002/1000.

Introduction

In order to know which preventive measures are appropriate for civilian or military personnel abroad, one must be aware of the risk of illness and death in this population. Such data on hepatitis A and B are not readily available. Using the limited, and usually not very recent, available data we shall nevertheless be able to present the order of magnitude of the risk of hepatitis A and B in travelers.

Retrospective and follow-up studies

Since it has become possible to differentiate hepatitis A from other serotypes, to our knowledge one retrospective study has been published that analyzed in detail the risk of travelers to import symptomatic hepatitis A (1). In this Swiss study, the numbers of patients with positive hepatitis A serology were correlated to the number of residents who had visited the respective destinations abroad. Not all cases could be identified, it was estimated that for various reasons some 50% of the cases were missed. Additionally, asymptomatic infections could not be identified by this method. Despite these biases, it appears that the hepatitis A attack rate exceeded 1 in 1000 and did not significantly differ between regions within the Third World (table 1). Hepatitis A clearly was most

Table 1: Hepatitis A attack rate at various destinations (n = 137)

Destination	Attack rate per 1000 journeys
Northern Europe	0.002
North America	-
Southern Europe	0.015
North Africa	0.6
Subsaharan Africa	
Near East	0.6
Middle East	
Far East	
Central America	0.5
South America	

frequently diagnosed serotype of hepatitis imported from developing countries. Roughly 60% of hepatitis cases in travelers returning from Africa, Latin America or from South and Southeast Asia were due to hepatitis A. Hepatitis B and hepatitis non A, non B were each diagnosed in about 15% of the cases, while some 10% remained unclassified at the time when just hepatitis A and B could be differentiated. In contrast, hepatitis A played only a minor role in infections acquired from within Europe.

How does this study compare with older studies which analysed either all cases of imported hepatitis, or of imported non-B hepatitis only (2-5)? As shown in table 2, the Swiss and the Danish studies show similar, but markedly lower rates than both Swedish surveys, despite the fact that the latter did not include hepatitis B cases. This can partly be explained by the fact that residents of Sweden have the lowest prevalence of anti-HAV (6). As shown on the bottom line for the Swiss study, trampers, such as hitch-hikers through India, had a much higher risk compared to other travelers, 1 in 50 got infected.

Table 2: Risk of imported hepatitis (any serotype) per journey abroad

Imported	Risk per 1000 travelers				
	Retrospective studies (only imported cases)				
to / from	Zurich n=221 (a) 1971-76	Copenhagen n=105 (b) 1976-78	Zurich n=137 (c) 1977-81	Göteborg n=80 (d) 1980	Stockholm n=85 (e) 1982
Northern Europe	0.006	0.005	0.01	N/A	
Southern Europe	0.1	0.03	0.04	0.2	0.05
North Africa	1.0	0.5	0.6	1.4	1.9
Developing countries, other	0.6-2.9	0.8	1.1	10.0	8.3
- Trampers	20.0	N/A	N/A	N/A	N/A

In the only follow-up study 8 out of almost 8000 travelers to various developing countries were diagnosed as having symptomatic hepatitis A (7). The majority of these patients were tourists who had stayed in hotels, often in particularly decent ones. Their mean duration of incapacity to work was about one month. Another 9 patients were classified as non A, non B hepatitis, but some of these cases may have been Fansidar-induced toxic hepatitis. Two professionals working in the tropics had hepatitis B, and 4 patients had unclassified hepatitis. Since the average duration of the stay abroad was 19 days, the incidence rate per month on the basis of these few cases can be estimated to be 1.6/1000. If we assume that 40% of travelers have anti-HAV by infection or immune globulin, the corrected rate for unprotected travelers would be about 3/1000. Again, asymptomatic infections were disregarded.

Seroconversion studies

To our knowledge, only a French group has published a hepatitis A seroconversion study in travelers (8). The researchers investigated 233 volunteers working in the bush of Central or West Africa in 1979/80, of whom 125 (54%) had anti-HAV prior to departure. The remaining 108 received no immune globulin for their stay, and 19/1000/month seroconverted, the majority with jaundice.

Professionals working in developing countries may additionally be at considerable risk of hepatitis B: of 219 agricultural, community or medical volunteers working in Africa 23 (10.3%) developed HBV infection during a stay abroad of 18-30 months, that means a rate of 5/1000/month. Only 7 patients with HBV infection and both with combined HAV/HBV infection were jaundiced, thus leaving (61% asymptomatic (9).

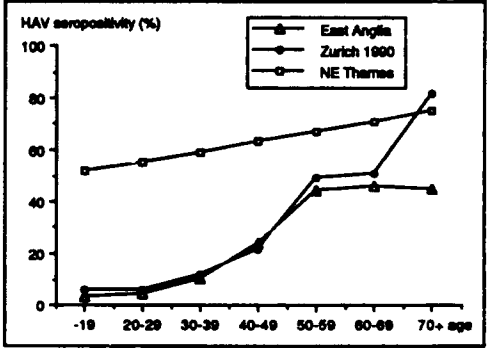
Conclusions on the Incidence of Hepatitis A and B

From all the available retrospective, follow-up and seroconversion data we conclude that the incidence rate of symptomatic hepatitis A for a one month journey from an industrialized country to a developing country is probably 3 (at most 6)/1000 for the usual non-immune traveler. This applies also to vacationers staying in renown hotels, as illustrated by the abovementioned study and a thesis conducted at the CDC (10). The incidence rate seems to be, however, sixfold higher in trampers or other persons, who eat and drink under bad hygienic conditions.

Assuming a linear rate of infection over time we can from the above mentioned data additionally estimate a monthly incidence of symptomatic or asymptomatic hepatitis B of 80-420/100,000 in expatriates working in developing countries.

Older publications indicated that a major proportion of adult travelers would be protected by anti-HAV. The major study based on blood donors, which may well be more often anti-HAV positive as they at least in some countries may rather belong to lower income classes. Recent data suggest that in various European countries only persons born during or before the World War II have a reasonable chance to be anti-HAV positive. In persons aged 50 or less HAV antibodies were exceptional (figure 1), however other studies in possibly less affluent sections of the society still showed a high proportion of seropositivity (11-14).

Figure 1: Recent HAV-seropositivity studies (11-14)



Comparison with other vaccine preventable diseases

How do these data compare with other vaccine preventable infections? Symptomatic hepatitis A is the most frequently occurring immunizable infection in travelers (table 3). Hepatitis B shows incidence rates which are lower, but the mortality estimates are in the same order of magnitude. American and European data suggest that hepatitis B is rare in vacationers and that in most cases it affects expatriates. In unprotected travelers, hepatitis A occurs 40 times more frequently than typhoid fever and 800 times more frequently than cholera (15). Similarly to hepatitis A,

Table 3: Morbidity and mortality of immunizable diseases in 1,000,000 non-immune travelers visiting developing countries

Infection	Incidence/rate per month	Case fatality rate (%)	Mortality/rate per month
Hepatis A	3000(-6000)	0.1	3(-6)
Hepatis B, expenetes (symptomatic or asymptomatic)	800(-2400)	2	16(-48)
Typhoid	30	1	0.3
-India, N/NW-Africa	300	1	3
Poliomyelitis			
- symptomatic	1	20	0.2
- asymptomatic	20(-1000)	-	? in contacts
Cholera	3	2	0.06

No data available for diphtheria, Japanese encephalitis, measles, meningococcal meningitis, rabies, tetanus, tuberculosis, yellow fever

these two gastrointestinal infections have a low case fatality rate in travelers. No epidemiological data, just anecdotal reports are available on other immunizable diseases, such as diphtheria, Japanese encephalitis, measles, meningo-coccal meningitis, plague, rabies, tetanus, yellow fever, etc. It is, however important to note that annually 2% of long term residents in the tropics are bitten by animals.

Immunization recommendations

From this information it is obvious that immunization against hepatitis A and hepatitis B are of primary importance. As hepatitis A vaccine will be marketed within the next few months, active immunization against both these infections should be offered to pilots serving in developing countries. Other vaccinations are far less important and should be recommended according to the risks anticipated during the mission (table 4). Otherwise we will create unnecessary St. Sebastian syndromes.

Table 4: Indication for immunization prior to a stay in a developing country

Duration of stay	*short*		*long*
Standard of hygiene	*high*	*low*	*any*
Poliomyelitis	+	+	+
Tetanus, Diphtheria	+	+	+
Hepatitis A	+	+	+
Hepatitis B	–	–	+
Typhoid fever			
- India, N/W-Africa	+	+	+
- other destinations	–	+	+
Rabies	–	–	(+)
Cholera	–	–	–
Meningoc. meningitis	in epidemics, trekkers, Sahel		

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VACCINATION AGAINST HEPATITIS B: THE ITALIAN STRATEGY

by

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Viral hepatitis type B is major worldwide public health problem. Infection with the hepatitis B virus (HBV) may progress to chronic liver disease including chronic active hepatitis, cirrhosis and hepatocellular carcinoma. Moreover it has been estimated that between 200 and 300 million individuals in the world are chronic carriers of HBV.
The availability of safe and effective vaccines allows the establishment of immunization programs aimed at the elimination of hepatitis B and the reduction of morbidity and mortality due to its sequelae.

Vaccines against hepatitis type B.
Currently available vaccines include plasma-derived vaccines and yeast-derived vaccines. Plasma-derived vaccines, introduced in 1981, were the first available vaccines to provide active protection against HBV. They consist of 22 nm spherical surface antigen particles (the noninfectious excess protein coat of the virus) purified from the plasma of HBsAg carriers, subsequently inactivated and alum adjuvanted. More recently, recombinant DNA technology has been used to express HBsAg in prokaryotic as well in eukaryotic cells such as yeast cells (Saccharomyces cerevisiae) (1). Much effort has been spent devising host cells able to succesfully express HBsAg, including mammalian cells, insect cells, vaccinia virus and hepatoma cells.
Concern has been expressed about the safety of vaccines derived from tumours cells, as well as for those employing vaccinia virus as a vector. The possibility of using other recombinant viruses as vectors (i.e. Adenoviruses) is being explored. *Saccharomyces cerevisiae* has proven to be a successful host and genetically engineered yeast-derived vaccines have been commercially available since 1986.
Despite the availability of licensed hepatitis B vaccines, much effort is still required to develop more potent and cheaper immunogens suitable for mass immunization programs.
Additional hepatitis B vaccines have been produced or are scheduled for production including : chemically sinthesized peptides, anti-idiotype vaccines and those containing additional antigens such as pre-S(1) , pre-S(2) and possibly HB core. Experimental vaccination with pre-S(2) peptide has induced protection in chimpanzees (2,3). In mice, the pre-S(2) protein has been demonstrated to enhance the immunogenicity of HBsAg (4). It was also shown that immunization of a «non responder» murine strain with both S and pre-S antigens can circumvent non responsiveness (5). Whether these results can be translated to man remains to be established.

SAFETY, IMMUNOGENICITY AND EFFICACY OF HEPATITIS B VACCINES.
Both plasma-derived vaccines and recombinant DNA vaccines have been administered to several million individuals with an outstanding record of safety and efficacy (6-22).

Adverse reactions are mild and largely confined to soreness, swelling and induration at the site of injection. Systemic reactions are uncommon (1).
Following the administration of a course of vaccination, the seroconversion rate to antibody against HBsAg (anti-HBs) is over 90% in healthy children and adults. The elderly, the immunodeficients (including those infected with HIV), hemodialysis patients generally show lower responses to vaccination and may require larger or additional doses of vaccine (1, 23-28).
To obtain an optimal response, the vaccine must be given, according to the manufacturer's instructions, intramuscularly (or subcutaneously) into the deltoid in adults and into the anterolateral thigh in neonates. Buttock injection as well as intradermal route are less successfull and the latter is presently not recommended because of its difficulty in administration and the relatively high incidence of side effects (29-30). Following vaccination, protection is virtually complete in individuals who respond to the vaccine, even though some HBV infections may occur. However, almost all such infections are subclinical and can be detected only through the appearance of antibodies to hepatitis B core antigen (anti-HBc) or a raised ALT level (15).
The duration of protection of hepatitis B vaccination is a crucial issue. Long-term immunogenicity studies have been performed (15,31-34). There is evidence that the persistence of anti-HBs, and hence the duration of protection, is directly related to the peak antibody response after completing a course of vaccination (35). But, the percentage of antibody decrease per period is independent of this peak. Thus, using a nomogram, it is possible to predict when the antibody level is likely to fall below 10 mIU/ml from the anti-HBs titer detected at the end of the of the vaccination course (36). Generally speaking, healthy adults vaccinated with a schedule consisting of three injections at time 0, 1 and 6 months, require an additional booster dose after 4-5 years, whereas vaccinees who receive four doses of vaccine at time 0, 1, 2 and 12 months should be re-boosted after 8-10 years. Loss of antibody does not necessarily mean loss of immunity, as immunological memory may outlast the presence of circulating anti-HBs. However, among people who respond to the vaccination, the risk of acquiring HBV infection increases as anti-HBs becomes undetectable, even if the risk of developing clinical hepatitis remains extremely low (15). Whether vaccinees should be tested for anti-HBs after completion of vaccination and boosted when antibody disappears, given booster vaccinations at regular intervals without determination of anti-HBs or simply not given booster doses and relying upon immunological memory is still a matter of debate. The final strategy depends on the cost of testing, cost of the vaccine and the risk of HBV infection. In this respect, ethical and medico-legal issues are also important players.

Individuals who do not respond to vaccine must be considered at risk of acquiring infection when exposed to the virus.
Some «non responder» individuals may respond after additional doses of vaccine.

STRATEGIES FOR CONTROL OF HEPATITIS B BY IMMUNIZATION.
Several options are available when we consider the prevention of hepatitis B by immunization. The strategy may vary from country to country depending on : a) HBV endemicity; b) predominant modes of infection (i.e. sexual, vertical/perinatal, early horizontal, needle sharing during drug abuse, etc.); c) age of infection; d) health-care resources.
Looking at the prevalence of the HBV markers, the world can be divided into areas of «high», «intermediate» and «low» endemicity (37). In areas of high endemicity most people become infected early in life. In Asia, where most HBsAg carrier mothers are HBeAg positive, vertical/perinatal infection is the major route by which HBV is transmitted, while in Africa (where most carrier mothers are HBeAg negative) most infections are aquired horizontally by child to child transmission. Infection during childhood is associated with an increased risk of developing the chronic carrier state and, later in life, cirrhosis and hepatocellular carcinoma (38-40). Therefore, in these countries, mass immunization of infants is the proper strategy for long-term control of hepatitis B (37).
The integration of hepatitis B vaccination into the framework of the WHO Expanded Program of Immunization (EPI) should be considered a priority.
In areas of «intermediate» endemicity infection occurs both in adults and children, but vertical/perinatal transmission is relatively uncommon because most carrier mothers are HBeAg-negative. Even in these countries, immunization of all infants may be considered the proper approach for long-term control of hepatitis B (37). Ideally, a combined program of infant and adolescent immunization, together with the immunization of individuals

at higher risk, should be applied where economically feasible and where an appropriate infrastructure for delivery exists.
In areas of «low» endemicity where most infections occur in adults through sexual contacts, sharing syringes during drug abuse and healthcare accidents, immunization programs have been initially aimed at screening of pregnant women and the immunization of high risk groups, including babies born to HBsAg carrier mothers (41). Unfortunately subject compliance to most such programs has been lower than that expected and the effect of lowering hepatitis B infection rates in the general population has been minimal (37,42). Extension of immunization to all neonates and possibly adolescents is being proposed in the USA.

HEPATITIS B VACCINATION IN ITALY.

Hepatitis B is endemic in Italy. 1.5 million carriers of HBsAg form a reservoir of infection corresponding to around 2.5% of the resident population. It is now estimated that 9000 individuals die each year from cirrhosis and/or hepatocellular carcinoma. Despite this dramatic reality, in Italy as in many other industrialized countries, the incidence of acute hepatitis B has been shown to progressively drop during the last 20 years (43). As recently reported (44), the low prevalence of HBV markers among children and young adults, confirms that the decreasing rates of incidence are real and not due, for example, to an increasing underreporting of acute hepatitis.
Socioeconomic factors and demographic changes, together with the improvement of general hygiene in the last decades, have clearly played an important role in changing the epidemiology of hepatitis B. As a consequence, HBV is spread less from child to child, while the sexual route and transmission of virus through needle sharing during drug abuse are becoming the most common modes of HBV infections.
As is shown in table, the actual incidence rate of acute hepatitis in youths of 15-24 years of age much higher than in subjects of 0-14 years of age and in adults over 25 years of age (table 2). Since 1984, a vaccination program to immunize individuals at higher risk of infection (i.e. healthcare personnel, household contacts of carriers, babies born to HBsAg carrier mothers, hemodialysis patients, hemophiliacs, drug addicts, etc.) has been launched on national scale.
A mass vaccination of all infants in a hyperendemic area of Southern Italy has also met with success (45). With reference to Italian law (46), the vaccination against hepatitis B has recently become compulsory in our country for all neonates and 12 years old adolescents, while the immunization of individuals at higher risk of infection remains facultative. Adolescents will be given 3 doses of vaccine , while two different schedules are available for babies. More in detail, babies born to HBsAg carrier mothers will receive HBIg plus vaccine at birth and then vaccine alone at 1,2 and 11 months of age, while those born to HBsAg negative mothers, the hepatitis B vaccination will be integrated into the routine infant immunization program which includes live antipolio, anti-tetanus and anti-diphtheria vaccines given at 3, 5, and 11 months of age. Twelve years after the start of this immunization program, all Italians under 24 years of age will be immune to HBV. From that time on, all 12 years old adolescents will only require a single booster dose to maintain immunity, as they were already vaccinated in infancy. This strategy of vaccination, combined with other effective infection control measures (i.e. education, «safe» blood and blood products, appropriate disinfection and sterilization, etc.) should finally provide a long-term control of HBV infection in the population.

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TABLE 1

INCIDENCE RATE (cases x 100.000) OF HEPATITIS B ACCORDING TO AGE AND YEAR OF NOTIFICATION (SEIEVA)

	AGE (years)		
YEARS	**0- 14**	**15- 24**	**25**
1985	6	41	7
1986	3	35	9
1987	2	31	8
1988	2	22	5
1989	2	19	5
1990	1	17	4

TABLE 2

Vaccination against hepatitis B in Italy (Law of 27 may 1991)

VACCINATION	POPULATION	SCHEDULE OF VACCINATION (N. of doses and time of injection)
MANDATORY	BABIES	3, at time 3,5,11 m
	ADOLESCENTS (12 years old)	3, at time 0,1,6 m
FACULTATIVE	INDIVIDUALS AT HIGHER RISK OF INFECTION	3, at time 0,1,6 m

Those born to HBsAg carrier mothers will be injected with HBIg plus vaccine at birth and then by vaccine alone at 2,5,11 months of age.

DISCUSSION SESSION I

HEPATITIS B (Papers 3 to 6)

F. AIUTI (IT): A question to dr. Rizzetto, related to the virus variance. Can this variance be transmitted to another subject? In this case, is it still aggressive or less aggressive than in the subject in which it has emerged?
A second question is related to the cofactors. Do you think that some cofactors are responsible for the emergence of this aggressive variance of hepatitis B or alternatively that a failure of the immune system, of neutralizing antibodies, may induce the emergence of this variance?

M. RIZZETTO (IT): These are very good points. Regarding the first, we don't know if the variance is infective in itself. There are experiments in chimpanzees, showing no infectivity of sera positive for the variant HBV. These experiments are very difficult to do, because we do not know how much variant or wild HBV are present. Experiments performed by dr Purcell at the NIH, on some anti-HBV positive sera given by dr Bonino of our group, show a lack of productive infection, only an abortive infection, with production of anti-HBs. The pre-core minus variant seems to be a selection product throughout the infection, possibly due to an escape from the immunological pressure mounted against HBeAg by the organism. But this may not be the full history. Also the significance of variance in itself must not be overemphasized in terms of virological versus immunological problems, because also in the normal acute hepatitis B there is at the end the selection of pre-core variants; so, the factors leading to the chronic liver disease associated to the variant are probably many and not the mutation in itself alone.

D.S. BURKE (USA): My question is also related to the variation of hepatitis B virus. A few years ago there was a report of antigenic variance of hepatitis B, that escaped neutralization in subjects immunized against HBV. Could you tell if this is, at current state, still a continuing problem?

M. RIZZETTO (IT): I think that dr Zanetti is much more ideal than me in replying.

A.R. ZANETTI (IT): We have signalled the emergence of our escape mutant of HBV in a baby born to an HBsAg/HBeAg carrier mother, who was vaccinated against hepatitis B and was also given HBIg. The virus showed a point mutation from guanosine to adenosine at position 587, resulting in an aminoacid substitution from glycine to arginine at position 145 in the a determinant of HBsAg. A similar variant has been described by Mc Mahon in a patient who underwent a liver transpantation and who was subsequently treated with monoclonal anti-HBs to prevent HBV reinfection of the homograft. More recently, the emergence of the same variant has also been found in Singapore in a baby who was given HBIg plus vaccine to prevent vertical HBV infection.

D. S. BURKE: These cases were originally discovered because they recurred in people that had been originally immunized.

A.R. ZANETTI (IT): Yes, during a study of immunogenicity and efficacy of hepatitis B vaccines in Italy, we have found a number of individuals who had apparently mounted a successful immune response, but nevertheless later became infected with HBV. These subjects were characterized by the coexistence of non-complexed anti-HBs and HBsAg. In most cases, there was only a transient appearance of HBsAg followed by anti-HBc and anti-HBe. However, in one child severe acute hepatitis occurred, followed by persistent elevation of ALT and establishment of the carrier state of HBsAg.

D. S. BURKE (USA): But has there been any indication, so far, that this form may spread as an epidemic form, replacing the current one?

A.R. ZANETTI (IT): I do not know how much, this kind of mutated virus is spread in Italy. However we are aware of other cases of HBV infection of anti-HBs-positive vaccinees and sequencing studies of the viruses involved are currently in progress.

R. STEFFEN (CH): A question to dr Parkinson. Have you any idea on the incidence rates of hepatitis A and B in your personnel stationed in developing countries or haven't you looked at these data yet?

M.D. PARKINSON (USA): Unfortunately we haven't looked at all these data yet, but we clearly intend looking into that data categorically, history of overseas assignment or no, and also continuously by total duration of time spent overseas all over the world. The US Army has a good project of immunizing troops stationed in Korea. We are beginning to ask if the same thing is possible on a selected target of USAF troops, which are generally at a lower risk for most infectious diseases, but we haven't yet looked at them specifically.

R. STEFFEN (CH): But I imagine that your troops stationed in developing countries all get immunoglobulins.

M. D. PARKINSON (USA): We do for deployments to developing countries. We do not however, routinely administer immunoglobulins to troops permanently stationed in many countries considered to be at high risk for hepatitis. For example, we did not administer immunoglobulins regularly to Air Force members in the Philippines.

M. RIZZETTO (IT): Now there is a new entity emerging as acute hepatitis in developing countries, the hepatitis E. Have you any data on the magnitude of the phenomenon of hepatitis E in developing countries, or in foreigners or in travellers as well?

M.D. PARKINSON (USA): Well, there is not yet any specific assay for enterically-transmitted non-A non-B hepatitis. However one thing that is very interesting is that 32% of our cases have no markers for testable hepatitides . We do not know whether among these patients some of them might have had an enterically transmitted non-A non-B hepatitis. Of course we can obtain further information demographically. There is a history of overseas exposure in areas where hepatitis E is endemic. But we do not have any outbreaks of hepatitis E in USAF personnel, of which we are aware of.

R. STEFFEN (CH): Sixty per cent of the imported cases of hepatitis were proven to be due to hepatitis A and about 15% to hepatitis B. The rest remains so far unclassified, and we don't know what proportion of this minority is really due to hepatitis E. So far we have anecdoctical, but no really firm, data on imported hepatitis E.

R.E. SPIER (UK): You talked about hepatitis B in developing countries and hepatitis A in developed countries. Is there any evidence on the proportion of the mode of transmission, sexually or by intravenous

drug abuse, poor hygiene or infected food or whatever?

R. STEFFEN (CH): Considering first hepatitis A, we don't have any evidence, except that this would be due to eating and drinking habits. We have to be aware of the fact that as well as tourists, business people and expatriates partly consume very risky foods in developing countries, including raw oysters. Then, with respect to hepatitis B there are some fascinating studies on missionaries, where everybody started to wonder about sexual habits, and there is evidence, not from studies on travellers but from survey of local native population, that there is some transmission which is not due to sexual behavior or to drug abuse, but hepatitis B is just transmitted by local wounds. You may be aware of the fact that seroconversion occurs in local population fairly frequently during childhood where the other risk factors are not present. For the other serotypes, as I mentioned before, I have no data.

R. D'AMELIO (IT): First of all I would like to congratulate all the speakers for this illustration of the problems of viral hepatitis.
I believe that the speech of prof. Steffen on the problems of travellers, is very important for the military environment, because military personnel are institutionalized travellers. Until now the only international regulation for travellers is the vaccination against yellow fever in some countries. What about the possibility of an up-to-date international regulation for travellers and, even more, for military personnel? The recent experience of the Persian Gulf War has been very instructive to this respect, because the troops sent there were often not up-to-date with the vaccinations, including the one against hepatitis B. I believe that this is a crucial problem and I would like to profit of the presence of all the experts here to have some insight on that.

R. STEFFEN (CH): Thank you for this suggestion. Actually, according to the international health regulations which you alluded to, as mentioned, only yellow fever vaccination is compulsory nowadays. Just Egypt require cholera immunization for travellers from Sudan. As we are also aware there is no regulation with respect to smallpox immunization. Well, as far as I know, the WHO policy (here there are official representatives of WHO, who may confirm or contradict me) is that there is no intention in issuing new firm regulations for compulsory vaccinations in travellers. There is a general feeling that freedom should dominate on this issue. However I think that the medical community has a very important task in stressing what is important in order to keep travellers or military personnel health and what is an important, but sometimes just antiquated, tradition.

M.D. PARKINSON (USA): I would like to make a comment on the new anti-hepatitis A vaccine. There are definite logistical problems with repeated administration of immunoglobulins every 3-6 months over a 3-5 year tour for troops in high risk areas. The introduction of a new hepatitis A vaccine would be a significant improvement over the current method of protecting such individuals.

R. STEFFEN (CH): Let me spend some words on the new anti-hepatitis A vaccination, of which not everybody is completely aware and that will see sunrise for Christmas in some countries. This SmithKline vaccine, which has been tested now in more than 1500 individuals, of which only one without seroconversion, is very well tolerated. Probably it will maintain the immunity for approximately 10 years according to the optimists and for about 5 years according to the pessimists. That is what we know so far.

J. A. BELLANTI (USA): I very much enjoyed the presentation of all the speakers and I would like to address a question to dr. Zanetti. You spoke about the compulsory immunization against hepatitis B in Italy and I understand that in USA we'll soon be following compulsory immunization for newborns. My question is related to any known adverse effects of immunization. It is important in fact to recognize not only the benefits, but also the adverse effects of vaccines. We have been burned several times in the past with mass immunization programmes, the most notable of course was the recent anti-flu immunization. We knew the safety of influenza vaccine being used for many years, but never for mass immunization or for a million people and there, as you know, we observed neurological complications, as Guillain-Barre' syndromes.
My question is: what is known about the side effects of hepatitis B vaccination, in terms of complications and No 2, what type of surveillance has been conducted, either by way of active or passive surveillance to assess these side effects? I certainly hope that there will be none, but, on the basis of past experience, this is something we have to look for.

A.R. ZANETTI (IT): All the reported studies confirm that the side effects are really minimal. They regard swelling, induration and soreness in the site of injections. Systemic reactions are really uncommon. In Italy we will have a Register for all the side effects of this vaccination, which started as a compulsory vaccination from May 1991.

J. A. BELLANTI (USA): Passive surveillance?

A.R. ZANETTI (IT): No, active surveillance.

M.D. PARKINSON (USA): Regarding hepatitis B vaccination, we are aware that in the US Army very good results have been obtained by plasma-derived vaccine intradermally, and it may be possible to use recombinant vaccine for short periods of protection. As pointed out by dr Zanetti, this is not recommended for intradermal use, but in USA, where tipically we pay 4 or 5 times as much for a vaccine, because, of our medicolegal system, it may be a promising way to deliver it for short periods of time.

A.R. ZANETTI (IT): Well, but the intradermal route is not recommended by the WHO, due to its difficulty in administration and the relative high incidence of side effects.

"HIV INFECTION IN THE NINETIES"

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SUMMARY

By the year 2000 a cumulative global total of 30 to 40 million men, women and children are projected to have been infected with HIV. This will represent a 3-4 times increase of the present total. Currently, it is estimated that about 5000 persons are newly infected daily.

Worldwide, the predominant and increasing mode of transmission is by heterosexual intercourse. Therefore, the number of infected women will equal that of men. Consequently, more infants will be infected by their mothers and more infants will be orphaned as their parents die of AIDS. By the end of the 1990's, over one million adult AIDS cases and deaths per year are expected, most of them in developing countries. Although the majority of HIV infections are currently occurring in Sub-Saharan Africa,the annual number of HIV infections in Asia is projected to exceed that in Africa during the 1990's.

Also in industrialized countries, the proportion of heterosexual transmission is increasing, and AIDS is becoming one of the predominant causes of death in young men and women.

In spite of promising scientific progress, vaccines and therapeutic drugs are not expected to have any major impact on the global development of the pandemic during the 1990's. WHO is promoting behavioural changes, condom use and control of other sexually transmitted diseases as the most important preventive measures.

INTRODUCTION

I am very pleased to have been invited to the Aerospace Medical Panel Symposium. In my presentation, I will try to give an insight into the dynamics of the HIV/AIDS pandemic and to our growing understanding of the main factors which are fuelling the continued and increasing HIV transmission. I will start with a short survey of the epidemiological background, the current trends and the forecasts of the directions of the epidemic during the next decade.

GLOBAL TRENDS OF THE PANDEMIC

The pace of the pandemic is now so rapid that the lethal virus will be transmitted globally to several hundred new individuals during the time of this short session; some 5000 persons are newly infected with HIV each day. Still, this is only the beginning. During the nineties, the transmission will increase dramatically and we will all be involved, professionally and personally.

The update on the global trends of the pandemic will begin with HIV infections (Table 1). WHO estimates that, by 1991, there is a cumulative number of 9-10 million adult men and women infected with HIV worldwide. About 60% of all HIV infections have occurred in sub-Saharan Africa, about 25% in North and South America and Australia, about 6% in Europe and about 10% in Asia. Thus, note that Asia is now estimated to have more infected persons than Europe.

Concerning AIDS, the total number of adults with AIDS is estimated to be above one million. The distribution of AIDS cases by region reflects that of the infections. However, Asia has only a low number of AIDS cases yet as the HIV infection was so recently introduced that most of the infections have not yet progressed to AIDS.

Furthermore, it is estimated that one million HIV-infected infants have been born to HIV-infected mothers. In addition, another 2.5 million non HIV-infected infants have been born to HIV-infected mothers, all of whom are potential "AIDS orphans" since their mother or both parents are likely to die from their infections.

The vast majority of HIV-infected children have already developed AIDS, half of which have died. This is because children develop AIDS more rapidly than adults. About 80% of children develop AIDS within the first five years of life. In contrast, in adults about 50% develop AIDS within 8-10 years. It should be underlined though, that the data in adults are based on studies mainly in men, in homosexuals and hemophiliacs, and in industrialized countries. There is not enough information on the progression rate to AIDS in women and in other populations to make a general statement on progression rate with reference to gender and ethnicity.

The pandemic has evolved along three broad, epidemiological lines. The determinants responsible for these different types of development are the period when HIV began to spread extensively and the major populations or risk factors involved.

In North America, Western Europe, Australia and New Zealand, extensive spread of HIV infection began during the late 1970s and the primary population group affected so far have been homosexual men and intravenous drug injectors. Although heterosexual transmission has to date accounted for a relatively small number of new infections, it is increasing at a greater rate compared to other routes of transmission in many places, particularly among teenagers, - a trend which is expected to continue.

In sub-Saharan Africa, extensive spread of HIV probably began at about the same time as in the USA, but is found predominantly among sexually active heterosexuals. In this area, HIV is increasingly spreading in rural areas where the majority of the population lives. In the hardest hit areas, as many as 20-30% of the sexually active population may be HIV infected, slightly more women than men.

Many countries in the Caribbean, Central and South America had an initial development similar to the USA. But by the mid- to late 1980s the pattern had shifted - sexual transmission among heterosexuals had increased extensively. There are rapidly increasing rates, e.g. in Brazil and Mexico; particularly in Brazil all the main routes of transmission being operative simultaneously and extensively; in hetero-, bi-, and homosexual men and in drug injectors and their sexual partners.

In Asia, Eastern Europe, North Africa, and the Middle East, HIV was introduced later, i.e. in the early to mid-1980s. The period of intense spread in Asia started only a couple of years ago. Right now, there is a continued dramatic spread of the epidemic in South and South-East Asia. No predominant mode of transmission was observed in Asia until 1988, when HIV transmission among intravenous drug injectors became prominent in at least four Asian countries located around the Golden Triangle; China, India, Myanmar (Burma) and Thailand. Rapid increases in seroprevalence rates have more recently been seen in female prostitutes, notably in India and Thailand. It seems clear that HIV infection has become firmly established among persons with high-risk behaviours and that transmission is predominantly heterosexual.

Due to the rapid spread, the current WHO estimate of cumulative adult HIV infections in South and South-East Asia is over one million. It is of particular note that the rate of increase of HIV infections in female prostitutes in urban India and Thailand between 1987 and 1990 is similar to that seen in the earlier stages of the HIV epidemic in sub-Saharan Africa. Thus, unless adequate control measures are taken now, in 5-10 years in some parts of Asia there may be the same high rate of HIV seroprevalence as currently seen in much of sub-Saharan Africa. This would mean a dramatic increase in the number of HIV-infected persons, as the population of, for instance, India alone is twice as large as that of sub-Saharan Africa. WHO projects that the annual number of HIV infections in Asia will exceed the annual number in Africa some time during the mid to late 1990's.

The potential impact of HIV and AIDS on overall adult mortality when a high percentage of young adults are infected, is upsetting. For example, in a population with an HIV seroprevalence rate of 20%, which is currently the case in many cities in Central and East Africa, adult mortality will increase two- to three-fold in only a 5-year period.

In New York City, AIDS was already in 1988 the leading cause of death in adult men and women 25-34 years of age, and is greater than that caused by drugs, cancer or homicide.

When HIV reaches 20% prevalence in pregnant women, there can be a 75% increase in mortality of children under five years of age due to perinatal transmission of HIV infection. As a result of child survival programmes, such as the expanded programme of immunization and diarrhoeal disease control, it was projected that child mortality would be reduced by one-third by 1995 in developing countries. However, because of paediatric AIDS cases, no decrease in childhood mortality is now expected to occur.

The first decade of the HIV/AIDS pandemic has drawn to a close. It is clear that this pandemic is still in its early stages and that its ultimate dimensions are difficult to predict. The most recent projections made by the WHO Global Programme on AIDS are shown in Table 2.

The dynamics of the AIDS pandemic in different regions of the world is illustrated graphically in Figure 1.

The annual incidence of AIDS is projected to peak in Europe and the USA during the mid 1990s, but continues to rise in Latin America, Asia and Africa, increasing the gap between the rich and the poor countries, between North and South, clearly indicating the link between AIDS and poverty!

HIV TRANSMISSION

Only three modes of HIV transmission have been documented: (1) via sexual intercourse (vaginal or anal); (2) by blood; (3) from a mother to her fetus/infant (perinatal transmission). Each mode involves exposure to infected body fluids such as semen, vaginal excretions, and blood.

Table 3 summarizes WHO's estimate of global HIV transmission by type of and efficiency of exposure, and percent of worldwide infections attributed to each as of 1991.

Transfusion of infected blood or blood products almost invariably leads to HIV infection: the likelihood of infection after such exposure is estimated to be greater than 90% Despite the very high probability that a large volume of infected blood will transmit infection to recipients, only an estimated 3%-5% of infections worldwide are due to such exposures because the number of persons at such potential risk is relatively small and blood in many countries since 1985 has increasingly been subjected to routine screening for HIV antibody.

The average probability worldwide that an infected woman will transmit HIV to her fetus or infant is estimated to be about 30%. Up to 10% of global HIV infections are estimated to be due to perinatal transmission.

HIV transmission through sexual intercourse has a markedly lower efficiency compared with blood transfusion or perinatal exposure. The risk of contracting HIV infection through sexual intercourse depends in part on the type and number of sexual contacts with infected persons. Studies have indicated that there is a greater probability that infected males will transmit infection to susceptible female partners as compared with the probability that infected females will infect susceptible male partners.

Accumulating evidence suggests that several contributing factors may increase HIV transmission via sexual intercourse. Several studies have implicated concurrent infection with other sexually transmitted diseases (STDs), especially those associated with genital ulcers such as syphilis and chancroid as significant "co-factors" in the spread of HIV, increasing the risk of infection by 5 times or more. Besides ulcerations of the mucous membranes, genital tract inflammation may enhance the risk of HIV transmission due to the increased number of the target lymphocyte cells in the genital tract.

Clearly then, considering the enhancing role of other STDs on HIV transmission, prevention and treatment of STDs is among the most urgent and doable interventions to contain the HIV pandemic. Also, as an STD is a sign of risk behaviour of the patient or the partner, the possibility should be considered that the patient may have been exposed to HIV as well, and the opportunity used to counsel the patient. This is likely to be the moment when a person is particularly receptive to counselling in order to be aware of the risk factors, the consequences of HIV infection and the need for behavioural changes relating to sexual practises and partners.

The probability of transmitting HIV infection from a single sexual act may be low - the reported range is 1/100 (1%) to 1/1000 (0.1%) in populations with low incidence of other STDs. However, as a result of very large numbers of sexual exposures which currently occur between HIV-infected and uninfected persons and the high incidence of other STDs in many groups and countries, sexual transmission is estimated, as of mid-1991, to account for up to 80% of global HIV infections; up to 70% of total infections have been due to heterosexual exposure and up to 10% to homosexual exposure. Due to the continuing increase among heterosexuals which constitutes the large population pool, the proportion of vaginal transmission will continue to increase.

CONCLUSION

The expectation is that HIV will spread more widely through populations where STD rates are high. Increasingly, heterosexual transmission will become the predominant mode of HIV transmission throughout the world. By the end of this decade, as many women as men will be infected. Invervention programmes to reduce the number of sexual partners, promote condom use and treat STDs will remain the key measures to control HIV infection. As an adjunct to health education efforts, vaccines against HIV would be an important contribution for AIDS prevention and control. However, in spite of promising scientific progress, a preventive vaccine will not be readily available before the turn of the century. There is still no cure against the HIV disease and no such cure is in sight.

HIV/AIDS has rapidly developed to be not only a medical and social problem, but also an economic and development problem of immense dimensions, threatening the very fabric of societies, as people in the most productive ages die, depriving society of their skills and manpower and leaving the very young and the elderly without support. Therefore, WHO is promoting multisectoral approaches and forceful, unprecedented response at the international and national levels. The efforts need to be sustained for decades to come.

TABLE 1

DISTRIBUTION OF ESTIMATED CUMULATIVE ADULT HIV INFECTIONS AND AIDS CASES

AREA	ESTIMATED HIV MID 1991	ESTIMATED AIDS END 1990
Africa	6 000 000	700 000
North America	1 000 000	200 000
Latin America	1 000 000	90 000
Asia	1 000 000	2 000
Europe	500 000	60 000
Oceania	30 000	3 000
TOTAL	9 500 000	> 1 000 000

TABLE 2

GPA PROJECTIONS OF HIV/AIDS BY THE YEAR 2000
(Updated, June 1991)

25-30 million cumulative adult HIV infections
8-10 million cumulative adult AIDS cases

PLUS

5-10 million cumulative paediatric HIV infections
4-8 million cumulative paediatric AIDS cases

TOTAL

30-40 million cumulative HIV infections in men, women and children
12-18 million cumulative AIDS cases in men, women and children

IN ADDITION

10-15 million children younger than 15 years old may be orphaned as a result of maternal AIDS

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Allergic, Immunological and Infectious Disease Problems in Aerospace Medicine

(Les Problèmes Causés par les Maladies Allergiques, Immunologiques et Contagieuses en Médecine Aérospatiale)

Papers presented at the Aerospace Medical Panel Symposium held in Rome, Italy, from 21st to 25th October 1991.

North Atlantic Treaty Organization
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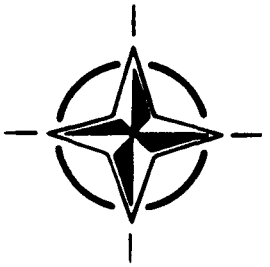

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ESTIMATED/PROJECTED ANNUAL ADULT AIDS

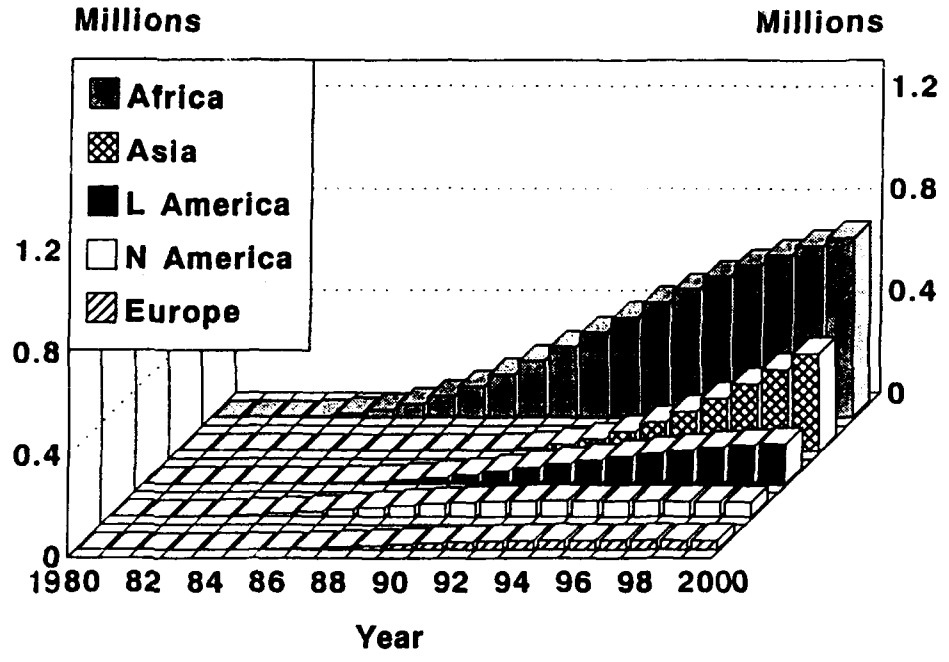

FIGURE 1

TABLE 3

HIV TRANSMISSION GLOBAL SUMMARY - 1991

TYPE OF EXPOSURE	EFFICIENCY PER SINGLE EXPOSURE	PERCENTAGE OF GLOBAL TOTAL
◯ Blood transfusion	>90%	3-5
◯ Perinatal	30%	5-10
◯ Sexual intercourse (vaginal) (anal)	0.1-1.0%	70-80 (60-70) (5-10)
◯ Injecting drug use - sharing needles, etc.	0.5-1.0%	5-10
◯ Health care - Needle-sticks, etc.	<0.5%	<0.01

AIDS/HIV IN THE U.S. MILITARY

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INTRODUCTION

«In the course of normal events, lessons learned in battle are lost from conflict to conflict. The Military Medical Department is not immune to this affliction. In the arena of medical disorders in the combat environment, sufficient historical perspective exists to allow global planning strategy to include a detailed analysis of disease as it may affect the U.S. military in any environment. Disease is woven intricately into the fabric of war. The story of one cannot be told without the other and yet, each succeeding generation of history, soldier and scholar alike, seems to be destined to repeat the errors of history and fail to perceive the impact of disease.» (1)

Acute diseases involving three organs of the body have most often affected military operations: the gastrointestinal tract, blood, and the genital tract, i.e. diarrhea, malaria and gonorrhea, etc. The Human Immunodeficiency Virus-1 (HIV-1), the cause of AIDS (Acquired ImmunoDeficiency Syndrome), infects two of these organ systems. *HIV is a fatal sexually transmitted disease that contaminated blood!* As such, its impact on medical readiness is broad and far reaching.

BACKGROUND

Human beings are constantly victimized by new infectious or communicable diseases which, at various times in history, have decimated the population (plague in the Middle Ages) or changed the course of history (diarrhea [shigellosis] during the campaigns at Gallipoli and Northern Africa). In the past 15 years, Legionnaires'Disease, Lyme Disease, Toxic Shock Syndrome and a score of other lesser known *new* infectious diseases have afflicted man. HIV infection (AIDS) is another. But unlike those noted above, HIV is much more diabolic. It is spread by means that have social, political and moral consequences (sex) or threaten the underpinning of modern medicine (blood). And it is always fatal.

There is now considerable evidence that HIV originated in Central Africa sometime during the last century. Its rapid spread was undoubtedly hastened by modern amenities, particularly air travel. In fact, its spread has been much more rapid to the wealthy industrialized nations in the Western Hemisphere than overland throughout Africa.

As noted above, HIV's primary mode of spread is through sexual contact. Hence, it follows the same epidemiologic pattern of all other sexually transmitted diseases (STDs), afflicting primarily young sexually active adults and increasing in incidence during time of social stress. Because it also contaminates blood, it can be efficiently spread through the exchange of blood - hence, the threat of contaminated blood transfusions, intravenous drug abuse or accidental injection. (HIV is an occupational hazard for all health care providers.) It cannot be spread in any other way (i.e. non-sexual or casual contact).

Not surprisingly, the first group in which HIV surfaced in the United States was among promiscuous homosexual men, the group in which the rate of sexually transmitted diseases (venereal diseases) has been highest in the United States for the past 20 years (the U.S. military was not far behind).

The next subgroup in which HIV infection (AIDS) has emerged is in urban intravenous drug abusers who share contaminated intravenous drug paraphernalia. The relationship between drug abuse and promiscuous sex is of course close, especially in women.

Thus, the stage is set for the inevitable spread throughout the active heterosexual U.S. population. This is a sobering thought when we consider the endemic equilibrium which existed for syphilis before the advent of penicillin, another sexually transmitted disease which contaminates blood. Approximately eight percent of the population of the United States was infected with syphilis before penicillin was introduced during World War II. How long it takes and what the endemic rate of HIV will eventually be is conjecture. But given the fact that human behavior has changed little over time, HIV is destined to influence modern day history in much the same way syphilis did. (Henry VIII, Sir Randolph Churchill, Ivan the Terrible and Ludwig Beethoven were a few of the notable individuals who were infected with syphilis.)

By following HIV rates in prostitutes, the relentless spread of the epidemic across the world can be traced. In some urban areas in Central Africa, up to 90 percent are now infected; in some U.S. cities, as many as 50 percent are infected; and in Bangkok, Thailand where only a handful of infected prostitutes were afflicted in the late 1980s, at least 15 percent are now infected,

The impact of HIV on a nation is similar to that of war. Young, productive, infected adults become disabled and dysfunctional, and die, leaving behind the young and old. Its devastating economic and political impact has already been felt in some African countries, and this unfortunate scenario is destined to be played out over and over again.

The virus kills in the most hideous of ways. It infects and eventually destroys the T-helper or CD4 cell, the very cell that is at the heart of our immune system. But it does so in a slow, relentless, steady manner over eight to 14 years. Thus, there is a long asymptomatic period during which the victim *feels well, looks well, performs well* and for all practical purposes *is well!* But, like the infamous Typhoid Mary, he/she is capable of spreading the infection. It is this infectious iceberg effect which makes the clinical diagnosis difficult and keeps the epidemic going. By focusing only on the end-stage of the disease (AIDS or WR Stage 5/6) (2) as the public health community has thus far been forced to do, what happened eight to 14 years earlier is revealed. It tells you nothing of the present infection rate, the threat lurking below the surface.

MILITARY CONCERNS

HIV infection potentially impacts the complete spectrum of military activities. It is becoming world-wide in distribution (Figure 1). Its burden on health care resources, especially in poor countries, will undermine and set back recent advances made in such vital areas as sanitation and public health. Its burden on the most productive portion of the population will reinforce the roots of poverty, despair, and dictatorial and despotic rule.

As the prevalence of HIV rises in the world, deployment of U.S. troops must be viewed in the context of its impact on strategic and tactical planning, especially with regard to landforces and their interaction with the local populace, be it buddy care or medical support to civilian casualties, protecting the blood supply, intimate civilian contact, unit morale or political concerns.

Sexually transmitted diseases have traditionally been two to

three times higher in U.S. military troops than in their civilian counterparts (up to 100 times higher in wartime). HIV infection is a new threat set in this mode. All diseases carry a political liability. But none as much as a sexually transmitted disease (social disease) that kills. In this regard, the military is caught in the classic catch-22 scenario; some foreign nations will view our military as the conduit that contaminates their populace (some countries have already stipulated that American troops are not welcome unless certified to be free of HIV); while the home front may view the military as the conduit that brings the problem home (many U.S. public health officials blamed the rise in penicillin-resistant gonorrhea in the 1980s on importation by military troops of such organisms from the Far East; most HIV disease in Cuba has been traced to Cuban soldier contact in Angola).

Furthermore, because HIV infects blood, a new dimension has been added. Modern warfare has stressed modern military medicine in many areas, but perhaps the most critical is the need to replace blood loss and correct blood clotting disorders with transfusions of uncontaminated freshly drawn blood. The requirement to reduce the risk of AIDS through blood transfusions to as low a level as possible (hopefully zero) is imperative. There simply are not enough blood reserves in our troops to cover transfusion requirements than for more than just a few casualties and we must rely on fresh blood obtained from the local civilian populace.
When as many as one in 10 or 20 is infected, the utility of that blood supply is obviously in question.

In addition, the rendering of care to civilian casualties would be severely compromised if even a small percentage were infected. Rendering medical assistance is important in winning their hearts and minds, and if callousness towards and withdrawing care from civilian casualties ensues, our morality is undermined. Thus, not only is our safe blood supply threatened, but the unit cohesiveness and trust necessary for an efficient combat force is undermined. In short, HIV potentially isolates us!

Finally, health care costs to the military, already stretched because of CHAMPUS costs, will be staggering. Even if no new cases were to occur, the 10-year projection in 1989 dollars for the Department of Defense (DoD) is $1.7 billion to $1.9 billion. At the present incidence rate of new cases, the cost is projected to be $2.7 billion to $3.0 billion. And these figures are based on only 60 percent of health care beneficiaries utilizing the military health care system (in other words the potential 10-year cost is close to $5 billion!).

THE ARMY (DOD) HIV RESEARCH PROGRAM

The Army, as «Lead Agent for Infectious Disease Research» for the DoD, has formed the framework for the military's entire HIV policy since its inception in 1985. It is multifaceted and comprehensive, emphasizing early diagnosis (screening and testing), patient notification and counseling, contact tracing, health education, compassionate and modern medical management of cases and medical research. It is the latter that will have the greatest effect at reducing the impact of HIV on the military. It emphasizes two main thrusts, epidemiology (tracking the epidemic world-wide and within the Army) and prevention. Prevention research follows two different thrusts, the development of a vaccine or prophylactic drugs and means to improve effective education to decrease risk in the soldier and better equip commanders to deal with this ubiquitous infection (Table 1).

Epidemiology

Tracking prevalence rates worldwide has an obvious benefit for military planning (Table 2). For example, cast in terms of recent events, it is recognized that infection rates in some Caribbean nations will soon approach those of Central Africa, about one in 10 young adults! Commanders must be equipped to address this largely invisible time-bomb from every perspective. They must understand the risk, convey it effectively to their troops, and decrease high risk behavior, but they must maintain the delicate balance of respect and compassion for the local populace.

By tracking the epidemic within the military, certain groups can and have been identified as being at a higher relative risk, which in turn allows us to modify and better direct our educational efforts at risk reduction. For example, the overall incidence rate in the Army over the past five years has fallen from 0.49/1000 (one in 2,000) to 0.29/1000 (one in 3,500) or 41 percent, but the rate in some minority subgroups and some military occupation skills has remained stable or increased. Why these subgroups have not been reached by Army educational programs thus far is unknown. To understand why will require intricate, sensitive and complicated research.

Furthermore, as part of this ongoing tracking effort within the Army, studies have been conducted regarding the performance of infected soldiers. Matching 573 infected soldiers with 2,292 uninfected soldiers with regard to age, military occupation, sex and length of service has revealed no differences in promotion rates, demotion rates, or disciplinary action. Infected soldiers match their uninfected brethren; some are good soldiers, most are average and some are misfits. However, given the spectrum of bodily dysfunctions caused by HIV infection, are there specific tasks which can be adversely affected by HIV? For example, there have been studies that suggest that some of these patients develop a slowed reaction time as measured in fractions of a millisecond; or a decreased ability to hit a 95-miles-per-hour Roger Clemens fast ball! In other words, the practical input of these findings is unknown. Research to address relevant military specific tasks is ongoing.

Finally, the demographic data generated by the Army is unique in the free world and has provided our nation with invaluable information surrounding the epidemic, especially with regard to civilian applicants for military service (Figure 2). Although biased, it is the only organized glimpse at the epidemic on which to base future (10-year) projections. For example, the rate in women applicants 17 to 20 years old is the same as that in men applicants 17 to 20 years old (about one per 1,000 applicants) and heralds the changing demographic profile of HIV infections in our nation.

Prevention

Reducing the risk to troops and making the Army safe requires a two-pronged approach: research efforts aimed at changing behavior of troops from high risk to low (zero) risk; and research efforts aimed at developing a vaccine or drugs to prevent the disease from occurring or which abate its lethal effects.

Based on past performances of our research efforts regarding other medical problems within the military, the former has been only moderately successful while the latter has been quite successful (the U.S. Army has been instrumental in the development of more vaccines than any other medical entity in the world). However, *effective education* that results in a change of behavior is all we have to offer at the present time, and given the complexity of the biology of the HIV infection, may be all we have to offer for sometime to come. Effective education on the prevention of sexually transmitted diseases requires close cooperation between commanders and the Medical Department. Since effective education is a benchmark of effective leadership, leadership training must acquire an added dimension. But this requirement is extremely difficult and complex because it embraces cultural differences, social mores, and individual standards.

The commander must understand how it is acquired (sex and blood), what risks an infected individual poses to others (buddy care, civilian casualties), what to expect from infected persons (civilian or military), and what the chance of acquiring the infection are under different circumstances, but at the same time he/she must be caring, compassionate and committed to overcome prejudices and biases towards HIV infected persons. For example, he/she must be able to articulate why the restrictions on OCONUS deployment are politically based and not medically driven. In short, commanders will be challenged to address their own sexuality on the one hand and to become surrogate public health proponents on the other. This should help commanders become more effective leaders by better understanding themselves and establishing the proper environment to maintain the requisite skills, knowledge and attitudes. Obviously, research that results in the development of truly effective means of behavioral change will be complicated, threatening and sensitive.

In contrast to behavioral research, biological research on HIV is less controversial but no less daunting. The Human Immunodeficiency Virus has an enormous repertoire to escape the time-honored means of developing an effective vaccine or drug treatment. (It integrates and becomes part of human cells and it destroys the very cell that is relied upon to combat and control infectious agents.)

Nevertheless, the United States Army Medical Research and Development Command (USAMRDC) has made significant strides (Table 3). It was the first research group to embrace the concept of immunotherapy as an innovative approach towards the development of an effective vaccine. This novel concept theorizes that a specific HIV vaccine can be given to infected persons that will augment or induce a protective immune response as evidenced by the induction of novel antibodies and augmented immunity that result in an alteration of the natural course of infection, with improved survivability and/or potential eradication of the virus. If the latter is accomplished in any way, an important step will have been taken towards developing a *protective vaccine* that can be given to all soldiers *before exposure,* be it sexual or post-wounding through contaminated blood. Early results have been extremely encouraging and have been extended to denovo protection in experimental animals!

However, because of the inherent long latency period from time of infection to full blown AIDS and death (eight to 14 years), it will take longer than that to prove true efficacy. Hence, even in terms of the most optimistic outlook, we are doomed to confront HIV for a long time!

Other

Less research investment, but just as intense efforts are ongoing in other areas; namely, the development of rapid diagnostic tests that can be used in the field and the testing of drugs that may be used as prophylaxis. The former is close to fruition and would have obvious enormous impact for medical personnel (buddy care) who could quickly determine whether a victim they are attending is infected or if a potential blood transfusion is contaminated.

The eventual development of prophylactic drugs is more problematic for the same reasons noted above for vaccine development. However, the development of a «transfusion or *morning-after pill*» would obviously alleviate much of our concerns.

Finally, the advancements made thus far by the USAMRDC have already had an enormous impact. For example, over 4 million civilian applicants have been screened and over 4,000 infected applicants have been excluded from military service. This translates to over $1 billion already saved! Furthermore, there have been no legal challenges with regard to a false or misdiagnosis. Our testing program runs efficiently and is cost effective (about $3.00 per person). It simply is the best screening program in the world!

Nevertheless, some have questioned the utility of the military's investment in AIDS research given the research efforts of the Department of Health and Human Services (HHS). But examination of the facts will convince the skeptics of the wisdom of the Army's research program.

The primary emphasis of the Health and Human Services Department is on the treatment (prolonging of life) of late stage disease (AIDS or WR Stage 5/6) (Table 4). This is an important, necessary and laudatory goal, but it fails to adequately address the military's needs. In contrast, the military research effort is on prevention and treatment of early disease. Hence, the military focus to make the Army safe emphasizes early diagnosis and early intervention and has formed the framework upon which DoD policies from recruit screening to staging have been built.

Furthermore, unbridled from the protestations and radical stands of certain interest groups, the military has been able to address HIV as one would any other communicable or public health menace, and its program has become the blueprint for the rest of the nation to follow. For example, early diagnosis, once the lonely posture of the U.S. military has now become a «National Public Health» priority. (Undoubtedly, over time, other public health priorities will come even closer to the military's.)

Because HIV infects human beings, and human beings make up the military, overlap between the DoD and HHS research programs will occur. But only the military medical establishment will have as its objective addressing military relevant issues (worldwide *surveillance, prevention, rapid field diagnosis, military* unique education). It is doubtful that the present DoD Applicant Screening Program would be in place or be as successful had not the USAMRDC undertaken that mission.

SUMMARY

HIV infection (AIDS) burst upon the scene a decade ago. Because it is a sexually transmitted disease that infects blood and kills its victim, it is military relevant and will impact on all aspects of the military. The USAMRDC as «Lead Agent for Infectious Disease Research» in the DoD has developed a comprehensive approach to address military concerns: surveillance of infection rates (intelligence) around the world and in the military; behavioral research to develop more effective means of education to change behavior; and biological research to develop a quick and easy field test, and a vaccine or drug to prevent the disease from occurring despite exposure. Its success will influence the success of the Army in the future.

NOTES

(1) A. Ognibene, BG, Mil Med 152:14, 1987.

(2) The Walter Reed Staging System classifies the infection based on the immunologic state of the patient. WR1 refers to the earliest stage when only antibodies are present; WR2, when a generalized enlargement of lymph nodes occurs (not seen in every patient); WR3, when the T4 helper lymphocyte count (CD4) falls below 400/ml; WR4 when the earliest functional immunologic defects can be seen, namely a decreased reaction to skin tests;
WR5, when a fungal organism, *Candida albicans,* which normally lives conformably in our bodies, causes an infection; and WR6, when opportunistic infections develop. AIDS is WR5/6.

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FIGURE 1

Trends in AIDS Cases by Geographic Region

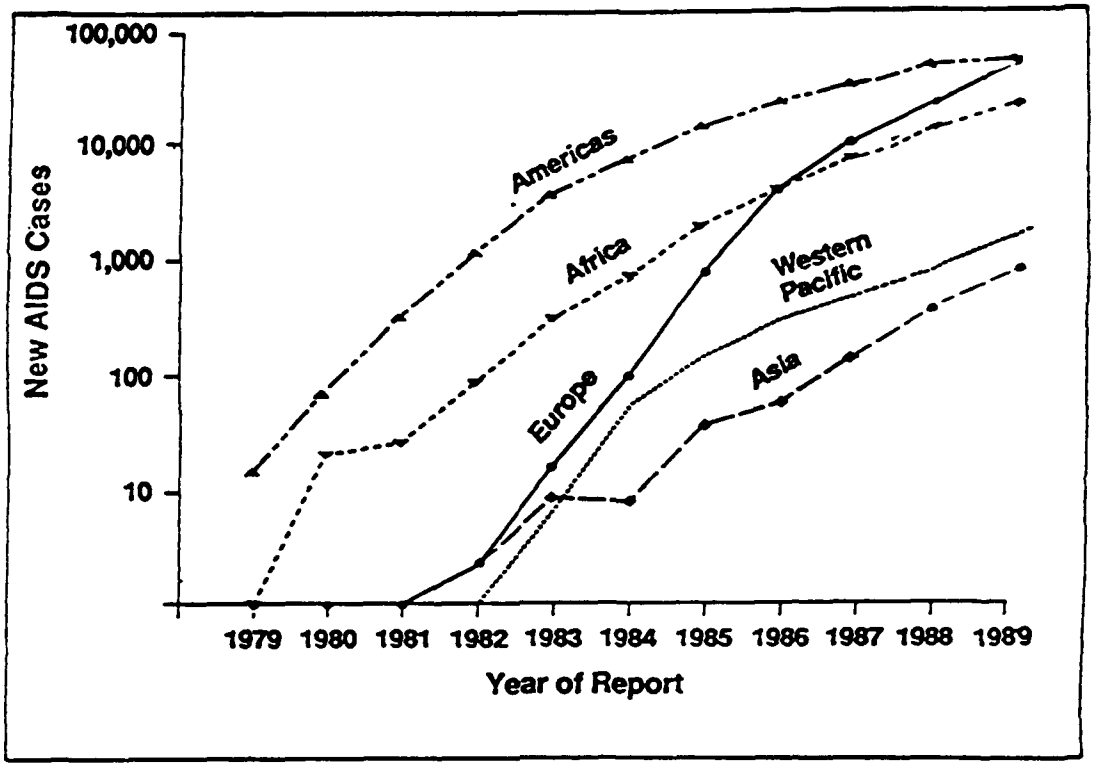

TABLE 1

MILITARY HIV RESEARCH PROGRAM

PRIORITIES AND OBJECTIVES

- IDENTIFY RISK FACTORS (INCLUDING OCONUS) IMPORTANT TO TROOP EDUCATION (CHANGE BEHAVIOR) AND HIV TRANSMISSION IN MILITARY POPULATIONS
- TEST AND EVALUATE VACCINE AND/OR PROPHYLACTIC DRUGS
- TEST AND EVALUATE DRUGS FOR EARLY INTERVENTION
- DEVELOP AN EFFICIENT, HIGHLY RELIABLE FIELD TEST
- EVALUATE THE COURSE OF INFECTION IN MILITARY POPULATIONS FOR DEFINING DOD POLICIES

TABLE 2

US MILITARY OCONUS RETROVIRUS RESEARCH PROJECTS

REGION	COUNTRY	ORGANIZATION
EAST ASIA	PHILIPPINES•	US NAVY
	THAILAND	US ARMY
	OKINAWA	US NAVY
SOUTH AMERICA	BRAZIL	US ARMY
	PERU•	US NAVY
MIDDLE EAST	EGYPT•	US NAVY
AFRICA	ZAMBIA•	USUHS
	ZAIRE	UNIV. PARIS
	SENEGAL•	HARVARD UNIV.

• DIRECTED BY USAMRDC

FIGURE 2

COUNTY-SPECIFIC PREVALENCES
OCTOBER 1985 – MARCH 1990
USING LOWER CONFIDENCE BOUND

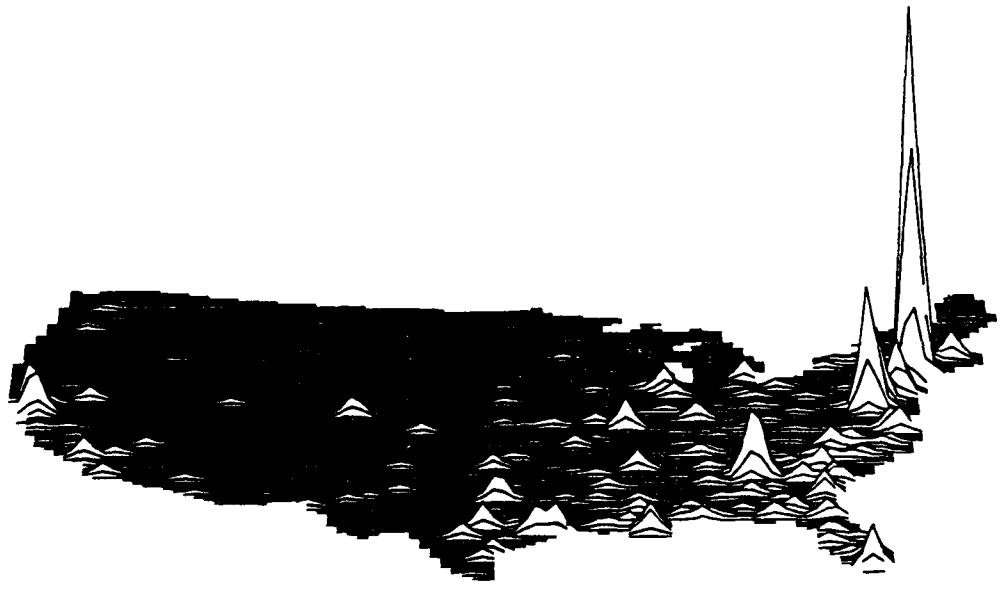

TABLE 3

ARMY HIV RESEARCH PROGRAM ACCOMPLISHMENTS (1990)

- ESTABLISHED WORLD'S HIGHEST QUALITY TESTING PROGRAM
- ESTABLISHED WORLDWIDE CLASSIFICATION STANDARD (WALTER REED STAGING SYSTEM)
- FIRST TO FOCUS ON EARLY DIAGNOSIS (NOW A NATIONAL PRIORITY)
- FIRST TO IDENTIFY HETEROSEXUAL TRANSMISSION IN USA POPULATION
- INSTITUTED RESEARCH OBJECTIVES OF IMMUNOTHERAPY
- PROVIDED SCIENTIFIC DATA BASE FOR AND DEFENDED DoD POLICY ON HIV (ENTRY SCREENING, EARLIER SEPARATION, TROOP EDUCATION, NO OCONUS ASSIGNMENT)
- PROVIDED COUNTRY WITH MOST MEANINGFUL DATA ON USA EPIDEMIC

TABLE 4

ARMY VS HHS PROGRAMS

THRUST AREAS	MILITARY	HHS
NATURAL HISTORY	MILITARY POPULATION	SUBGROUPS ONLY
EPIDEMIOLOGY	EARLY INFECTION (WR 1-4)	AIDS (WR 5-6)
DIAGNOSIS	EARLY INFECTION (WR 1-4)	AIDS (WR 5-6)
BLOOD PROGRAM	RAPID, FIELD	TIME INSENSITIVE
VACCINES	BASIC AND APPLIED RESEARCH	BASIC RESEARCH
DRUGS	PROPHYLAXIS	THERAPY
CHANGE BEHAVIOR	MILITARY POPULATION	SUBGROUPS
DATA BASE	MILITARY UNIQUE	NONE

ESTIMATES OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INCIDENCE AND TRENDS IN THE US AIR FORCE

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SUMMARY

Between early 1986 and February 1991, over 700,000 United States Air Force (USAF) active duty personnel had been screened for antibodies to the Human Immunodeficiency Virus (HIV). All HIV-infected patients are evaluated at Wilford Hall USAF Medical Center, and staged using the Walter Reed scheme.(1) Two total-force screenings were conducted prior to October 1990. The USAF case registry is maintained by the Epidemiologic Research Division. Computer support for incidence calculations is provided by the Defense Manpower Data Center.

During the first screening (February 1986-September 1988), 721 HIV-positive personnel were detected from a USAF population of approximately 607,000. The estimated seroprevalence was 1.2/1000.(2) From the second screening 206 positive individuals were detected among approximately 571,000 personnel. As of January 1991, among the total 942 HIV-positives, only 29 were female. Three hundred and fifty-one (37.3%) remained on active duty in the US; 296 (31.5%) were on temporary disability retirement lists; 193 (20.5%) had separated or retired; and 101 (10.7%) had died.

From June 1987 through June 1990, the estimated incidence of HIV infection in USAF personnel declined from 0.19 to 0.17/1000 person-years. The rate for males in June 1987 was estimated at 0.21/1000 person-years. In all age groups, incidence rates were highest among black males.

In October 1985, the US Department of Defense (DOD) mandated that all applicants for military service be tested for evidence of antibodies to the human immunodeficiency virus (HIV).(3) This testing continues, and if test-positive, applicants are denied entry.(4) Testing of those already in the US military services began soon afterward.(3) Between early 1986 and February 1991, over 700,000 active-duty US Air Force (USAF) personnel were tested for the presence of HIV antibodies.

In addition to two total-force (USAF, USAF Reserves, and Air National Guard) screenings prior to October 1990, USAF personnel have been tested in conjunction with: evaluation and treatment for other sexually transmitted diseases, routine and periodic physical examinations, enrollment in drug or alcohol rehabilitation programs, orders to permanent overseas assignments, and clinically-indicated medical reasons. This paper discusses 1987-1990 HIV incidence estimates in a relatively young, mobile male population by: age, ethnicity/race, sex, and occupational category at time of positive HIV antibody test.

METHODS

We identified all persons who served ≥180 days on active duty in the USAF between 01 July 1986 and 30 June 1990. From this population we have identified all who had a prior negative enzyme-linked immunosorbent assay (ELISA) for HIV antibodies, followed by a confirmed positive test at least 60 days later. The criterion for a positive test was a repeatedly reactive ELISA followed by a Western blot that exhibited at least two of the following bands: p24, gp41, and/or gp120/160.

From February 1986 to August 1986 in our laboratory, ELISA testing to detect the presence of antibodies to HIV in serum was accomplished using the Abbott Laboratory (Abbott Park IL) HTLV-III (HIV-1) Enzyme Immunoassay (EIA) test kit. Contractor (Blood Systems, Inc.; Scottsdale AZ) HIV screening began in August 1986 and employed the DuPont (Biotech Research Inc.) HIV-1 ELISA test kit. From August 1986 to July 1987, approximately 80% of USAF total-force screening was conducted using the DuPont kit; 20% using the Abbott EIA kit. Beginning in August 1987, all USAF screening was accomplished with the DuPont kit.

The USAF began using an ELISA method from a manufacturer different from the screening method ("alternate" ELISA) in August 1986, and the Biotech Research Laboratories, Inc. (Rockville MD) Western blot assay methodology to confirm initial positive results. Since March 1987, the Cambridge Bioscience Corporation (Worcester MA) recombinant DNA ELISA and an indirect fluorescent immunoassay have been used to resolve indeterminate or discordant results.

All individuals who test positive while on active duty are evaluated at Wilford Hall USAF Medical Center (WHMC); Lackland AFB TX; retested using both ELISA and Western blot assays, and classified clinically according to the Walter Reed (US Army) HIV-disease staging system.(1) Following this clinical confirmation and disease staging, data on these individuals are maintained in the Epidemiologic Research Division of the Aerospace Medicine Directorate, Armstrong Laboratory, Brooks AFB TX.

The at-risk period for each individual began with the last negative ELISA and continued until the first positive (Western blot confirmed) assay or the next negative ELISA. The time of seroconversion was assumed to be the point midway between the last negative ELISA and the first confirmed positive. The individual was included in the numerator if this midpoint fell within a period of active duty. The age of the individual at seroconversion was calculated as the Julian date of the "seroconversion" midpoint, minus the Julian date of birth.

Person-years-at-risk were calculated using dates and results of all ELISA and Western blot assays from the total-force screening (including USAF Reserve and Air National Guard) and ancillary testing programs. Dates and results of HIV antibody tests (DOD Reportable Disease Data Base) for all USAF personnel are subsets of data in personnel files at the Defense Manpower Data Center (DMDC).

Incidence rates of HIV seroconversion (01 July 1986 through 30 June 1990) were calculated using person-years at risk for the denominators.(5) Person-years-at-risk included only time on active duty for persons tested as members of the National Guard, Reserves, or retirees. The software programs for these calculations were developed by Defense Eligibility Enrollment Reporting System (DEERS) personnel at DMDC in Monterey CA. Ninety-five percent Poisson confidence intervals were calculated for each rate. (6)

RESULTS

During the first screening (February 1986-September 1988), 721 HIV antibody positive personnel were detected from a USAF population of approximately 607,000. The estimated seroprevalence was 1.2/1,000.( ) The second screening detected 206 seropositive individuals among approximately 571,000 personnel. As of 31 December 1990, among the cumulative 942 HIV seropositives from the USAF, only 29 have been females.

Table 1: As of 31 December 1990, the marital status of USAF cumulative HIV cases by sex.

	Married	Not married	Total
Female	12	17	29
Male	278	635	913
Total	290	652	942

Marital status was self-reported at initial interview after positive test result. Female cases are more likely to have been married at time of test.

Table 2: Active duty HIV "cases" in the USAF by calendar year (CY) of positive test and sex.

CY	Female	Male
1985	0	32
1986	8	203
1987	13	370
1988	1	130
1989	2	101
1990	4	60

Table 3: USAF positive HIV cases by calendar year and whether the first or subsequent HIV test.

CY	First test	Prior negative
1985	32	0
1986	208	3
1987	364	19
1988	101	30
1989	19	84
1990	10	54

The "prior negatives" were the first HIV incident cases identified.

Table 4: USAF cases by calendar year and grade or class (enlisted vs officer).

CY	Enlisted	Officer
1985	27	5
1986	192	19
1987	346	37
1988	110	21
1989	93	10
1990	54	10

Table 5: The first 190 USAF incident HIV cases, by grade and sex.

	Officer	Enlisted	Total
Female	0	7	7
Male	17	166	183
Total	17	173	190

Table 6: USAF incident HIV cases by marital status and sex.

	Married	Not married	Total
Female	1	6	7
Male	60	123	183
Total	61	129	190

Note that in female incident cases, the proportion of "married" is much smaller than in the total female cases.

Table 7 displays the HIV seroconversion rates in the USAF for July 1986 through June 1990 by 10-year age groups. Table 8 displays these rates for males by the same age groups. Numerators for 5-year age groups were very small for those aged 35-50. Note that overall rates and rates for total males have dropped slightly over time: 0.21 to 0.18, and 0.19 to 0.17, respectively.

Table 9 illustrates the HIV seroconversion rates in USAF males for July 1986 through June 1990 by Caucasian/non-Caucasian and 10-year age groups. While overall rates in Caucasian males have remained stable, and rates in non-Caucasians have declined, the changes within age groups and/or between races have not been consistent. Rates in females were not calculated because of the small numbers within each age group and each time interval. Rates in males $\geq$40 appear to be increasing.

As of 31 December 1990, there were 22 HIV-positive aeronautically rated officers: 9 were pilots; 4 were navigators; 4 were airweapons officers; 4 were landbased missile officers; and 1 was a space operations officer.

As of 31 December 1990, there had also been 22 HIV-positive officers identified from the medical career fields: 8 were physicians (4 surgeons, 2 internists, 1 pediatrician, and 1 family practitioner); 7 were nurses (4 ward/clinical nurses, 1 operating room nurse, 1 mental health nurse, and an administrative nurse); 3 were dentists (2 general dental officers and 1 periodontist); and 4 others (podiatrist, social worker, pharmacist, and health care administrator).

DISCUSSION

The HIV and consequent disease problems in the USAF are concentrated in young (20-35 year old), unmarried males. However, rates in older men appear worrisome. Numbers are small. It is difficult to generalize these findings to the US population. However, some civilian studies also note increasing rates in older men. The HIV seroconversion rates in the USAF male and female populations are markedly different. Numbers of HIV-positive USAF females are so small, we can't have confidence in estimates of rates. Such differences are not due to differences in case definition or ascertainment; all sample submission and data analyses were performed in identical manners. Self-selection and policy factors within the USAF probably result in a population that is behaviorally different from demographically similar civilians. As in the US Army, infection of health care providers doesn't point to an occupational risk from the HIV agent. Aviators and crews are not at increased risk.

Information regarding high-risk behaviors (male homosexual or bisexual, anal sex among heterosexuals, or illicit/intravenous drug use) is difficult to obtain in the US military population. More detailed discussions of age-, race-, or occupation-specific risk factors are therefore impossible. If USAF rates of HIV seroconversion have stabilized, as these data suggest, it is even more important that the medical units continue to ensure that HIV-prevention educational programs are presented to all USAF personnel. Continued follow-up of USAF personnel, for HIV seroconversion, will reveal more about the dynamics of this epidemic.

Table 7
HUMAN IMMUNODEFICIENCY VIRUS (HIV)
SEROCONVERSION RATES*,
US AIR FORCE ACTIVE DUTY

	01 Jul 86 - 30 Jun 88 n/person-yrs (rate) 95% C.I.**
Age group	
<20	7/54531.6 (0.13) .05-.27
20-29	81/339328.6 (0.24) .19-.30
30-39	18/153685.2 (0.12) .07-.19
≥40	3/ 38289.8 (0.08) .02-.23
Total	109/585835.2 (0.19) .16-.23
"At risk"***	520,981

	01 Jul 88 - 30 Jun 89
Age group	
<20	1/ 28627 (0.03) 0.00-.17
20-29	55/241211.1 (0.23) .17-.30
30-39	16/134796.3 (0.12) .07-.19
≥40	2/ 39513.2 (0.05) .01-.18
Total	74/444147.6 (0.17) .13-.21
"At risk"***	522,047

	01 Jul 89 - 30 Jun 90
Age group	
<20	1/ 15600.5 (0.06) 0.00-.33
20-29	30/118703.6 (0.25) .17-.36
30-39	5/ 69944.8 (0.07) .02-.16
≥40	3/ 20146.6 (0.15) .03-.44
Total	39/224395.5 (0.17) .12-.23
"At risk"***	377,553

* per 1000 person-years
** Poisson 95% Confidence interval
*** Individuals contributing time in the denominator

Table 8
HUMAN IMMUNODEFICIENCY VIRUS (HIV)
SEROCONVERSION RATES*, US AIR FORCE
ACTIVE DUTY MALES

	01 Jul 86 - 30 Jun 88 n/person-yrs (rate) 95% C.I.**
Age group	
<20	7/ 44822.5 (0.16) .06-.33
20-29	80/287437 (0.28) .22-.35
30-39	17/135233.7 (0.13) .08-.21
≥40+	3/ 36510.3 (0.08) .02-.23
Total	107/504003.4 (0.21) .17-.25
"At risk"***	449,773

	01 Jul 88 - 30 Jun 89
Age Group	
<20	1/ 23161.6 (0.04).001-.22
20-29	54/204387.1 (0.26) .20-.34
30-39	16/118320.8 (0.14) .08-.23
≥40+	2/ 37822.4 (0.05).006-.18
Total	73/383691.9 (0.19) .15-.24
"At risk"***	448,895

	01 Jul 89 - 30 Jun 90
Age Group	
<20	1/12325.4 (0.08).002-.45
20-29	26/99083.1 (0.26) .17-.38
30-39	4/60992.2 (0.07) .02-.18
≥40+	3/19192.2 (0.16) .03-.47
Total	34/191592.8 (0.18) .12-.25
"At risk"***	323,483

* per 1000 person-years
** Poisson 95% Confidence interval
*** Individuals contributing time in the denominator

Table 9
HUMAN IMMUNODEFICIENCY VIRUS (HIV)
SEROCONVERSION RATES*, US AIR FORCE
ACTIVE DUTY MALES, BY RACE

01 Jul 86 - 30 Jun 88
rate (95% C.I.)**

Age group	Caucasian	Non-Caucasian
<20	0.08 (.02-.23)	0.49 (.13-1.25)
20-29	0.22 (.16-.29)	0.49 (.33- .70)
30-39	0.08 (.03-.16)	0.27 (.12- .51)
≥40	0.07 (.01-.25)	0.15 (.00- .84)
Overall	0.16 (.12-.21)	0.40 (.29- .54)
"At risk"***	354,785	94,988

01 Jul 88 - 30 Jun 89

Age group	Caucasian	Non-Caucasian
<20	0.00 (.00-.16)	0.25 (.01-1.39)
20-29	0.17 (.11-.25)	0.61 (.40- .90)
30-39	0.11 (.05-.20)	0.21 (.08- .46)
≥40	0.03 (.00-.17)	0.16 (.00- .89)
Overall	0.13 (.09-.18)	0.41 (.28-. 57)
"At risk"***	354,277	94,318

01 Jul 89 - 30 Jun 90

Age group	Caucasian	Non-Caucasian
<20	0.00 (.00-.30)	0.46 (.01-2.56)
20-29	0.20 (.11-.32)	0.47 (.23- .86)
30-39	0.06 (.01-.18)	0.07 (.00- .39)
≥40	0.12 (.01-.43)	0.31 (.01-1.73)
Overall	0.14 (.09-.21)	0.32 (.17- .55)
"At risk"***	256,116	67,367

* per 1000 person-years
** Poisson 95% Confidence interval
*** Individuals contributing time in the denominator

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SILENT HIV INFECTION

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Summary

The period of latency between infection by the human immunodeficiency virus type-1 (HIV-1) and the production of specific antibodies to viral antigens may be prolonged and, occasionally, may last for years. This condition of seronegative infection could represent a serious risk of viral transmission from subjects who are unaware of their status. However, whether these individuals are actually infectious, especially through body fluids, has not been clarified. We have performed a prospective study in 65 high risk individuals seronegative for HIV-1 antibodies for a prolonged period of time. Twelve of them (18%) were shown to be carriers of HIV-1 proviral sequences by the polymerase chain reaction (PCR). The virus was isolated from mitogen-stimulated peripheral blood lymphocytes (PBMC) in five out of ten subjects tested since the first positive PCR. In two of them, virus could also be isolated from cell free plasma, subsequently they remained seronegative during 10 months of follow-up. These data indicate that delayed seroconversions may be associated with productive infection, suggesting that mechanism(s) other than viral latency may be responsible for the absence of antibody responses to HIV-1 proteins. Furthermore our findings suggest that prolonged seronegative individuals can transmit HIV-infection through their body fluids.

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) infects CD4+ cells leading to CD4+ T depletion, immunedysfunction and AIDS (1,2). However, the period after viral infection necessary to induce production of specific antibodies,immunological defect and conclamate disease is variable and probably depends upon the characteristics of both the virus and the host (3-6). It has been estimated that approximately 95% of infected individuals seroconvert within 6 months from infection (7). However anti-HIV-1 antibodies may appear later, up to and after 36 months (8,9). Indeed the actual frequency and duration of these late seroconversions, and the mechanism by which HIV-1 may escape the immunesurveillance remain, at present,to be established. Therefore, it is of great importance to determine to what extent persons who remain seronegative for prolonged periods can transmit HIV-1 through blood cells or cell-free body fluids (11,12). Here we report a prospective study in a group of individuals who remained HIV-1 seronegative although they had been continously exposed to HIV-1 infection by high risk activities such as intravenous drug abuse or prolonged sexual partnership with seropositive individuals. Polymerase Chain Reaction (PCR) and virus isolation from blood cells and cell-free plasma were simultaneously performed to determine the duration of antibody latency, the state of viral replication, and the potential infectiousness during this period.

2. Patients and Methods

2.1 Patients

Sixty-five seronegative subjects at very high risk of infection were selected for this study. Thirty-six of them had been intravenous drug abusers.Inclusion criteria were:intravenous drug abuse and sharing of syringes for more than 3 years, suspension of this risk behaviour for at least one year and admission to a strictly controlled therapeutic community. The 29 seronegative sexual partners chosen had had unprotected sexual intercourse with their seropositive partners for more then one year before our investigation.

All the 65 individuals were persistently negative for anti-HIV-1 antibodies by ELISA and Western blot analysis and p24 HIV-1 antigen during one year of follow-up, before the present study. Clinical examination and routine laboratory analysis demonstrated their normal clinical status.Thirty seropositive patients,classified according to their clinical status (CDC criteria), and 24 normal blood donors were selected as controls.

2.2 Serology

The presence of anti-HIV-1 antibodies was assessed by two enzyme linked immunosorbent assays (ELISA,Ortho Diagnostics,Raritan, N.J. and DuPont DeNemours). Both assays were performed for each serum or plasma sample and the results were confirmed by Western Blot analysis (DuPont DeNemours). HIV-1 p24 antigenemia was also determined by two different enzyme immunoassays (DuPont DeNemours and Abbott Laboratories) in

the serum, plasma and supernatant fluids of infected cultures.

2.3 Polymerase chain reaction

Density gradient (Ficoll-Hypaque) purified peripheral blood mononuclear cells were lysed by a standard technique (13). One ug of genomic DNA was used as a template for each amplification assay. The oligonucleotides utilized as primers were: SK68/69 and CO1/2 (env gene) (15), SK38/39 (gag gene) (15), P3/P4 (pol gene) (16). The PCR was carried out with the Taq1 polymerase (Perkin-Elmer Cetus) as previously described (14). After 30 cycles of denaturation (1',92 C) primer annealing (2',55 C) and polymerisation (2',72 C), DNAs were extracted, electrophoressed in 3% agarose gel, and then transferred to nylon membrane (Zeta-Probe, Biorad). Filters were incubated for one hour at 37 C in prehybridization solution (6X SSC, 5X Denhart's solution, 0.5% SDS. 20 mg/ml salmon sperm DNA, 50%formamide) and hybridized to a 32P nick translated HIV-1 full length probe (BH10 clone) (19,20) at 37 C overnight.Filters were washed twice with 2 x SSC 0.1% SDS solution for 15 minutes at 50 C and once with 0.5 x SSC 0.1 % SDS for 15 min. at 65 C and autoradiographed over-night at 80 C. The sensitivity of PCR was assessed by testing progressive dilutions of HIV-1 infected H9 cells as reported in detail by Ensoli et al. (10).

2.4 HIV-1 isolation

Plasma obtained by whole blood centrifugation at 3000 x g for 15 min., was passed through a 0.45 um filter. Two ml of the filtered plasma were cultured with 4 x 10 phytohemaglutinin-activated PBMC from healthy blood donors, in 2 ml of RPMI-1640 medium with 15% fetal calf serum and 10% (vol/vol) interleukin-2 (Cellular Products, Buffalo,N.Y.). Cultures were then monitored on day 5, 10,15 and 20 for the presence of HIV-1 p24 antigen in supernatant fluids by a standard assay (Abbott Laboratories). Patient's PBMC were isolated by Ficoll-Hypaque gradient centrifugation. 4 x10PBMC were activated by phytohemaglutinin and cultured in 2 ml of RPMI 1640 medium with 15% FCS and 10% (vol/vol) interleukin-2. Cultures were monitored for HIV-1 p24 antigen expression as described above.

3.RESULTS

3.1 Polymerase Chain reaction

Out of 65 individuals at high risk for HIV-1 infection, negative for HIV-1 antibodies and HIV-1 p24 antigen, 12 (18%) were positive by PCR (table 1). Only the patients in which, at the initial determination, HIV-1 sequence could be detected with at least two of three different primer pairs, and in at least two separate experiments, were considered positive. Because the primer pairs SK38/39 (gag) and SK68/69 (ENV) gave consistent results in repeated experiments, they were the most used. The percentage of positivity was slightly higher among ex intravenous drug abusers (22%) compared with the heterosexual partners of seropositive individuals (13%).Thirty seropositive subjects, included in this study, free from antiviral therapy were positive at the PCR analysis, however p24 antigenemia was detectable in the serum of only 6 of them (20%). All the 24 seronegative healthy blood donors were consistently PCR negative.

3.3 HIV-1 isolation

HIV-1 was isolated from mitogen-activated peripheral blood mononuclear cells of 5 out of 10 PCR positive/antibody-negative subjects (table 1). Furthermore infectious virus could also be recovered from cell-free plasma in two of 10 individuals examined. In both cases plasma viremia was detected twice, once at the first PCR and then after 6 to 8 months, while p24 antigenemia was always undetectable. In contrast to the reported association of plasma viremia with low CD4+ cell counts (17,18), our cases had normal CD4 cells for several months after the first determination of viremia. Both cases remained seronegative 10 months after the determination of plasma viremia (table 2), suggesting that factor(s) other than nonproductive infections are responsile for a delayed antibody response in some individuals.

3.4. Serological follow-up

Table 2 summarizes the serological monitoring of the 12 PCR positive/ antibody-negative subjects, followed for a period of 2 to 18 months. HIV-1 p24 antigenemia remained negative in all these individuals.One of them became seropositive 4 months after PCR assay and after 16 months of serologically negative follow-up). Of the remaining 11, 7 were reevaluated after 10 to 14 months and all were found to be still negative. It was possible to test again only 2 of them at 16 and 18 months, respectively. They were still found seronegative.

4. Discussion

Infection by HIV-1 may precede the appearance of antibodies by many months (3-10). It has been reported that 95% of the individuals evaluated after a known exposure date had seroconverted within 6 months from infection (7). However, some of them remained seronegative for up to 36 months although HIV-1 could be isolated from blood cells (9). It has been suggested that this state of delayed seroconversion may be due to virus latency and lack of viral antigen stimulation thus escaping the immune-surveillance. In all the previous studies, HIV-1 was detected either by PCR (6,7,9) or by virus isolation from PHA-activated PBMC (3-5,8,9). With both techniques, it is not possible to distinguish between *in vivo* latent or productive infection. By contrast we could isolate HIV-1 from cell-free

plasma of 2 subjects who were still seronegative after 10 months from the first virus detection. Plasma viremia appears to be a marker of viral replication in vivo, and correlate with the clinical stage of infection and of the viral burden (17,18,21).The evidence that late-seroconverters can have productive infections suggests mechanism(s) other than non productive infections are responsible for a delayed antibody response in some individuals. The suppression of viral expression and,thus,unavailability of antigen,may prevent seroconversion, but since it is likely that the infectivity of plasma reflects a viral replication above the threshold for the stimulation of the immune system, our data argue against this single mechanism. An alternative explanation is that a genetic background could be associated with low antibody responsiveness to HIV-1 antigens.The reported association of some HLA angens with the likelihood of seroconverting after exposure to HIV-1 (22,23) lends some support to this interpretation. A study of HLA antigens in late-seroconverters is needed to explore this issue.

A second issue concerns the degree of infectiousness of an individual during a period of prolonged seronegativity. Imagawa et al (9) could isolate HIV-1 from mitogen-stimulated, but in no instance from unstimulated blood cells of late seroconverters, and suggested that non-productive infections are the rule in these individuals. Thus, the capacity of these persons to transmit infection, by routes other than the direct transfer of cells carrying provirus, remained undetermined. By contrast, we provide direct evidence that blood can harbour infectious virons at least 10 months before seroconversion. Our findings strongly point to the possibility that plasma of some individuals with prolonged seronegativity may transmit infection. The concept that blood of all HIV-1 seropositive individuals should be considered infectious (18,21) should, therefore, be extended to the individuals with the evidence of HIV-1 infection as detected by PCR, irrespectively of their seronegativity and its duration (11,12).

A routine PCR screening of individuals at high risk for HIV infection might be of great value in limiting the inadvertent transmission of HIV, although the high costs of such program at present, represent a strong limitation. Larger scale studies are, therefore necessary to conclusively determine the actual frequency, duration and prognosis of the silent-infection state.

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TAB.1

VIROLOGICAL FINDINGS IN SERONEGATIVE INDIVIDUALS WITH POSITIVE PCR

	P C R (*)			VIRUS ISOLATION	
	GAG	POL	ENV	PBMC (§)	PLASMA (#)
DRUG ABUSERS					
1) C.P.	+	-	+	N D	-
2) P.G.	+	-	+	-	N D
3) V.A.	+	+	+	-	-
4) B.F.	+	-	+	+	-
5) N.C.	-	+	+	+	-
6) T.C.	+	-	+	-	-
7) C.B.	+	-	+	+	+
8) A.P.	+		+	-	-
SEXUAL PTRS.					
9) F.P.	+		+	-	-
10) R.R.	+	+	+	+	-
11) C.A.	+		+	-	N D
12) M.M.S.	+		+	N D	-

(*):HIV-1 DNA detection by polymerase chain reaction.

(§):HIV-1 isolation from peripheral blood mononuclear cells.

(#):HIV-1 isolation from cell-free plasma.

TAB.2

MONITORING OF SEROLOGICAL RESPONSE TO HIV-1 ANTIGENS IN PCR POSITIVE SUBJECTS

MONTHS (§):	0	2-4	6-8	10-14	16-18
DRUG ABUSERS					
1) C.P.	-			-	
2) P.G.	-	+/-	+	+	
3) V.A.	-			-	
4) B.F.	-			-	-
5) N.C.	-		-	-	
6) T.C.	-		-		
7) C.B.	-		-	-	
8) A.P.	-		-		
SEXUAL PTRS.					
9) F.P.	-	-			
10) R.R.	-		-	-	-
11) C.A.	-	-			
12) M.M.S.	-		-		

(§):Serological follow up started one year before the time 0, that correspond to the time of the first positive PCR.

HIV VARIABILITY AND PERSPECTIVES OF A VACCINE

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Since human immunodeficiency virus (HIV) was identified as the causative agent of AIDS the pressing challenge that researchers are facing is the development of a vaccine against this disease. Although progress has been made in the study of the biology of HIV faster than for any other virus, the development of an effective vaccine has been slowed by the peculiar features of HIV.

The difficulties standing before a rationale approach to vaccine design are: 1) the ability of the virus to rapidly change its genome sequence; 2) its spreading from cell to cell; 3) its ability to establish latent infection integrating its genome into that of the target cell without expressing any viral genes and thus remaining hidden inside the infected cell; 4) its insidious attack to the immune system upon which a vaccine depends in order to be effective.

The simplest way to design a rationale approach to a vaccine is the use of whole inactivated virus as it was designed for other viral infections. Partially successful experiments using whole inactivated SIV (a distinct virus but closely related to HIV), that is able to infect monkeys, have already been reported (Ref 1-4). However, concern about the safety of an inactivated viral preparation (residual infectivity, presence of nucleic acid) would be the major obstacle for the use of this type of vaccine in man. Furthermore, there are evidences that several components of HIV can mediate immunosuppression in the absence of viral infection (Ref 5-7) and that antibodies against some regions of the HIV envelope can even enhance the infection of monocytes and macrophages that are considered a reservoir of the virus in vivo (Ref 8-10). Therefore, it may be advantageous to avoid in the vaccine preparation those viral regions that can induce a harmful immune response or even facilitate the HIV infection.

Possibly, the most correct approach to AIDS vaccine is: first, to identify those viral regions that can induce both humoral and cellular protective immunity without raising immune responses that could be potentially harmful and, second, to design subunit and/or peptide vaccines that are safe because they do not contain contaminating live virus and/or nucleic acid.

The target for immune protection against different viruses has been the external envelope of the viral particle. Similarly, a large majority of studies with regard to AIDS vaccine development have been focused on HIV envelope proteins, gp120 and gp41 (Ref 11-13).

In particular, a fragment of HIV-1 gp120, lying in the carboxy-terminal half of this protein, has been shown to contain a Principal Neutralizing Determinant (PND) and to induce high-titered neutralizing antibodies. This region (V3 region) is highly variable and lies in the third major variable domain of gp120. Reports from a number of laboratories (Ref 14-16) demonstrate that neutralizing antibodies raised against peptides representing the V3 region are type-specific, being directed mainly to the homologous isolate or to strictly related heterologous isolates.

The V3 region is thought to exist as a loop structure because of the presence of two highly conserved residues of cysteine. It was shown that, besides the neutralizing epitopes, V3 loop includes sites that bind antibodies without subsequent virus neutralization.

Different functional roles in natural HIV infection have been proposed for this domain. It has been found (Ref 17) that the domain plays a role in the association of gp120 with gp41. Otherwise, the V3 region may interact with two other relatively conserved domains within gp120 and may facilitate the exposition of the fusogenic domain in gp41 (Ref 18) after the binding of gp120 to CD4 cellular receptor. More recently, it has been suggested (Ref 19) that the V3 loop could be cleaved by a cellular protease to activate the fusion reaction. Finally, recent reports (Ref 20) indicate that the V3 region might play a critical role in mediating the penetration of HIV-1 virions into specific target cell populations.

It is obvious, therefore, how so many efforts for the development of a vaccine have been directed to this region, which seems to be necessary component of it.

As already mentioned, the V3 region is however highly variable in terms of its aminoacidic sequence. In fact, animal sera raised to synthetic peptides repres-

enting the V3 sequence of a given variant can neutralize predominantly in an isolate restricted fashion and rarely they cross-neutralize highly divergent variants.

However, although the hypervariability in the PND could seem a major block in developing a vaccine that will be effective against all strains of HIV, it may not be as insurmountable as thought. In fact, in a serological study it has been shown that nearly 80% of HIV positive human sera in USA recognize a synthetic peptide representing the V3 region of one isolate (MN strain) (Ref 21). This suggests that the bulk of the virus genotypes in the field shows some homology with sequences that fall in the MN V3 domain.

These data are confirmed in a recent work by La Rosa and coll. (Ref 22) in which 249 field and prototype HIV-1 isolates are compared following PCR amplification, cloning and sequencing of the V3 regions. The results clearly show that the majority of the field isolates is quite similar to MN strain in their V3 sequence. The homology was most apparent in the central portion of the loop with 44% of the isolates yielding an IGPGRAF sequence.

Therefore, the MN V3 sequence might represent an important vaccine component as it might elicit antibodies capable to neutralize a different isolates of HIV-1.

Immunization studies in chimpanzees have revealed that the initial humoral immune response to the viral inoculum is directed against the type specific V3 loop. However, after repeated immunizations, a more group-specific antibody response to a HIV variants, develops (Ref 23).

Nevertheless, as so many field isolates show minimal homology within the V3 sequences, several approaches could be devised to overcome that heterogeneity.

The first solution, and also the most obvious one, is to use a cocktail of different immunogens, such as synthetic peptides, representing the V3 regions of the majority of known isolates. A vaccine containing several of these sequences would elicit antibodies capable of neutralizing more than 85% of all known isolates (Putney S., personal comunication).

A second approach, that is a consequence of the first one, is the use of hybrid immunogens containing sequences of more than one isolate. At present, a number of laboratories are studying whether such hybrid peptides (synthetic or recombinant) could give a better response than a cocktail of individual peptides.

A final approach is the identification of relatively conserved sequences in the V3 region. As already reported (Ref 22), a conserved sequence is present in the tip of the V3 loop in a significant percentage of the virus isolates: this sequence, GPGRAF, is contained in about 60% of the studied isolates. If it will be possible to elicit antibodies that bind this sequence, the majority of field isolates will be neutralized. Synthetic peptides composed of repeated GPGRAF are now under study in animals for their ability to induce broad neutralizing antibodies.

The efficacy of V3 region as component of a vaccine is already under testing. Marc Girard and coworkers (Ref 24) immunized chimpanzees with whole inactivated virus or purified recombinant proteins and then boosted them with HIV-1 V3 loop peptide coupled to a carrier. Neutralizing antibodies were induced and partial protection (two out of three chimpanzees in the experiment were protected), following a challenge with homologous live HIV, was obtained.

Protection of chimpanzees against HIV infection was also achieved by immunization with the envelope glycoprotein gp120 but not with gp160 (Ref 25).

The efficacy of anti-V3 antibodies in protecting from natural infection is also suggested by Rossi et al. (Ref 26) and Devash et al. (Ref 27), who found that antibodies to V3 loop in pregnant women can predominantly block the transmission of the virus to their offspring.

Taken together, these results give us some reasons to believe that the presence of high titres of neutralizing antibodies to the V3 loop can be protective, at least against the corresponding strain of HIV-1.

However, to be considered effective, a vaccine would have to protect against infection by a large majority of HIV strains an individual could be exposed to.

A different approach for the control of HIV infection is the possibility to induce a kind of intracellular immunity. The possibility that a cell harboring a defective HIV provirus can become resistant to HIV superinfection was firstly investigated by Trono et al. (Ref 28), who demonstrated a reduction of the released superinfecting HIV-1 from cells harboring a gag-mutant defective HIV-1 provirus. We have focused our studies on a cellular clone (F12) that we obtained from Hut-78 cells infected with the supernatant of cultured lymphocytes from an AIDS patient (Ref 29). This cell clone, in spite of the presence of a full lenght HIV-1 provirus and the production of some viral proteins is not able to release viral particles. Moreover, F12 cells are fully resistant toward superinfection with any HIV-1 or HIV-2 isolates. We studied at which step the homologous viral interference occurs in F12 cells and in productively HIV-1 infected cells. As characteristic of HIV-1 infected cells, F12 cells showed a down-regulation of CD4 receptors. This could imply that the observed interference was the result of receptor competition between infecting viruses. On the other hand, the ability of superinfecting HIVs to cross the F12 cell membrane was demonstrated by 1H-NMR analysis. By this technique, transient

modifications in intensity of the signals from fatty acid chains, as a consequence of rearrangement of membrane structure following HIV entry into susceptible cells, have been observed (Ref 30, 31). It seems, therefore, that the homologous interference observed in F12 cells is due to an intracellular block in the replication of superinfecting HIV. The intracellular fate of superinfecting HIV-2 was followed at different hours after superinfection of F12 cells by DNA-PCR technique. We used HIV-2 specific primers to discriminate between the DNA of the superinfecting HIV-2 and that of the HIV-1 constitutively present in F12 clone. The results obtained suggest that the superinfecting HIV-2 is able to enter F12 cells and to retrotranscribe, at least in part, its genome. By using a series of PCR primers designed to detect certain steps of the reverse transcription process, it results the inability of the superinfecting HIV-2 reverse transcriptase to complete full retrotrancription of the input viral genomic RNA. This block was found to be operative also after superinfection of HIV-1 infected producer cells. The non producer cell clone (F12) could be efficiently transfected with an HIV-1 infectious molecular clone, indicating that the inhibition of replication of superinfecting HIV is not operating after the integration step.

What it is peculiar of F12 cells is that they are not releasing infectious virus and therefore it could be possible to reproduce the homologous viral interference by transfering the clones F12/HIV genome into HIV susceptible cells and render these cells resistant to HIV infection.

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DISCUSSION SESSION II

HIV INFECTION (Papers 7 to 11)

J. FIRTH (UK): In this practical Meeting and in the light of the Hollywood and San Francisco experience one has firstly to refute the WHO suggestion that AIDS is caused by poverty, unless it be moral poverty? What evidence is there that normal vaginal intercourse in the absence of ulceration has ever allowed HIV transmission? Secondly, aircrew make the point, very strongly, that the Israeli Defence Forces had the answer to this disease well before Christ. The answer to AIDS is morality - codes of social conduct. Unless the virus mutates radically, unless you are given infected blood or unless your mother already has it, AIDS is a voluntary disease, like suicide. Thirdly, what is the present, agreed specificity and sensitivity of the screening methods that were presented here today? Unless one defines the criteria, screening is meaningless.

L.O. KALLINGS (CH): I did not quite follow the meaning of the first comment about poverty. It is true that the first cases from the Western World were often wealthy persons as actors and so on among white homosexuals. But it has gradually been a disease of underprivileged people, as we have seen for instance in New York city and in many other places of the USA. The spreading is now more prevalent in people of colour and of low socio-economic conditions in urban slum areas. Then in the developing countries, it might have started among populations with enough money to travel and to have many girlfriends but it has been spreading more and more among average populations, which, in the developing countries, are poor.
Concerning the evidence of spreading transmission of HIV vaginally, without a simultaneous other sexually transmitted disease, among the first studies showing this , are those from hemophilic males, by definition males, who infected the female partners. In this population there is not a high incidence of ulcerating venereal disease. Another situation is in Africa, where there are more females than males infected and of course not everybody has a sexually transmitted disease.

D.S. BURKE (USA): There was also the contention that this was a voluntary disease of behavior and that perhaps the appropriate answer was a more appropriate moral standard rather than public health.

J. FIRTH (UK) : This suggests that moral standards and public health are two different things, yet up to now or until very recently in the 1960's they were synonymous. Old Testamental Morality was largely an expression of concern for the public health.

D.S. BURKE (USA): It can be said that they intercept, but I am not sure that they are synonymous.

L.O. KALLINGS (CH): I could add there is a fair chance HIV can be transmitted even in a steady relationship, where one of the partners might have been infected from the beginning. So it's not true that mutual fidelity will protect people if not HIV tested initially and both are negative. There won't be any children if people would not continue with sexuality. In the effort to decrease the number of sexual partners, which is one of the objectives of programs to prevent HIV infection, moral standards of course have a place. In this context, the different religions are of importance. WHO has links with the world religions and has discussed these things, but morality is not primarily a business for UN authorities.

F. AIUTI (IT): International data report that sensitivity and specificity are from 98 to 99% in ELISA tests and almost 100% in WB and according to WHO and CDC a positive test is defined when at least 2 bands are present in WB against the *env* and the *core* regions. Of course we should also consider the specificity and sensitivity of other tests that are important for detecting HIV infection, such as isolation of the virus from plasma or peripheral blood, but this is not a routine test. According to recent data of the literature, in asymptomatic carriers of HIV infection stage I and II, isolation was also possible in 90-95% of subjects, and in the later stages of AIDS it is almost 100%. PCR, as I said, is a very difficult method to perform and there are a lot of false positive results, so I think it is impossible to give a number for specificity and sensitivity, since PCR is still a method for research. Concerning transmission, I would like to give some data. I studied 142 couples in which one of the partners was positive and the other negative (this data is from a paper in press in "AIDS") and we found that the transmission rate is 42%. As reported in the literature, this figure is higher from males to females than from females to males. Concerning mucosal lesions, we found that they were present in 30%. However there are also a lot of other data that can be discussed, because in our series and in others too, anal intercourse has been reported, so it is very difficult to calculate which is the most important cofactor: anal intercourse, mucosal lesions or CD4 number or stage of the seropositive partner.

J. FIRTH (UK): A point that has to be made is that a sad feature of social behaviour in some parts of Africa is that anal intercourse is a preferred method of contraception and an answer to the calendar. These African populations' practices are designed to destroy them by HIV infection. This is the real social, medical and moral challenge.

D.S. BURKE (USA): We did publish in "The New England Journal of Medicine", about 3 years ago, a specific study to look at the issue of specificity in the screening programme. We did review all the positives, and we found 1 false positive among 135,000 persons tested. Subsequently, we introduced improvements in specificity and now we calculate 1 false positive among half a million-1 million individuals tested. The price is sensitivity and sensitivity of the system is not perfect. We acknowledge that there will be individuals that are missed, partly because there is a window and those can not be identified by any antibody-detecting system. But, in addition to that, we intentionally sacrified a small part of sensitivity, to assure specificity. This is a general problem for the screening of many pathological conditions, such as colon cancer, breast cancer, hypertension, where 98-97% of sensitivity would be considered superb.

J. FIRTH (UK): May I ask the Chairman whether his experience over the last three years confirms the published data?

D.S. BURKE (USA): We did not identify any false positive in these last 3 years, but this does not mean that the problem does not exist.

L.O. KALLINGS (CH): If I could address a question again regarding anal intercourse in developing countries, I don't think that it is a major contributing factor. The concern for preventing pregnancy does not seem to be so big, at least in some African

countries. For instance Kenya has one of the highest population growths in the world (4%). It is difficult to institute the idea of family planning. Neither other methods for preventing pregnancy, nor anal intercourse seem to be prevalent.

R.E. SPIER (UK): May I ask Dr. Verani a couple of questions? The first is related to the specificity of CD4 receptor. This is something that it is overplayed in the AIDS business to quite a considerable degree. Quite a lot of cells in the body, particularly the macrophage cell lines and other cell lines can get infected by HIV and they do not show any signs of CD4 receptor. May be that CD4 receptor happens to be there and happens to be the thing that the virus attaches to in some circumstances, but there is, if you like, no necessary relationship between CD4 receptor and virus infection. It is a misleading concept of people to think that it is an important thing and particularly shows that the work that has been done to produce soluble CD4 as a possible antagonist of AIDS is not likely to be a successful way to combat this disease. That's one thing. Second question. You mentioned in your speech, madam, that it was unlikely that one would use inactivated viruses as a possible vaccine. It seems to me that the only data we have got as a possible successful vaccine was on chimpanzees, and they are based upon inactivated viruses of the SIV model. Would you like to comment and perhaps reverse this particular thought?

P. VERANI (IT): As far as the first point of CD4 receptors, there are several reports of HIV infection of human cells which are not exhibiting CD4 receptors and are lacking of detectable CD4 mRNA synthesis. This would point to the existence of alternative models of HIV entry. However we cannot exclude the presence of very low amounts of CD4 below the sensitivity of the detection method (usually flow cytometry analysis). In any case, CD4+ cells are easily infected by HIV, due to the high affinity between gp120 and CD4. The possibility that some cells get infected even if they do not express CD4 could in part explain why therapy with soluble CD4 does not completely prevent infection.
The second point was related to vaccine with whole inactivated virus. The disadvantages associated with the possible use of this type of HIV vaccine in humans are for its safety. Protection against SIV infection has been obtained in monkeys vaccinated with inactivated whole virus vaccine. However, it was recently published in "Nature" that some protection was also obtained in some monkeys receiving uninfected human cell preparations and challenged with SIV grown in the same cells. Therefore, it is necessary to confirm if the protection is mediated by immune response to viral antigens or to heterologous cells. Although several attempts to induce protection against experimental HIV-1 infection in chimpanzees by active immunization have failed, recently, however, protection of chimps was obtained by immunization with recombinant HIV-1 gp 120.

G. GRAY (CA): A question to dr. Warner. One of the concerns about HIV is the possibility (and there are a couple of interventions in the next session) of the Central Nervous System (CNS) involvement and neuropsychiatric sequelae. My question is: what do you do with seropositive pilots, regarding acromedical disposition, and with other personnel, such as medical personnel?

R.D. WARNER (USA) The USAF practice is that a seropositive pilot is removed from the cockpit, independent of clinical CNS involvement or not. Regarding medical personnel, the USAF policy is to remove medical personnel from contact with patients, allowing other activities, such as education, research and administrative duty (see the later comments of W.H. Stigelmann, discussion session II HIV infection, papers 12 to 16).

D.S. BURKE (USA): The US Army position is to decide case by case. But medical personnel is normally not removed from patient contact, in this area too the decision is taken case by case, generally allowing in-patient contact.

G. GRAY (CA): In Canada there is not yet a general policy for seropositive pilots, but they normally are not grounded and they are allowed to continue to fly with some restrictions, also depending on the clinico-immunological examination.

D.S. BURKE (USA): Probably we'll be able to discuss in more detail the performance in the next session.

R.E. SPIER (UK): I'd like to come back to CD4 and macrophage. Macrophage may come into contact with HIV more than through gp120-CD4 link, through phagocytosis of HIV by macrophage.

F. AIUTI (IT) : As an immunologist I want to defend the role of CD4 in the binding. CD4 is in fact important not only for T helper cells, but also for monocyte, macrophages, that have small quantities of CD4 receptors. Recently dendritic cells have been demonstrated infected by Haseltine and in these dendritic cells very small quantities of CD4 molecules have been found. So the entry of the virus in these cells is through CD4 too. And the CD8 cells are not infected at all until the end of the disease, and they do not expose CD4.

R.E. SPIER (UK): I am not arguing against the fact that CD4 cells do not get infected, but just the fact that there is a necessary causal relationship between these two things. In fact muscle cells and intestinal cells can get infected. What I want to say is that there is a physico-chemical environment leading to the infection of cells by HIV. The key point is the immunoprophylaxis, that probably is misleading.
The second point is going back to the macrophage: if you have a situation where you induce antibodies against gp120, when you get an antibody-antigen complex, which is met by the macrophage, this macrophage says: "thank you for your work, you made my job easier for me" and it may ingest the complex. This is extremely dangerous, because this antibody may enhance infectivity and this may result in a wrong vaccine.

R. D'AMELIO (IT): I would like to profit in having many scientific authorities collected here along this table, including the representative of the WHO. I believe we have to reflect on a problem. Before this meeting, I performed a careful survey among NATO countries about the present policy regarding the screening for HIV. Well, among 14 NATO countries, only one, the USA is performing generalized screening and 3 other countries, Belgium, Denmark and Greece, are performing screening for selected categories, including pilots. I think we have to meditate on these data, because my friend Donald Burke demonstrated that the serological tests for HIV present a very high specificity and an acceptable sensitivity. So, we have in our hands methods able to screen seropositives with very good precision. In addition, in the military there are many stimuli, such as vaccinations with live agents, like smallpox, yellow fever, BCG, oral polio virus (I stressed this point in the opening remarks). I think we all here remember the case, described by Col. Burke's group in "The New England Journal of Medicine", of one seropositive military man, whose condition was unknown in the Department of the Army, who developed a generalized vaccinia after smallpox vaccination. We heard this morning from dr. Cogoli that physico-psychological stress may impair the immune system; consequently a seropositive military recruit is facilitated in the

progression to clinical disease by the stressing environmental conditions linked to the military life. Lastly, another peculiar aspect of the military, that has been already reported this morning, is the frequent opportunity military have in travelling all over the world. These travels are often performed to developing countries. This creates an additional risk, arising from the endemicity of many severe infectious and parasitic diseases.
Considering what has been above reported, I would like to know the position of the different chairmen and speakers at the podium, mainly the position of Prof. Kallings, as the representative of WHO.

L.O. KALLINGS (CH): Concerning compulsory screening, WHO is not recommending any mandatory screening other than for blood donors. Generally, - I am not talking particularly about military condition - if there is mandatory screening, people who may be at high risk are likely to try escape from the testing and go under ground. The most important thing is to remain in contact with these people in order to diminish their high risk behavior. So, mandatory screening is considered to be counterproductive, because it may prevent possibility in interacting with these people. I would like to add that WHO is concerned with many developing countries, and their Armies, where HIV prevalence might be as high as 50% and the annual incidence in recruits is 6-7%, when they come from the rural areas. By these figures one may understand that screening with exclusion of HIV positive person from service would rule out most of their Forces.

D.S. BURKE (USA): I disagree with prof Kallings on the issue of compulsory or mandatory testing for military population. There are in fact many aspects in military life that are mandatory and I think that HIV testing is one of these special situations, which is part of mandatory military existence. So the decision has been made in the best of interests. So I think that it is appropriate that countries, on the basis of public health and cost-benefit considerations, go ahead with testing under those circumstances. I agree that those aspects of society where choice is universal may be unappropriate to enforce mandatory HIV testing, but in those elements of the society where aspects of life are already mandatory, as happens in the military or in prisons, it is appropriate for other persons to make and enforce the decision.

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IMMUNOLOGICAL PARAMETERS IN CURRENT AND FORMER US AIR FORCE PERSONNEL

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INTRODUCTION

Major advancements have been made in the laboratory assessment of the immune system over the past decade. There has been a proliferation of sophisticated techniques to measure the number and functional capacity of subsets of lymphocytes and other cellular elements of the immune system. Unfortunately, the pace of this technology has made it difficult for many practicing physicians to develop and maintain an understanding of these new tests. Additionally, appropriate reference values for these tests in "normal" populations are unclear and little has been done to assess the effects of factors such as age, race and lifestyle on these measures of immunity.

Laboratory assays are available to assess both the counts of the various cellular elements of immunity in the peripheral blood as well as the functional capacity of many of these cells. Tests of delayed cutaneous hypersensitivity are based on techniques used in allergy testing and tuberculosis control. After antigens such as Trichophyton or Candida are injected intradermally, the skin's reactivity correlates with the recall sensitivity of the T cells to each antigen used. Counts of total T lymphocytes (CD2 cells) and B lymphocytes (CD20 cells) are decreased in immune deficiency conditions and are elevated with lymphoproliferative diseases. The helper/inducer T lymphocytes (CD^4 cells) are commonly decreased in progressive HIV infection and are increased in autoimmune disorders. The suppressor T cells (CD8) are increased in some viral infections and immunodeficiency states. The CD4/CD8 ratio is a commonly used measure of immune system capability. Tests of activated T lymphocytes (CD25 cells) assess the presence of stimulated T lymphocytes. Unstimulated T cells do not react to this test. This is a useful test in identifying the presence of lymphoproliferative disorders. Counts of total lymphocytes are also made.

Tests of the functional status of the cellular components of the immune system assess the response of the cells in the peripheral blood to stimulation with various mitogens such as phytohemagglutinin. These tests measure the cells' capability to become activated by external stimuli. The natural killer cell assay measures the body's lytic response to foreign tissue cells both before and after stimulation with interlukin. This response is decreased with impaired natural immunity.

Standard quantitative measurements of immune globulins (IgA, IgG and IgM) are also useful in the assessment of immune functions. They measure the ability of specific B lymphocytes to secrete specific classes of antibody.

Table 1 shows a useful approach to the categorization of several of the quantitative and functional tests of immunity. The tests described in the table provide a comprehensive assessment.[1]

As part of a comprehensive epidemiologic study of current and former Air Force personnel, an extensive assessment of the immune system was conducted by a single immunology reference laboratory using stringent quality control standards.[2] All of the tests described above were used in this study. The demographic characteristics of these study subjects are displayed in Table 2. All of the subjects were middle-aged males, most of whom did not currently smoke tobacco. Nearly 80% of them currently drink alcoholic beverages.

RESULTS

Based on the results of the immunologic tests on these subjects[3], mean values and the 95% range for each immunologic test were calculated. These values, along with the sample size for each assay, are shown in Table 3.

It is evident that the range of values for many of these tests is quite broad, making it difficult to interpret any single test result. A better approach would be to look for patterns in the results of the immunologic test battery. However, the impact of factors such as age, race and lifestyle must be considered in any interpretation. It is standard procedure in most immunology laboratories to use medical students, nurses and laboratory technicians as the pool to develop "normal" results. This group is probably not representative of the general patient population and the "normal" results from this selected pool may not be appropriate to the general population.
As part of the Air Force study, statistical analyses were performed to determine

the effect of age, race and cigarette and alcohol consumption on each of the immunologic tests. The results of these analyses are shown in Tables 4 - 7. It is clear that current use of tobacco has a profound effect on these assays, with variability of 20% or more commonly seen. Similar results but smaller shifts were seen when lifetime use of tobacco was used in the analyses. Smoking tended to increase all of the cell count tests, but decreased the functional capability of the cells to perform (PHA, natural killer cell function and immunoglobulins). Conversely, alcohol tended to reduce the cell counts but stimulated the functional studies. Race had little effect on the tests but age had a significant effect.

CONCLUSION

An excellent battery of sophisticated tests is available to assess the quantitative and qualitative status of the immune system but the practicing physician must use care when evaluating the results of these tests. These findings of this study highlight the importance of using age-specific ranges and consideration of lifestyle factors in the interpretation of immunologic test data.

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Table 1

IMMUNOLOGICAL PROCEDURES
FUNCTIONAL AND QUANTITATIVE STUDIES

	CELL TYPES			
TESTS	T Cells	B Cells	Natural Killer Cells	Macrophages
Functional				
In vivo	Skin tests			
In vitro	PHA		NK Assay NK-1 Assay	
Cell Counts	TLC Total T (CD2) Helper (CD4) Suppressor (CD8) CD4/CD8 Ratio Activated T (CD25)	TLC B Cells (CD20)	TLC	Monocytes (CD14)
Immune Globulins		IgA IgG IgM		

Table 2

DEMOGRAPHIC FACTORS

Mean Age	49 Years
Race	6% Black; 94% Non-Black
Smoking History	
Never Smoked	27.4%
Less than 10 packyrs	27.6%
More than 10 packyrs	45.0%
Current Smoking	
None	66.9%
1 pack/day or less	17.0%
More than 1 pack/day	16.1%
More than 4 packs/day	0.1%
Alcohol Use History	
Never	9.0%
0-40 Drink yrs	68.3%
More than 40 drink yrs	22.7%
Current Alcohol	
0-1 drinks/day	79.4%
More than 1, less than 4	17.4%
More than 4 drinks/day	3.2%

Table 3

NORMAL VALUES AND RANGES

Variable	Sample Size	Units	Mean Value	95% Range
Total T cells (CD2)	878	Cells/cu.mm.	1708.8	771 - 2971
Helper cells (CD4)	883	Cells/cu.mm	981.9	417 - 1841
Suppressor cells (CD8)	882	Cells/cu.mm.	531.4	174 - 1200
CD4/CD8 ratio	881	--------	2.2	0.7 - 4.5
Monocytes (CD14)	887	Cells/cu.mm.	41.5	7.4 - 124
B cells (CD20)	884	Cells/cu.mm.	174.8	48 - 453
Activated T cells (CD25)	883	Cells/cu.mm.	15.6	0.0 - 77
Total lymphocytes	2289	Cells/cu.mm.	2019.6	950 - 3550
Natural killer cells	869	Counts/minute	429.0	91 - 884
Nat. killers (interlukin)	876	Counts/minute	821.3	440 - 1272
Phytohemagglutinin	880	Counts/minute	2240.6	654 - 5754
IgA	2291	Mg/dl	228.5	85 - 471
IgG	2291	Mg/dl	1041.7	635 - 1550
IgM	2291	Mg/dl	125.1	45 - 291

Table 4

COVARIATE RELATIONSHIPS
T CELL COUNTS

Immunologic Parameter	Age	Current Alcohol Use	Current Smoking	Race
Total T cells (CD2)	-5%		+22%	
Helper Cells (CD4)	-8%		+36%	
Suppressor cells (CD8)	-2%	-1%	+12%	
CD4/CD8 ratio		-5%	+22%	
Activated T cells (CD25)				
Total lymphocytes	-3%		+28%	

All indicated correlations are statistically significant

Table 5

COVARIATE RELATIONSHIPS
B CELLS AND MACROPHAGES

Immunologic Parameter	Age	Current Alcohol Use	Current Smoking	Race
Total lymphocytes	-3%		+28%	
B cells (CD20)	-13%	-4%	+39%	+31%
Monocytes (CD14)	+17%		+66%	-25%

All indicated correlations are statistically significant

Table 6

COVARIATE RELATIONSHIPS

IMMUNE GLOBULINS

Immunologic Parameter	Age	Current Alcohol Use	Current Smoking	Race
IgA	+8%	+9%	-5%	+7%
IgG			-8%	+23%
IgM	-3%	+<1%		-13%

All indicated correlations are statistically significant

Table 7

COVARIATE RELATIONSHIPS

FUNCTIONAL STUDIES

Immunologic Parameter	Age	Current Alcohol Use	Current Smoking	Race
Skin tests				
Phytohemagglutinin	-12%			+5% *
Natural killer cells				
with IL2	+3%		-7%	-2%
without IL2	+5%		+.7%	-11%

All indicated correlations are statistically significant
* Total packyears causes 5% decrease

Early Markers of HIV Infection and Subclinical Disease Progression

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ABSTRACT

Human Immunodeficiency Virus (HIV) infection in U.S. Air Force personnel between 1985 and 1989 was examined through a mandatory serologic survey, and through annual examination of infected patients. CD4+ cell counts were determined by flow cytometry; β_2 microglobulin and neopterin were measured by immunoassay. During this period 933 cases were found, of which 161 were documented seroconversions, giving an incidence rate of 15.6/100,000 person-years. For patients with >400 CD4 cells/μl, the rate of initial occurrence of opportunistic infection was 1% and 4% at 1 and 2 years, respectively. HIV infected persons with < 400 CD4+ cells/μl, in contrast, had rates of 21% at 1 year and 36% at 2 years.

In a cross-sectional study, β_2 microglobulin concentration was shown to increase in both the serum and spinal fluid of patients infected with HIV as their blood CD4 numbers declined. Neopterin levels in serum and spinal fluid showed a similar trend, with significantly lower neopterin concentrations in the group that had >1000 CD4+ T cells compared to the 0-600 CD4+ cell group.

Longitudinal studies included correlation of HIV p24 antigen with CD4 counts over a one year period. The p24 antigen-positive group had a 21% decline in CD4+ T cells, while the antigen negative group had a 14% decline. Specific helper T-cell subsets were also examined over a 6 month period. A significant decline was seen in the CD4+\CD29+, CD4+\CD45R+, and overall CD4+ subsets which was not seen in AZT treated patients. A significant increase in the CD4+\CD29+ memory T-cell subset, which is responsible for response to recall antigens and is capable of γ interferon secretion, was noted in the AZT-treated group.

INTRODUCTION

Infection with HIV is typically characterized by an acute mononucleosis-like syndrome (1), followed by a prolonged, relatively symptom-free period. Subsequently, as the number of CD4+ T cells falls below 200\$\mu$l or 20%, opportunistic infections begin to occur. With the onset of this late phase of illness the quality of life for the patient can fall as symptoms become more varied and more frequent. During the prolonged latent phase, however, an HIV-infected individual might enjoy a 5-10 year period (2) in which he is fully capable of performing at his premorbid level. Markers of disease progression are important, therefore, to offer prognostic information during the period of subclinical infection so that informed decisions regarding prophylactic therapy for opportunistic infections, antiviral therapy, and determination of

fitness for duty may be accomplished. Likewise, these markers may represent parameters which may show variance over a much shorter time period than clinical endpoints such as onset of opportunistic infection or death (3). The ability to make these measurements becomes increasingly more significant as newer forms of pharmacotherapy and immunotherapy are developed and require evidence of efficacy. In this study data from 1985 to 1989 is reviewed from Wilford Hall United States Air Force Medical Center, where all U.S. Air Force HIV evaluations took place on an annual basis.

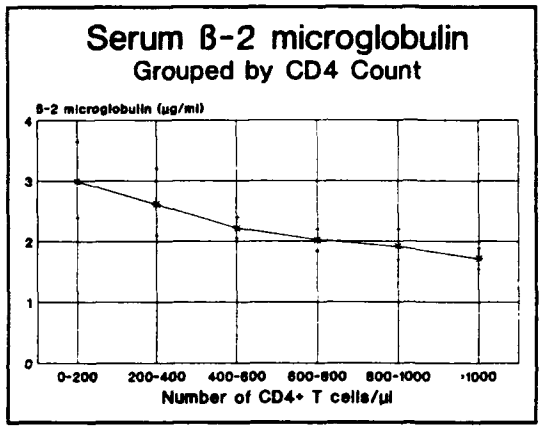

Figure 1. Serum $ß_2$ microglobulin levels with 95% confidence intervals for 163 patients.

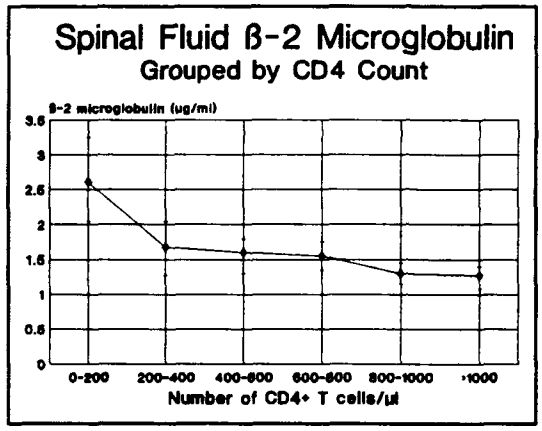

Figure 2. Corresponding spinal fluid values for figure 1 (with 95% confidence intervals.)

METHODS

Cases of HIV infection were detected by a mandatory annual screening program for all active-duty personnel, or by testing when clinically indicated. Identified cases were confirmed with Western blotting and were evaluated annually at Wilford Hall. The data discussed in this paper were obtained between 1985 and 1989, during which time AZT was routinely given to HIV-infected patients with CD4+ T-cell counts less than 200\µl. Lymphocyte phenotyping was done using 2-color flow cytometry with gating set by the use of isotype and fluorochrome matched monoclonal antibodies. $ß_2$ microglobulin and HIV p24 antigen were measured using an enzyme-linked immunosorbent assay (ELISA). Neopterin levels were evaluated using a radioimmunoassay (RIA). Data were analyzed using an analysis of variance or the Wilcoxan rank-sum test.

RESULTS

There were 933 cases of HIV infection during the study period, including 161 documented seroconversions. Of the seroconvertors, 157, or 97%,had CD4+ T-cell counts >400 \µl. The overall incidence rate was 15.6/100,000 person-years. A clear difference was evident in the rates of initial opportunistic infection when

patients were stratified by number of CD4+ T cells. Patients with >400 CD4+ T cells \μl incurred initial opportunistic infections at a rate of 1% at 1 year and 4% at 2 years, while those with <400 CD4+ T cells \μl had a rate of 21% at 1 year and 36% at 2 years.

Measurements of $ß_2$ microglobulin and neopterin were made in paired serum and cerebrospinal fluid samples from several patients. Figure 1 shows the concentration of $ß_2$ microglobulin in serum of patients grouped by CD4 counts. In these 163 patients, $ß_2$ microglobulin levels were significantly higher in subjects with <400 CD4+ T cells than in the group with >1000 CD4 cells. A similar pattern is seen in figure 2, where $ß_2$ microglobulin levels in spinal fluid show an increasing trend as the number of CD4+ T cells fall.

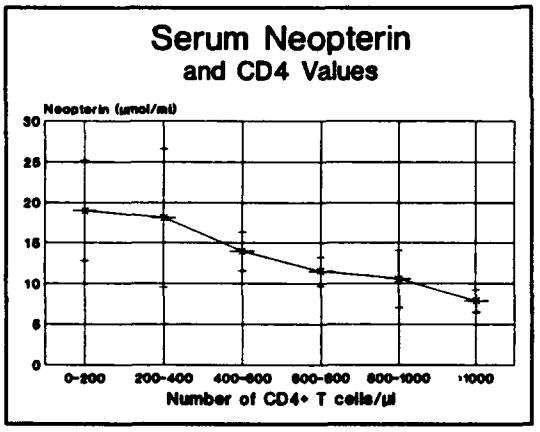

Figure 3. Mean serum neopterin concentration with 95% confidence interval for 159 patients.

Neopterin was likewise found to increase in both serum (figure 3) and spinal fluid (figure 4) as the numbers of CD4+ T cells in peripheral blood decreased. In a

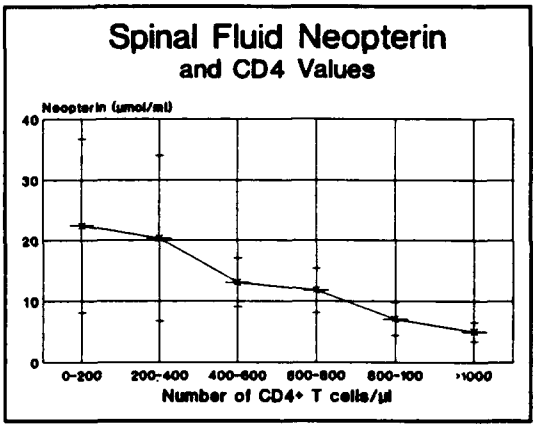

Figure 4. Spinal fluid neopterin levels and 95% C.I. corresponding to figure 3.

pattern that paralleled $ß_2$ microglobulin findings, the groups with the highest CD4 counts had significantly lower neopterin levels in serum and spinal fluid than the group with the lowest number of CD4+ T cells.

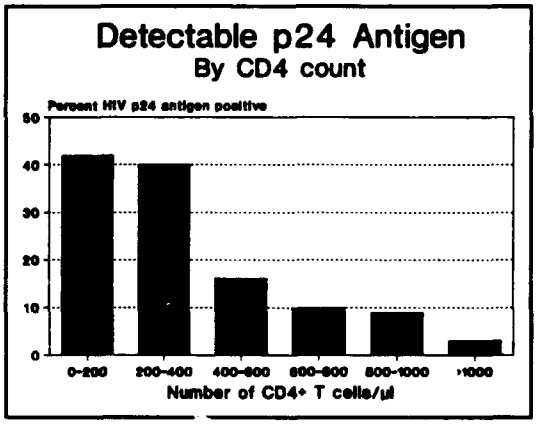

Figure 5. Percentage of patients with detectable HIV p24 antigen

The percentage of patients with detectable p24 antigen is shown in figure 5

grouped according to CD4 counts. Again noted is the fact that as the number of peripheral blood CD4+ T cells falls, the proportion of patients with detectable HIV p24 antigen rises, appearing in the serum of 42% of the <200 CD4+ cells\μl group. Twenty-nine of the p24 antigen-positive patients with >200 CD4+ T cells\μl were followed for 1 year and compared with a corresponding group of 169 p24 antigen negative individuals regarding rate of change of CD4 counts. The p24 antigen positive group had a mean of 605 CD4+ T cells\μl initially and 479 at 1 year for a 20.9% decrease. The p24 antigen negative cohort had 746 CD4+ T cells\μl at the first assessment but fell only 13.6% to 645 CD4+ T cells\μl during the same period.

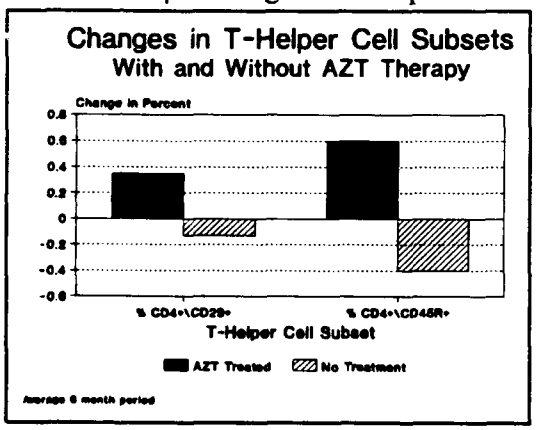

Figure 6. Percentage changes in T helper-cell subsets with AZT.

The effect of AZT on helper T-cell subsets was examined in figures 6 and 7. Over an average of 6 months, the percent of CD4+\CD29+ T cells rose significantly ($p<.05$) in AZT treated patients, while it fell significantly in those who did not receive antiretroviral therapy. Comparing values from groups with to those without AZT treatment,significant differences favoring AZT were evident for the

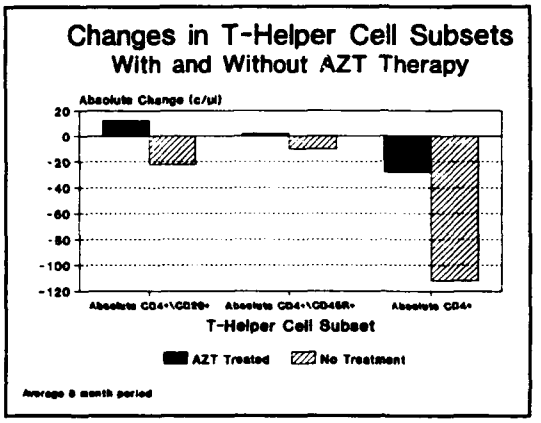

Figure 7. Absolute value changes in helper T-cell subsets with and without AZT.

following variables: %CD4+\CD29+, %CD4+\CD45R+, and absolute CD4A count . While AZT did not prevent the fall in the absolute CD4 count, it did slow it appreciably ($p<.01$).

DISCUSSION

Several of the Laboratory parameters discussed here, ($ß_2$ microglobulin, HIV p24 antigen, and neopterin) show a similar theme, namely a steady, gradual increase as the number of CD4+ T cells falls during the course of HIV infection. The clear relationship between the concentration of CD4+ T lymphocytes and the time to initial opportunistic infection was observed here as well as in other studies (4,5). Specific subsets of the helper T-cell group include those identified by the monoclonal antibody 4B4 (CD29+), and those labeled by 2H4 (CD45R+). The former represent "primed" or "memory" cells which are capable of producing a recall response to

soluble antigen; the latter represent "naive" T cells. HIV has been shown to cause an early, preferential loss of the CD4+\CD29 T cells (6), which our data show is reversed, at the 6 month sampling period, by AZT. This finding may have significance in conjunction with other strategies which involve immunomodulation, or vaccination and response to recall antigens.

$ß_2$ microglobulin is a subunit of the class I major histocompatibility complex (MHC) of antigens that are present on the surface of all nucleated cells. Its concentration in serum is felt to relate to lymphocyte turnover and activation. In the San Francisco General Hospital study patients with <200 CD4+ T cells\$\mu$l and $ß_2$ microglobulin levels $>5\mu$g\ml had a 95 probability of progression to AIDS over a 3 year period, while those similarly matched for CD4 cells with $ß_2$ microglobulin concentrations $<3\mu$g\ml only had a 56% likelihood of progression to AIDS (4). Our population demonstrated a stepwise increase in $ß_2$ microglobulin values as the CD4 count decreased during HIV infection.

Presence of HIV p24 antigen at the time of seroconversion has been associated with an accelerated time course of disease due to HIV (7). The data shown here establish an increased rate of initial opportunistic infections in the p24 antigen positive cohort (with CD4 cells >200\$\mu$l) over a 1 year follow-up period. Although the low prevalence of p24 antigenemia has limited its utility as a marker for disease progression, one study using acid pretreatment of sera raised the fraction with detectable p24 antigen from 12.4% to 50.6% (8). This antigen, as well as $ß_2$ microglobulin and neopterin were recently shown to decrease after only 8-12 weeks of AZT therapy, suggesting a possible role of these tests as surrogate markers in HIV treatment interventions (3).

Neopterin is a metabolic product of γ interferon activated macrophages, and levels >20 μmol\ml are shown to correlate with an increased risk of developing AIDS over a 3 year interval (9). The increase in cerebrospinal fluid neopterin and $ß_2$ microglobulin parallelled the serum test results for CD4 grouped subjects. These findings suggest that lymphocyte and macrophage activation and turnover probably occur in the central nervous system during HIV infection in a similar pattern to that seen in peripheral blood, either as a result of primary HIV pathogenesis or due to a coinfectious process. Alternatively diffusion or transport across the blood-brain barrier may play a role in the increased local concentration of these immune products in the central nervous system of late HIV-infected patients.

In a 1990 editorial, Polis and Masur suggest that the combination of CD4 counts, HIV p24 antigen, and either $ß_2$ microglobulin or neopterin levels would be an appropriate constellation of tests to follow on HIV-infected patients to obtain the best laboratory-based prognostic determination (10). The data presented here agree that stereotyped changes in these individuals do occur during the natural history of HIV infection which could be prognostically useful. These tests could also act as sensitive surrogate markers of drug efficacy, and may provide an enhanced assessment of subclinical disease progression.

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ANALYSIS OF DISEASE PROGRESSION FROM CLINICAL OBSERVATIONS OF US AIR FORCE ACTIVE DUTY MEMBERS INFECTED WITH THE HUMAN IMMUNODEFICIENCY VIRUS: DISTRIBUTION OF AIDS SURVIVAL TIME FROM INTERVAL CENSORED OBSERVATIONS

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1. SUMMARY

A nonparametric estimator of the AIDS survival time (after developing AIDS) is computed for the AIDS data set from the US Air Force (USAF). Survival times are unobservable. They are censored by the screening mechanism. The Armstrong Laboratory's Epidemiologic Research Division maintains data on over 940 active duty US Air Force (USAF) individuals who tested positive for Human Immunodeficiency Virus (HIV) antibodies. Many have been clinically evaluated six times since 1986. The HIV-positive individual is classified in seven stages of the disease complex as time progresses. Exact times of transition from one stage to the next are unknown. It is known that transition occurred between two consecutive evaluations. The aim of this study is to analyze distributions of the times that individuals spend in each stage of the HIV disease complex.
We will discuss methods used to obtain nonparametric estimators of the distributions of times that individuals spend in stage 6. Finally, we hope to model the median time spent in each stage of the disease. This, along with incidence and separation data, will allow us to predict the impact of HIV disease on USAF individuals and medical care systems.

2. INTRODUCTION

The US Air Force data file for HIV/AIDS contains screening information on 954 infected individuals who have been evaluated at least once since 1986. Some individuals have been clinically evaluated as many as 7 times. The number of infections and evaluations per individual will increase as time passes. At each evaluation the infected individual is classified into one of seven stages of the disease. The different stages are the Walter Reed (WR) classes 1 to 6 and finally, death. The description of the different WR stages is progressive:

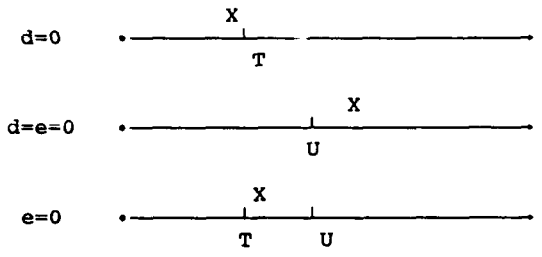
Stage 1: Infected, but no symptoms.
Stage 2: First signs of infection such as swollen lymph glands.
Stage 3: Abnormally low number of T-helper cells (with or without symptoms).
Stage 4: Low response to skin-test battery for body's ability to fight disease.
Stage 5: No response to skin-test battery, or yeast infection in the mouth.
Stage 6: AIDS.

Our primary interest is to study the progression of the disease from one stage to the next within an infected individual. The aim is to obtain nonparametric estimators of distributions of the times that an infected individual spends in each stage of the disease. The exact transition times from one stage to the next are unknown. It is only known that transition occurred within an interval of time, a situation known as interval censoring. Since the origin (infection) for each individual is also unknown, we are considering first the backward process and restrict the analysis to those individuals that provide information about stage 6; 92 observations. From these 92 individuals, 76 are dead. For the deaths, the origin of this backward process is the date of death. For those that are alive, the origin of the backward process is the date of the last medical evaluation. From this subset of the USAF data we estimate (nonparametrically) the distribution of the AIDS survival time (time that an infected individual spends in Walter Reed stage 6) for those infected individuals who develop AIDS.
A nonparametric estimator of a survival distribution was introduced by Kaplan and Meier (1958) for right censored data. Turnbull (1974) developed the expectation-maximization (EM) algorithm for doubly-censored data which is an iterative method based on the self-consistency concept introduced by Efron (1967). Groeneboom (1990) presented an alternative method called iterative convex minorant (CM) algorithm for interval censored observations. The method used here is an adaptation of the iterative CM algorithm by Aragon and Eberly (1991).

3. METHODS

Let X_i be the time that i^{th} infected individual spends in stage 6 and let (T_i,U_i) be the interval censoring the variable X_i. The random variable X_i is unobservable. We only observe T_i,U_i and the indicators $d_i = I_{(X_i \le T_i)}$ and $e_i = I_{(T_i < X_i \le U_i)}$. Note that $d_i = 1$ corresponds to a left censored observation and $d_i = e_i = 0$ corresponds to a right censored observation. Therefore U_i is not needed if $d_i = 1$ and T_i is not needed if $d_i = e_i = 0$.

Figure 1 illustrates the three cases.



It is assumed that X_i and (T_i,U_i) are independent.

We follow the same approach as in Groeneboom. The likelihood function, conditional on those who develop AIDS, is given by

$$(2.1)\quad L(F) = \prod_{i=1}^{n} F(T_i)^{d_i} \left(F(U_i) - F(T_i)\right)^{e_i} \left(1-F(T_i)\right)^{1-d_i-e_i}$$

where F is the cumulative distribution function of X_i and n is the sample size.

The nonparametric maximum likelihood estimator (NPMLE) of the distribution F is the function F_n maximizing Equation 2.1. Note that only the values of F at the points T_i's or U_i's matter for the maximization problem.

If we let $y_i = F(T_{(i)})$ where $T_{(i)}$ is the i^{th} order statistic of the set $(T_1,\ldots,T_n, U_1,\ldots,U_n)$, the maximization problem is formulated as follows.

Maximize the (conditional) loglikelihood

$$(2.2)\quad \phi(y) = \sum_{i\in I_0} \log(y_i) + \sum_{i\in I_1} \log(1-y_i) + \sum_{i,i'\in I_2} \log(y_i - y_{i'})$$

over the simplex $S=\{y \in IR^m : 0 \le y_i \le \ldots \le y_m \le 1\}$

where $\{1,\ldots,m\} = I_0 \cup I_1 \cup I_2$ is a disjoint union, and where $i' < i$ is an index dependent on the choice of i.

The algorithm to solve this concave programming problem, called iterative damped CM algorithm, is described with detail in Aragon and Eberly. It is an adaptation of the CM algorithm developed by Groeneboom in order to have global convergence. There are no convergence results for the CM algorithm of Groeneboom.

4. RESULTS AND DISCUSSION

The 92 observations corresponding to the infected individuals from the USAF data who developed AIDS are listed in Table 1, Walter Reed stage 6 data from U.S. Air Force.

t	u	d	e
0.00	35.36	0	0
0.00	18.00	0	0
0.00	18.11	0	0
0.00	14.43	0	0
0.00	17.18	0	0
*****	******	*	*
22.25	24.96	0	1
16.32	38.93	0	1
11.89	48.04	0	1
24.36	37.82	0	1
7.93	24.89	0	1
*****	*****	*	*
13.11	0.00	1	0
7.57	0.00	1	0
6.29	0.00	1	0
12.32	0.00	1	0
8.00	0.00	1	0

The first 16 observations correspond to individuals who are still alive. For this group, the value of u is computed as the time interval between the first and last evaluations in which the individual was classified in stage 6. The unit of time used is months (28 days per month). For the remaining 76 observations the value of t (if positive) is computed as the time interval between the date of death and

i) the last date in which the individual was classified in stage 5, if d = 0.

ii) the first date in which the individual was classified in stage 6, if e = 0.

The value of u (if positive) is computed as the time interval between the date of death and the last date in which the individual is classified in stage 5.

The estimated distribution is given in Table 2 and Figure 2.

Table 2. Estimated cumulative probabilities.

Time (months of 28 days)	Cumulative prob.
2.07	.16
6.29	.21
7.32	.25
18.93	.31
22.96	.51
24.89	.65
26.43	.83
37.82	.90
52.61	.90

From Figure 2 we can see that the median survival time in stage 6 is approximately 23 months which is consistent with the results provided by some authors in the literature. Longini, et. al. (1991) estimated, from US Army data, a mean time of 1.3 years for individuals older than 30, and 2 years for the age group 25 or younger.

It is clear from the graph of the estimated distribution that the USAF sample size is not large enough to provide a reliable estimate. This will improve with time as more information becomes available. The data do not provide information on treatment or reporting delays for deaths. This may be a problem since it is likely that there are differences among treatments, and

reporting delays of deaths may be a source of bias.
One last observation is that the clinical evaluation which defines the censoring mechanism may not be independent of the survival time. The fact that infected individuals are not evaluated at random times, and some of them decide on their own when to show up for an evaluation, suggest that an informative censoring mechanism is more appropriate. If this is the case, special care should be taken when interpreting the estimator of the survival time distribution.

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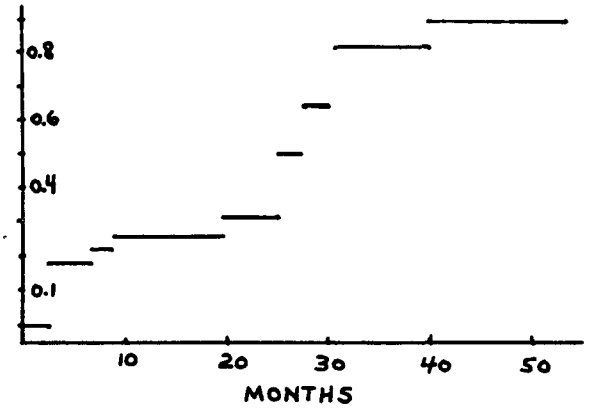
Figure 2. Cumulative probability distribution of survival after AIDS diagnosis; USAF 1990 data.



RELATING COGNITIVE FUNCTION TO MILITARY AVIATOR PERFORMANCE IN EARLY HIV INFECTION

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SUMMARY

There has been controversy about whether cognitive changes occur in early human immunodeficiency virus (HIV) disease. In those studies reporting cognitive changes, findings are typically subclinical, and their relationship to daily and/or occupational functioning has not been addressed. The potential effects of changes may vary as a function of occupational demands. This is germane to military performance, where occupational demands cover a wide spectrum of complexity. In particular, such effects are important to consider in the many cognitively-demanding specialties associated with military aviation.

This paper will explore ways in which possible HIV-related military performance decrements in aviators may be measured empirically. First, studies from Walter Reed Army Medical Center (WRAMC), which have shown cognitive changes in early HIV disease, will be described. This will be followed by a summary of presentations and discussions at a November, 1990 conference, entitled, "HIV and Military Performance: Assessment Methodologies," held at WRAMC. The third section of the paper will describe a program of research, which is developing measures to detect cognitive difficulties in civilian aviators. The application of measures from this research to research on HIV will be discussed. Finally, the paper will conclude with presentation of a research program being developed to examine the possible impact of HIV-related cognitive changes on military aviator performance.

1 BACKGROUND

There is controversy over whether cognitive changes occur at the early stages of HIV infection. Although some researchers have reported neurobehavioral changes in asymptomatic individuals at the early stages of disease (Ref 1, 2, 3, 4, 5, 6, 7), others have not (Ref 8, 9, 10, 11, 12, 13, 14). Those researchers reporting deficits have found changes in attention, speed of information processing, learning/memory, and sensorimotor skills. However, *the relationship of these statistically significant cognitive deficits to daily and/or occupational functioning remains to be established.* This issue is particularly germane to military performance. For example, a 50 millisecond (ms) reaction time decrement would probably have little impact on the performance of a clerk or cook, but this might not be the case for the performance of an aviator, where split-second reactions are necessary.

2 FINDINGS FROM RESEARCH AT WALTER REED ARMY MEDICAL CENTER

Data reported previously (Ref 2, 3, 15, 16, 17), collected in laboratories at WRAMC, have suggested that cognitive changes may occur at the early stages of HIV disease. Fifty-two HIV-seropositive (HIV+) subjects were evaluated and compared to 15 HIV-seronegative (HIV-) non-psychotic psychiatric subjects with a diagnosis of Adjustment Disorder (AD) and 18 HIV- normal control subjects (NC). HIV+, AD, and NC control subjects were matched on age, education, ethnic background, and military rank. Eighty four percent of HIV+ subjects were at the early stages of infection (Walter Reed Stages 1-3; Ref 18). The HIV+ subjects performed significantly more slowly on measures of speeded information processing and attention, and exhibited difficulty with spatial abilities, verbal learning, and verbal memory, relative to both HIV- groups. The HIV+ and AD subjects did not differ in self-reported symptoms of depression (Beck Depression Inventory, Ref 19; Means: HIV+ 14.9, AD 14.4). Yet, 48% of the HIV+ subjects were impaired on more than 10 of the 51 neuropsychological measures examined, while only 7% of the AD group showed similar changes. Thus, cognitive changes could not be accounted for by depression alone.

Particularly notable were findings of slowed speed of information processing. HIV+ subjects were significantly slower than HIV- subjects on simple and choice reaction time tasks; mean group differences were approximately 50 ms. Further, when results for HIV+ subjects were examined only for those at Walter Reed Stages 1 and 2, these significant group differences in reaction time remained. Presence of constitutional symptoms and T4-lymphocyte level also did not account for results. For each group, the number of subjects who were two or more standard deviations slower than the mean of HIV- control subjects was calculated. For each reaction time task, this value was approximately 90 milliseconds slower than the mean. For the HIV+ subjects, 20% and 35% were classified as exceeding this cutoff on the simple and choice reaction time tasks, respectively. For the HIV- group, these values were 9% and 3% on the two tasks.

In addition to the results obtained at baseline assessment, a subset of the original HIV+ subjects were re-evaluated at six month intervals, up to 18 months. A subset of the original NC group was re-evaluated once, at six months following baseline evaluation. As a group, the HIV+ subjects exhibited progressive slowing of cognitive and motor performance. In contrast, no significant change was found in the NC group. Progressive changes in the HIV+ subjects over the first six month period were not correlated with changes in mood state, measures of immunological functioning (i.e., CD4 count, CD4/CD8 ratio), or presence of constitutional symptoms.

Finally, at the initial evaluation, 66% of the HIV+ subjects were found to have abnormally elevated levels of quinolinic acid (QUIN), an endogenous neurotoxin and NMDA-receptor agonist, with a two to three fold increase relative to reported normal values (Ref 20). In addition, the progressive motor and cognitive slowing noted in the HIV+ subjects over time was correlated with initial levels of QUIN ($r=.41$, $p<.05$), as well as with increasing levels of QUIN obtained on repeat evaluation ($r=.85$, $p<.01$; Ref 3).

In summary, a significant minority of HIV+ subjects in this sample displayed slowing of information processing speed, relative to matched HIV- control subjects. Progressive slowing of reaction time occurred from the first to the second testing session. Subjects also showed elevations in quinolinic acid levels, and progressive increases in these levels correlated with progressive slowing of reaction time, suggesting a mechanism for cognitive changes.

3 HIV AND MILITARY PERFORMANCE: ASSESSMENT METHODOLOGIES

Although a relation between slowing of information processing and performance in cognitively-complex military tasks, such as aviation, might be hypothesized, empirical work has yet to demonstrate the presence of such a relation. In November, 1990, a conference was held at WRAMC to discuss ways of measuring the possible effects of HIV-related cognitive changes on military occupational performance (Ref 21). The goals of this meeting were to 1) Discuss methodologies which have been used to assess human performance in military and civilian settings, 2) Determine which methodologies might be most useful to study the effects of HIV on military performance, and 3) Establish specific priorities in the pursuit of such research.

The first two days of the conference consisted of topical presentations. Plenary sessions addressed the neurobehavioral effects of HIV, taxonomies of human performance, modelling human performance, neuropsychological evaluation of fitness-for-duty, applications of military performance assessment batteries (PABs) and other types of computerized skill testing, and the mediating effects of stress on human performance. Presentations were followed by a panel discussion of the most effective ways to measure performance.

On the third day of the conference a small working group met, which included representatives from the Army, Navy, Air Force, and civilian settings. A variety of issues were addressed including the population to be studied, subject selection and experimental design, types of data needed to answer questions about performance, assessment measures for pre- and post-HIV infection functioning, use of simulators, paradigmatic differences in neurobehavioral and human performance research, and constraints on research.

The difficulty of establishing criterion measures for job performance was discussed. Although neuropsychological evaluation has been used for fitness-for-duty evaluations, it was acknowledged that these measures, although sensitive to brain-behavior changes, lack ecological validity and may not be the best measures to use. Hence, it was concluded that other criterion measures, more closely related to job performance, needed to be used and/or developed. Measures discussed included the following:

Pre-HIV Functioning

- Armed Services Vocational Aptitude Battery (ASVAB; or comparable measures)
- Officer Tests
- Expected pre-HIV functioning based upon neuropsychological predictors

Post-HIV Functioning

- ASVAB (repeated)
- Job performance ratings
- Neuropsychological measures
- Military performance assessment batteries (PAB)
- Knowledge tasks
- Hands-on tasks
- Computerized skill testing
- Non-promotion based performance ratings (to be developed)
- Simulators (Air, ground, individual, group)
- Computer modeling based on the above as predictor and criterion variables

It was noted that Army Project A has developed a number of such measures, but only for a small number of job specialties and at a considerable expense of personnel, money, and time (Ref 22). The enormity of the task was appreciated, and it was recommended that 1) tasks be developed/used which have application across specialties 2) particular attention should be given to specialties in which possible HIV-related changes could place others at risk (e.g., aviation-related specialties), but 3) research should also focus on the specialties which have large numbers of HIV-infected individuals.

The following model research program was outlined:

1) Determine the occupational specialties of HIV+ individuals in the three services
2) Perform task analysis of each specialty, identified according to criteria above, to determine cognitive demands, using a taxonomy of human performance developed for this procedure (Ref 23)
3) Select PAB/neuropsychological tasks based upon task analysis
4) Recruit HIV- controls matched to HIV+ subjects on demographic variables
5) Collect medical, neurological, neuropsychological, and PAB data, and job performance ratings
6) Develop and administer criterion measures of job performance to all subjects
7) Analyze data by group (HIV+ vs. controls) and develop models to predict job performance based upon collected data
8) Evaluate the ability to predict military job performance

It was acknowledged that it would be necessary to break down the model program into a series of smaller studies. In particular, the sensitivity of computerized cognitive measures, such as the PAB, to HIV-related changes in the central nervous system would have to be demonstrated. It would also be necessary to spend time researching and developing better criterion measures of job performance.
A number of constraints on such a study were identified, including:

1) Lack of subject testing time and "test fatigue"
2) Lack of interest of HIV- individuals in serving as controls
3) Reluctance of subjects to return for repeated visits
4) Differing organizational research priorities
5) High cost

At the end of the meeting, resources were identified, and preliminary plans were made for collaborative study.

4 DEVELOPMENT OF COMPUTERIZED COGNITIVE MEASURES TO EVALUATE AVIATOR PERFORMANCE

As a first step in the proposed research program, it was decided to explore which cognitive measures might be sensitive to changes in aviator performance. Although there have been no studies of the relationship between cognitive *function* (as measured by standardized neuropsychological measures or PABs) and actual aviator performance (Ref 24; see below), a number of studies have examined the ability of cognitive measures to improve aviator selection in training programs. In a series of studies, Carretta (Ref 25,26,27) examined the ability of several computerized measures (the Basic Attributes Test, BAT) to predict success in Undergraduate Pilot Training (UPT) and classification for post-UPT advanced flight training. The degree to which measures from the BAT added to predictions provided by other measures was also examined. Although a number of BAT measures had no predictive utility, others were able to predict outcome at a statistically significant level, but only to a modest degree (the additional variance accounted for by BAT measures was quite small). BAT measures were most useful when used to predict whether those completing training were assigned to advanced training for bomber/transport planes vs. fighter/attack planes. Measures had little utility in prediction of those who successfully completed training, although this may have been due to the restricted high range of ability in those who were admitted to training. Measures which were most predictive were Digit Memory (a visual span task), Decision Making Speed (a choice reaction time task), Item Recognition (the Sternberg task, a measure of short term retention), and Mental Rotation. Studies on the utility of a modified BAT are continuing.

Kay and Horst (Ref 24) reviewed other studies of cognitive function in pilots. They reported that a number of cognitive batteries, computer-based and non-computer based, had been developed to assess cognitive skills assumed to be important to aviation. Many of these tasks had been selected by using a performance taxonomy developed by Fleishman and colleagues (Ref 23), selecting *a priori* those skills which appeared important to aviation, and developing tasks to measure these skills. The extensive review of the literature by Kay and Horst (Ref 24) indicated that PABs had been used primarily to predict success in flight training or to examine the impact of flight-related variables (e.g., G forces, the presence of multiple cognitive demands) on performance. They reported, however, that there were no studies in which test batteries had been validated against actual flight performance of experienced pilots. Further, based upon work by Fleishman (Ref 23), they concluded that it was likely that the skills required to *train successfully as a pilot* were different than those skills *used by experienced pilots when actually flying*. Hence, batteries used for selection and training might not be relevant to answer questions about fitness-to-fly in experienced pilots.

Kay and colleagues have developed a program of research to examine fitness-for-duty in civilian pilots, using computerized cognitive measures. These measures are based upon a taxonomy of human performance abilities (Ref 23), and have been theoretically linked to cognitive components of a number of military occupational specialties. The measures are administered in a standard manner, provide measures of response accuracy and time, and are relatively immune to practice effects, rendering them useful for repeated measurement.

Several studies have been completed by Kay and colleagues using the computerized tasks. Horst and Kay (Ref 28) investigated the ability of mental status tests, neuropsychological measures, and computerized performance measures for cognitive assessment, to discriminate between 60 licensed civilian pilots and 60 mildly brain damaged patients (etiologies included head trauma, seizure disorders, tumors, multiple sclerosis, cerebrovascular disorders, dementia, alcoholism, central nervous system infection). Short mental status tests did not discriminate between the two groups. As expected, neuropsychological tests were able to distinguish between the groups. Finally, computerized measures were as sensitive to the presence of brain dysfunction as were traditional neuropsychological screening tests. In fact, some of the computerized measures were more sensitive than any of the traditional clinical measures.

Subsequently, the results of this study were applied to the development of COGSCREEN, a 12-test, 45 minute computerized cognitive screening battery, designed for use in the Federal Aviation Administration's medical recertification process. COGSCREEN was shown to be highly sensitive to the presence of brain dysfunction in a study of 40 pilots, 40 non-pilot normal subjects, and a heterogeneous sample of 40 patients with mild brain dysfunction (Ref 29). At present, COGSCREEN is undergoing further development, and a modified and enhanced version of COGSCREEN is being administered to 450 commercial aviators. A planned study will examine the relationship between COGSCREEN performance and skilled pilot performance in a full-motion flight simulator. Another planned study will examine COGSCREEN performance in Navy pilots, and will eventually relate COGSCREEN performance to flight simulator performance.

In summary, COGSCREEN has been shown to differentiate pilots from subjects with mild brain damage. Because tasks were selected specifically to measure skills believed to be important in aviation, it was believed that COGSCREEN would provide a starting point for building a foundation for the measurement of performance in HIV-infected military aviators. It was also believed to be a useful set of measures for several other reasons 1) theoretical work has associated these measures with aspects of military performance (Ref 23), 2) the cognitive domains which these measures assess have been those noted to be affected in early HIV disease, and 3) all include measures of information processing speed, shown to change in early HIV infection. It is hoped that these measures may be established as reliable and valid measures of HIV-related neurobehavioral changes, may eventually provide a shorter group of cognitive measures to be used in subsequent studies, and may provide a foundation for the measurement of aviation performance.

5 CURRENT STATUS

A research proposal to study early neurobehavioral changes in HIV-infected aviators received initial approval in May, 1991 and is undergoing final review. It is hoped that studies will begin in the Fall or Winter of 1991. The purpose of the study is to build a foundation for measuring possible HIV-related changes in military aviator performance by first demonstrating the sensitivity of COGSCREEN to HIV-related neurobehavioral changes. The study will examine neurobehavioral function in HIV-infected aviators in the Navy and Air Force. In this select group of individuals, the possibility of pre-HIV neurobehavioral difficulties has been carefully ruled out, because of job requirements. Groups of HIV- control subjects will be established for each service. Two groups of control subjects will used: one group will be on active flying status, and the other group will be aviators not on flying status, due to reasons other than psychiatric or neurologic disorder. The latter group will be used to control for the psychological effects of being grounded. HIV+ individuals will be studied carefully using neurological evaluation, psychological/psychiatric evaluation, neuropsychological evaluation, MRI scan, SPECT scan, long-latency event-related potentials, and CSF analyses. Job status, flying status, and employment satisfaction will be monitored, and will be related to collected data. HIV- subjects will be studied using all measures except lumbar puncture.

Two methodological limitations have been identified, and these are being addressed. First, the sample size is limited, and may make it difficult to detect subtle differences between groups. To enlarge the sample, individuals in aviation-related tasks, such as air traffic controllers, radar operators, and missile silo controllers, may be included. In the future, those in other cognitively-demanding "positions of risk" may also be included. Second, it may be very difficult to recruit HIV- control subjects, particularly those on active flying status. Pilots are very reluctant to engage in an evaluation which might reveal abnormalities which could impact on flying status. To facilitate participation, individuals at the Navy Bureau of Medicine and Air Force Office of the Surgeon General have been briefed, and are considering ways of recruiting HIV- control subjects. In addition, HIV+ subjects may be asked if friends who are aviators might be willing to participate.

In summary, the planned study will begin to answer empirically 1) whether neurobehavioral changes associated with early HIV infection be detected in a select, high-functioning group of military aviators, and 2) if such changes are detected, whether they may impact on aviator performance or are benign. At the end of the first stage, it

is hoped that a group of computerized cognitive measures will be established as a screen for whether early cognitive changes are present. Development of such a tool will assist with establishing policy regarding the flying status of HIV-infected aviators, who are at the early stages of infection and are otherwise physically asymptomatic.

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NEUROPSYCHIATRIC MORBIDITY IN EARLY HIV DISEASE: IMPLICATIONS FOR MILITARY OCCUPATIONAL FUNCTION

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SUMMARY

The Military Medical Consortium for Applied Retroviral Research's (MMCARR) Behavioral Medicine Human Immunodeficiency Virus (HIV) Research component is conducting a tri-service, comprehensive, and longitudinal research study in military HIV-infected personnel at all stages of infection. Identification of neuropsychiatric and psychosocial outcomes and their determinants will help the military minimize the impact of the HIV epidemic on military readiness and function.

Neuropsychiatric and psychosocial findings are among the most common complications seen in early HIV Disease and among the most likely to have an adverse impact on military readiness and function. The study has demonstrated that the average HIV-infected service-person experiences at least transient military occupational difficulty following notification of HIV status. More than 15% at any given time have levels of clinical or subclinical anxiety or depression that are referrable for mental health intervention. Ten percent of study subjects have a current major mood disorder and 5% have a psychoactive substance use disorder. Finally, 17% of study subjects have experienced serious suicidal ideation or behaviors at least once since notification of seropositivity.

Fortunately, however, data also indicate at least partial effectiveness of current primary, secondary, and tertiary preventive efforts. Only about 1% of Air Force HIV-infected persons are discharged for psychiatric reasons prior to eventual medical discharge. Further, a large majority of active-duty patients demonstrate solid military occupational and social performance. Though military HIV neurobehavioral research is still in progress, preliminary data identify social support and pre-HIV psychiatric predisposition as important factors associated with current neuropsychiatric status.

I. INTRODUCTION

When neuropsychiatric findings occur in HIV-infected individuals, they may be due to pre-existing neurologic or psychiatric disorders, primary CNS effects of HIV or associated phenomena such as opportunistic infections and neoplasms, psychosocial stressors, or secondary gain. Previous work in the military setting (1) by our group has demonstrated the complexity and extent of neurologic, psychiatric, medical, psychosocial, and immunologic inter-relationships. For example, in 98 randomly selected HIV-infected U.S. military patients, high Hamilton Anxiety Rating Scale (HARS) (2) scores were found to correlate with cerebrospinal fluid (CSF) nucleated cell count ($r=.56$, $df=15$, $p<.05$) and absolute CD4a count($r=.51$, $df=16$, $p<.05$). High Hamilton Depression Rating Scale (HDRS) (2) scores were found to correlate with CSF nucleated cell count ($r=.49$, $df=19$, $p<.05$), and between HDRS score and CSF protein ($r=.47$, $df=19$, $p<.05$). Other viral (EBV and CMV) titers were not correlated with HARS or HDRS scores. These results tenuously advance the hypothesis that there is an association between effects of HIV infection in the CNS and high anxiety or depression levels. However, cause-effect relationships are clearly not straightforward and cannot be established because data relevant to other potential causes of high HARS and HDRS scores are unavailable.

Psychosocial factors also have clear relevance and etiologic significance with regard to production of neuropsychiatric syndromes. Social support has been shown to buffer the effect of stress (3-6), decrease psychological distress (7,8), and help maintain physical and emotional well-being (5,9,10). Social support networks may play an instrumental

role in better coping strategies (11, 12) and in reducing the number of stressful events to which an individual is exposed (10,13). There is considerable evidence that reduction in both the amount and quality of social support is associated with increased depression (14,15), poor physical health (16-18), and increased mortality (19). Optimal recovery from psychiatric disorders is correlated with the perception of social support and its availability (20,21).

Social support has been related to decreased HIV transmission among gay men (22). In Acquired Immunodeficiency Syndrome (AIDS) patients, satisfaction with social support has been linked with lower levels of psychological distress (23). Little information is available, however, on the effects of social support in individuals with early asymptomatic stages of HIV infection.

Suicide rates in individuals diagnosed with AIDS have been reported to be 17-36 times higher than that of demographically similar populations (24-26). Suicidality is one indicator of level of psychological distress in a population. In previous work (27), we calculated a suicide rate in Air Force HIV-infected personnel 21 times that of the U.S. Air Force in general, a rate consistent with that found in civilian HIV studies. Little is known, however, about actual determinants or the meaning of suicidality in early stages of HIV Disease or about the methods most likely to be used by HIV-infected suicide attempters.

In this paper, we will discuss the complexity of relating neurologic, psychiatric, psychosocial, medical, immunologic, and behavioral outcomes and their determinants in early HIV Disease. Then we will present data to date from the TSBS and its predecessor studies. The findings' relevance for future research and for primary, secondary, and tertiary preventive intervention development in the military occupational setting will be discussed.

A. Neuroimmunology, Neuropathology, and HIV Disease

Disordered immune function may affect the central nervous system and alter its function. CNS autoimmune and viral processes associated with neurologic or systemic disease states may elicit behavioral pathology or neuropsychological impairments (28-30). In addition to HIV Disease, classic examples of this phenomenon include CNS systemic lupus erythematosis and multiple sclerosis.

Important techniques and methods in neuropathological study of HIV Disease include autopsy, electron microscopy, and immunohistological procedures.

Neuropathological changes in early HIV Disease are characterized by the presence of macrophages in the brain tissue not related to opportunistic infections (28-30). In early asymptomatic HIV encephalopathy there is typically perivascular macrophage infiltration, particularly in subcortical areas and white matter. As CNS infection progresses, there may be perivascular infiltration by not only macrophages but microglial cells and multinucleated giant cells, as well as the appearance of detectable or significant numbers of macrophages in the neuropil of subcortical structures (30). The monocyte-macrophage cell line is extremely important with regard to pathogenesis and either produces or is responsible for the appearance of a number of neurotoxic substances including some cytokines and proteolytic enzymes. Microglia and macrophages either resident in or migratory to the CNS are sites of replication for HIV in vivo (31). In very severe HIV CNS disease there also may be astrocytic hyperplasia, astrocytic hypertrophy, glial nodules with multinucleated giant cells, and progressive diffuse encephalopathy.

Cerebrospinal fluid (CSF) studies of early asymptomatic HIV-infected persons reveal frequent abnormalities, providing evidence that HIV enters the CNS early in the disease (29). A high proportion of early patients have abnormalities that include CSF pleocytosis, elevated protein, increased CSF immunoglobulin, or oligoclonal bands.

Neuropsychological findings most frequently associated with HIV-related CNS involvement are those involving subcortical, integrative, and executive functions: visuospatial integration, visuospatial memory, reaction time, verbal fluency, nonverbal fluency, problem-solving, conceptual skills, visual processing tasks, set shifting, design fluency, fine and gross motor control, concentration, speed of mental processing, sequencing, and initiation/inhibition/ mental flexibility (32-36). Language and related general intellectual skills tend to be spared.

Anatomic and functional changes and their neuropsychiatric clinical correlates in HIV Disease are preferentially localized to white matter in subcortical regions and limbic system (30). Subcortical and limbic system areas have many important cognitive, motor, and behavioral functions (37).

B. The Tri-Service HIV Biopsychosocial Study

Since 1985, the United States military services have conducted periodic force-wide HIV screening. All active duty U.S military personnel with positive

results on both screening (ELISA) and confirmation testing (Western Blot) are evaluated periodically at a major military medical center on a specialized, dedicated research and clinical HIV unit. The nature and quality assurance of the military testing program have been described elsewhere (38).

Four HIV research units (Wilford Hall U.S. Air Force Medical Center, Walter Reed Army Medical Center, San Diego Naval Hospital, and National Naval Medical Center--Bethesda) are collaborating in a multi-center tri-service study of neuropsychiatric and psychosocial factors related to military occupational function and HIV transmission potential of HIV-infected Army, Air Force, Navy, and Marine Corps personnel. The Tri-Service Biopsychosocial Study (TSBS) integrates medical, immunological, psychiatric, psychosocial, neuropsychological, and HIV transmission risk factor data in an attempt to resolve many of the cause-effect attribution dilemmas inherent in biopsychosocial research.

The largest single TSBS study site is Wilford Hall U.S. Air Force Medical Center, Lackland Air Force Base, TX (WHMC). Many occupationally relevant aspects of the TSBS are of particular interest to the Air Force because of the high percentage of Air Force personnel who are engaged in cognitively demanding tasks as part of their work effort. Performance of these tasks may be adversely impacted by neurological and psychiatric disorders, neuropsychological deficits, and psychosocial duress, regardless of etiology. HIV Disease is one potential etiology. The highly technological nature of many Air Force career fields makes an understanding of predictors and extent of neuropsychiatric and psychosocial complications of HIV Disease important.

The WHMC TSBS site has conducted mission-relevant studies since 1983, when the Air Force first began to identify personnel with HIV-related medical and neuropsychiatric disorders. The pace of research increased with the advent of periodic HIV screening in the Air Force. Preliminary and pilot studies conducted there between 1987 and 1989 (1,27,39-44) provided the basis for development of the current TSBS.

This remainder of this paper will describe the TSBS research design with a focus on its applicability to military occupational factors of particular relevance to the unique U.S. Air Force mission and to Aerospace Medicine. Neuropsychiatric TSBS data from the first wave of the prospective study will be presented. Neuropsychiatric disorder prevalence data will be compared to a large, seronegative community-based sample for both lifetime and current disorders. Additional laboratory and clinical estimates of neuropsychiatric and medical status will be presented, including measures of cerebrospinal fluid status, immunological status, depression, anxiety, general occupational and social function, and suicidality.

II. METHODS

Once confirmed to be HIV-infected, U.S. military medical beneficiaries are referred to one of the designated HIV research and clinical centers for evaluation. Reevaluation occurs every 6-18 months, depending on a patient's administrative status. All HIV-infected U.S. military medical beneficiaries followed at one of the four collaborating TSBS study sites are eligible for the protocol. Data from the TSBS is analyzed in the context of important relevant data from other protocols and clinical evaluations when available: clinical neurological information, medical data, immunologic data, and imaging data. Individuals who consent to participation in the study are enrolled in its five core areas of data collection:

A. Standardized Structured Psychiatric Examination: The Structured Clinical Interview for the DSM-III-R (SCID) (45), modified for use with an HIV-infected population, is administered by fully trained clinicians (psychiatrists, psychologists, and supervised senior psychiatric residents). The source of diagnostic criteria for all SCID-derived psychiatric diagnoses is the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (46). During SCID examinations, subjects are also administered the Structured Interview Guide for the Hamilton Depression and Anxiety Scales (SIGH-AD) (2).

Investigators administering the SCID received standard training either from one of the authors of the instrument (M. First, M.D.) or from one of the TSBS Principal Investigators. Regular meetings are held to assess interrater reliability. Data are checked for accuracy prior to data entry and all discrepancies resolved with the examiner. Finalized data are double entered by two independent technicians. The two data files are then merged and all inconsistencies resolved by referring to the original SCID scoring documents.

B. Self-Report Psychosocial Questionnaires: These instruments include the Spielberger State-Trait Anxiety Scale (47), SCID questionnaire for Personality Disorder Assessment (Axis II) (45), UCLA Loneliness Scale (48), AZT Compliance Questionnaire, Multidimensional Health Locus of Control (49), Profile of Mood States (50), and Symptom Check List-90-Revised (51).

C. Neuropsychological Testing: A three hour neuropsychological testing battery is administered, which includes standard clinical neuropsychological measures. Addenda to the TSBS have been developed to study sub-populations of TSBS subjects with more detailed computerized cognitive performance tasks, clinical neurological exams, CSF examination, neuro-imaging procedures, and self-report ratings of job performance, satisfaction, and status.

D. HIV Transmission Risk Factor Assessment: The questionnaire comprising this core protocol area is currently anonymous, but is eventually designed to be linked to other protocol data. The Transmission Risk Factor Assessment Core Area assesses knowledge, attitudes, beliefs, and practices related to HIV transmission by seropositives.

E. Stress and Coping Assessment: Information is collected during the protocol relating the nature and impact of specific stressors occurring during the course of HIV Disease to defined ways of coping and maintaining adequate occupational and social function in the military context.

In order to focus attention on issues most relevant to the Air Force and Aerospace Medicine, most data analyses in this paper exclude Army, Navy, and Marine subjects. For the Air Force study sample, 472 patients (442 men, 30 women) consented to participate during year one of the study. Of the entire group approached to enroll in the study, only one patient declined to participate, and no patients withdrew their consent. Because of the low numbers of women enrolled to date, many data analyses presented here include only men. Data specific to military women are di ed elsewhere (39,41). Some subjects hav mpleted two waves of longitudinal data collection to date; only Wave 1 data is included in this report.

Staging of HIV infection by infectious disease specialists at the four study sites followed the conventions of the Walter Reed Staging System (WR), in standard use throughout the Department of Defense and described elsewhere (52). Comparisons of current and lifetime psychiatric diagnostic frequencies were made, when applicable, to U.S. community-based estimates of psychiatric disorders (past month and lifetime) as recorded during the U.S. National Epidemiological Catchment Area (ECA) surveys (53-55). Data comparisons are possible for adult males between the ages of 18 and 44, stratified into two groups, ages 18-24 and ages 25-44.

Psychiatric morbidity data will be expressed by the variables current psychiatric diagnosis (including comparisons to the ECA reference population) and lifetime psychiatric diagnosis (to address pre-seroconversion contributions to current diagnoses and baseline population differences between the Air Force cohort and the ECA reference population). In addition to psychiatric diagnoses, five additional domains of neuropsychiatric status of HIV-infected persons in the military occupation/social setting are reported: A) Global Assessments of Functioning (GAF, DSM-III-R Axis V) (46), including estimates for current level of functioning, lowest and highest levels of functioning since HIV-seropositivity notification, highest level of functioning in the past year, and lowest level of functioning in the past month; B) Anxiety rating scale scores (2); C) Depression rating scale scores (2); and D) Suicidality data, both before and after knowledge of HIV seropositivity; and E) Social support perception. These data were also analyzed by age group and Walter Reed stage when available.

In order to explore the possibility that social support perception is significantly associated with neuropsychiatric findings in military HIV-infected patients, current and historical psychiatric diagnostic data collected in 95 randomly selected military HIV-infected subjects were related to data from the Perceived Social Support Scale for Friends (PSS-Fri) and Family (PSS-Fam) (56). This measure has both high test-retest reliability and internal consistency. The HDRS, HARS, and Michigan Alcoholism Screening Test (57) were also administered to these subjects.

The Chi-Square Test was used to analyze relationships among categorical data, except in situations where the number in one or more statistical cells was less than five. In those instances, Fisher's Exact Test was used. When means were compared, two-tailed Student's t-Tests and analysis of variance (ANOVA) tests were used. Correlative relationships were assessed with Pearson's product-moment correlation coefficient. We chose the conservative level of $p = .01$ to determine statistical significance for psychiatric disorder comparisons between the TSBS subjects and ECA subjects. For other data analyses $p = .05$ is our designated level of statistical significance.

III. RESULTS

A U.S. Air Force sample of Wave 1 TSBS subjects consisting of 442 men who were, or had been, on active duty at the time they were notified by the military of their seropositive status has been considered separately because of unique demographic and occupational specialty characteristics relevant to U.S. Air Force missions

TABLE 1. GLOBAL ASSESSMENT OF FUNCTIONING: MEAN GROUP VALUES

GAF Variable	N	Mean	SD
LOWEST PAST MONTH	415	73	11
HIGHEST PAST YEAR	414	86	7
LOWEST SINCE HIV+	414	63	12
HIGHEST SINCE HIV+	413	86	6
CURRENT	261	79	11

and to Aerospace Medicine. Racial/ ethnic composition of this group is: White 56.1%, African-American 34.6%, White Hispanic 7%, Black Hispanic 0.5%, Asian/Pacific Islander 0.7%, other 0.9%. 86.7% of the group is employed. All patients had at least 12 years of formal schooling; an additional 28.1% reported between 13 and 15 years of education, 5.7% 16 years, and 5.0% more than 16 years.

Current rank or rank at time of medical retirement was skewed toward the middle enlisted ranks: 4.1% were E-3 or lower, 39.1% were E-4, 32.1% were E-5, 10% were E-6, and 14.5% were E-7 or higher, including 10.2% who were officers. The majority of patients had never been married (55.9%); 19.5% reported they were engaged or married, 8.8% were separated from their spouse, 15.4% were divorced, and 0.2% widowed. Forty-nine patients were in the 18-24 age range (11%), and 393 (89%) in the 25-44 age range. A large majority of Air Force study subjects were in early asymptomatic stages of infection (WR 1-2). Staging data are available for 394 (89%) of the subjects: WR stage 1 = 33.8% (N=133), WR 2= 42.4% (N=167), WR 3= 6.6% (N=26), WR 4= 3.3% (N=13), WR 5= 10.4% (N=41), and WR 6= 3.5% (N=14).

Global Assessment of Functioning (GAF) Scale (46) scores are indicators of general occupational and social functioning. The maximum GAF scale score is 90 for individuals with no social, psychological, or occupational dysfunction. Lower scores indicate higher degrees of dysfunction. For example, a GAF score of 65 indicates fair general occupational/social function with specific areas of difficulty, such as occasionally missing work. A GAF score of 45 indicates serious occupational/social disturbance that incorporates such behavioral anchors as serious suicidal ideation, inability to keep a job, and lack of friends. A GAF score of 10 indicates an acute and persistent danger of recurrent destructive behaviors such as serious suicide attempts and recurrent violence. Table 1 summarizes group mean GAF scores and standard deviations.

Standard anxiety (HARS) and depression (HDRS) rating scale scores, continuous measures of psychological distress and psychiatric status, obtained during the clinician-administered SIGH-AD (15), are detailed in Tables 2 and 3 by age group. Mean values and standard deviations are listed. Lower scale score values represent lower levels of reported and observable anxiety and depressive signs and symptoms. Scores $\geq$ 16 generally represent clinically significant levels of anxiety or depression, although we have used a threshold of $\geq$ 11 to indicate the possibility of subclinical, but relevant, levels of distress.

Current psychiatric disorders are listed in Table 4. Where comparable, ECA diagnostic point prevalences for males 18-44 years old are included. The ECA community surveys, using a related standard psychiatric diagnostic assessment instrument, the Diagnosic Interview Schedule (58), did not evaluate for all diagnoses assessed by the SCID. When ECA data are not included in Table 4, insufficient comparability for diagnostic categories or methodologies exists between the two instruments. "Major" psychiatric disorders exclude simple phobias, adjustment disorders, sexual dysfunctions, personality disorders, and all potential foci for psychiatric treatment not attributable to a mental disorder (e.g. normal grief). The most frequently diagnosed major psychiatric diagnoses are major depression, simple phobia, social phobia, and alcohol use disorder.

More than one-fifth (21.7%) of the Air Force sample was diagnosed with a personality disorder, defined as lifelong social and occupational impairment due to lifelong combinations of stable maladaptive personality characteristics. These disorders by diagnostic definition existed in almost every case prior to notification of HIV seroconversion. The most prevalent of the personality disorders were obsessive-compulsive (5.9%), paranoid (5.0%),

TABLE 2. HAMILTON ANXIETY RATING SCALE SCORES

SCORE (range)	18-24 (N=44)	25-44 (N=370)
0-5	59.1%	69.7%
6-10	20.4%	17.3%
11-15	4.6%	7.1%
>15	15.9%	5.9%
MEAN SCORE:	6.2	4.8
STAND. DEV.:	7.4	5.8

TABLE 3. HAMILTON DEPRESSION SCALE SCORES

SCORE (range)	18-24 (N=36)	25-44 (N=310)
0-5	52.8%	64.2%
6-10	19.4%	20.6%
11-15	11.1%	7.5%
>15	16.7%	7.7%
MEAN SCORE:	8.4	5.4
STAND. DEV.:	8.3	5.6

avoidant (5%), Not Otherwise Specified (4.8%), and borderline (2.9%).

Table 5 follows the same conventions as Table 4 in listing the lifetime prevalences of psychiatric disorders for the Air Force seropositive sample and the ECA sample (54). By definition, "lifetime" includes those disorders present currently as well as any SCID-verified prior psychiatric disorders. The majority of noncurrent lifetime diagnoses antedated HIV seroconversion, and in many cases, antedated military service as well. Statistically significant differences between the seropositive Air Force sample and the ECA sample are in the direction of greater prevalences in the Air Force HIV-infected sample, with the exception of simple phobias and agoraphobia.

To assess potential associations between perception of social support and neuropsychiatric status, 95 randomly selected subjects in a TSBS pilot study were divided into three social support perception categories according to scores on the PSS-Fam and PSS-Fri measures. Subjects were defined as having "poor" perceived social support (PSS) if PSS scores were below the median for both PSS-Family (PSS-Fam) (16.2, range 0-20) and PSS-Friends (PSS-Fri) (16.4, range 0-20). Perception of "good" social support was defined as both scores being above the median. "Mixed" perceived social support was defined as one score above and one score below the median.

When overall (family plus friends) perceived social support is divided into good, mixed, and poor categories (Table 6), a number of statistically significant associations emerge. Perception of poor overall social support is significantly related to greater frequency of Axis I diagnoses, HARS scores $\geq$ 11, and higher frequency of suicidal ideation. Poor or mixed overall perceived social support was significantly related to major Axis I diagnoses, Alcohol Abuse/Dependence, HARS score $\geq$ 11, multiple psychosocial stressors, and decreased libido.

Suicidality, both past and present, is an indicator of psychosocial distress and duress. This parameter was standardly assessed during each subject interview with careful attention to distinguishing between ideation, plan, intent, and attempt (Table 7). The "total past" suicidality composite represents the proportion of patients who expressed either suicidal ideation or reported at least one attempt or gesture prior to learning of their seropositive status. The "total suicidality since HIV+" composite

TABLE 4 CURRENT PSYCHIATRIC DISORDERS: USAF HIV-SEROPOSITIVE MEN VERSUS ECA COMMUNITY SAMPLE

DIAGNOSIS	HIV-SEROPOSITIVE			ECA SAMPLE		
	18-24 (N=49)	25-44 (N=393)	18-44 (N=442)	18-24 (N=923)	25-44 (N=1664)	18-44 (N=2587)
ANY AXIS I DX	42.9	38.2	38.7	---	---	---
MAJOR AXIS I DX	26.5	17.8	18.8	---	---	---
AFFECTIVE D/O	16.3**	9.2**	10.1**	3.4	4.5	4.2
MAJOR DEPRESSION	12.2**	5.6**	6.3**	1.5	2.2	2.0
DYSTHYMIA	2.0	2.5	2.5	2.2	2.8	2.6
BIPOLAR D/O	2.0	1.0	1.1	0.4	0.5	0.5
ANXIETY D/O	12.2	11.7**	12.9	4.9	4.7	4.8
SIMPLE PHOBIAS*	6.1	3.8**	4.1	3.6	3.5	3.5
SOCIAL PHOBIA	4.1	3.6	3.6	---	---	---
AGORAPHOBIA	0.0	0.3	0.2	---	---	---
PANIC DISORDER	0.0	1.5	1.4	0.4	0.3	0.3
GENERALIZED ANXIETY D/O	0.0	2.5	2.3	---	---	---
OBSESSIVE-COMPULSIVE D/O	2.0	1.3	1.4	1.7	1.2	1.4
SUBSTANCE USE D/O, ALCOHOL	10.2	2.8	3.6	6.0	6.2	6.1
SUBSTANCE USE D/O, NONALCOHOL	4.1	1.3	1.6	4.8	2.3	3.2
ADJUSTMENT D/O	2.0	3.8	3.6	---	---	---
SEXUAL DYSFUNC	14.3	22.7	21.7	---	---	---
ANY ORGANIC D/O	2.0	1.0	1.1	---	---	---

* FOR ECA DATA, ALL PHOBIAS ARE COMBINED
**SIGNIFICANTLY HIGHER PROPORTION THAN ECA SAMPLE, P<.01

TABLE 5 LIFETIME PSYCHIATRIC DISORDERS: USAF HIV-SEROPOSITIVE MEN VERSUS ECA COMMUNITY SAMPLE

DIAGNOSIS	HIV-SEROPOSITIVE			ECA SAMPLE		
	18-24 (N=49)	25-44 (N=393)	18-44 (N=442)	18-24 (N=1397)	25-44 (N=3722)	18-44 (N=5119)
ANY AXIS I DX	71.4	69.5	69.7	---	---	---
MAJOR AXIS I DX	51.0	52.2	52.0	---	---	---
AFFECTIVE D/O[1]	28.6*	26.5*	26.9*	6.4	6.6	6.6
MAJOR DEPRESSION	24.5*	22.1*	22.4*	5.3	8.7	7.8
DYSTHYMIA	2.0	2.5	2.5	2.1	3.8	3.3
BIPOLAR D/O	4.1	1.8	2.0	1.3	1.6	1.5
ANXIETY D/O	14.3	14.5	14.5	---	---	---
SIMPLE PHOBIAS	6.1	4.1	4.3	11.0	12.4#	12.0#
SOCIAL PHOBIA	4.1	3.8	3.9	---	---	---
AGORAPHOBIA	0.0	0.3	0.2	5.4#	6.3#	6.1#
PANIC DISORDER	2.0	2.5	2.5	0.9	2.2	1.9
GENERALIZED ANXIETY D/O	0.0	2.5	2.3	---	---	---
OBSESSIVE-COMPULSIVE D/O	2.0	1.3	1.4	2.8	3.4	3.2
SUBSTANCE USE D/O, ALCOHOL	30.6*	29.0*	29.2*	13.4	17.7	16.5
SUBSTANCE USE D/O, NONALCOHOL	18.4	19.6*	19.5	13.3	8.1	9.6
ADJUSTMENT D/O	18.4	19.6	19.5	---	---	---
SEXUAL DYSFUNC	14.3	23.9	22.9	---	---	---
ANY ORGANIC D/O	4.1	1.5	1.8	---	---	---

1 AGE GROUPS ARE 18-29 AND 30-44 IN ECA SAMPLE
SIGNIFICANTLY HIGHER PROPORTION THAN USAF SEROPOSITIVES, P<.01
* SIGNIFICANTLY HIGHER PROPORTION THAN ECA SAMPLE, P< 01

TABLE 6. IMPACT OF PERCEPTION OF POOR, MIXED, AND GOOD SOCIAL SUPPORT ON PSYCHIATRIC STATUS

Category	Poor (N=27)	Mixed (N=40)	Good (N=28)	p1^=	p2^=	p3^=	p4^=
Any Axis 1 Disorder	18(66.7%)	22(55.0%)	11(39.3%)	.242	.152	.038*	.122
Any Axis 2 Disorder	12(44.4%)	10(25.0%)	7(25.0%)	.081	.615	.109	.178
Major Axis 1 D/O	12(44.4%)	12(30.0%)	0 (0.0%)	.081	.001*	.001*	.001*
Major Depression	4(14.8%)	1 (2.5%)	0 (0.0%)	.081	.588	.050*	.029*
Adjustment D/O	5(18.5%)	9(22.5%)	4(14.3%)	.470	.300	.476	.695
Alcohol Abuse	3(11.1%)	6(15.0%)	0 (0.0%)	.471	.035*	.111	.109
Sexual Disorder	1 (3.7%)	8(20.0%)	3(10.7%)	.055	.249	.305	.751
Simple Phobia	6(22.2%)	6(15.0%)	5(17.9%)	.330	.502	.473	.372
Psy Fac/Phy Cond.	2 (7.4%)	2 (5.0%)	0 (0.0%)	.533	.342	.236	.219
Organic Ment. D/O	3(11.1%)	3 (7.5%)	0 (0.0%)	.462	.197	.132	.219
HARS Score > 11	7(25.9%)	8(20.0%)	0 (0.0%)	.389	.010*	.044*	.019*
HARS Score > 15	4(14.8%)	2 (5.0%)	0 (0.0%)	.172	.342	.050*	.071
HDRS Score > 11	8(29.6%)	7(17.5%)	3(10.7%)	.192	.339	.078	.192
HDRS Score > 15	3(11.1%)	1 (2.5%)	2 (7.1%)	.176	.380	.482	.356
MAST > 4	6(22.2%)	9(22.5%)	3(10.7%)	.611	.177	.216	.416
Suicidal Ideation	9(33.3%)	8(20.0%)	3(10.7%)	.172	.248	.043*	.117
Suicide Attempt	2 (7.4%)	0 (0.0%)	0 (0.0%)	.159	.999	.236	.376
3 or more Stressors	12(44.4%)	24(60.0%)	9(32.1%)	.158	.021*	.254	.072
Wants out of A.F.	9(33.3%)	18(45.0%)	8(28.6%)	.242	.131	.464	.348
Decreased Libido	8(29.6%)	19(47.5%)	5(17.9%)	.113	.011*	.149	.034*
Age > 30	11(40.7%)	15(37.5%)	7(25.0%)	.494	.207	.170	.420
Fath Died/Childhood	3(11.1%)	3 (7.5%)	5(17.9%)	.462	.178	.374	.420
Moth Died/Childhood	1 (3.7%)	4(10.0%)	2 (7.1%)	.374	.519	.540	.625

^= p1--Significance level of poor vs mixed social support perception
p2--Significance level of mixed vs good social support perception
p3--Significance level of poor vs good social support perception
p4--Significance level of poor vs mixed vs good support perception

*=Statistically significant at the p<.05 level.

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TABLE 7. SUICIDALITY IN HIV-SEROPOSITIVE USAF MEN

PARAMETER	18-24 (N=49)	25-44 (N=393)	18-44 (N=442)
NONE (LIFETIME)	75.5%	68.2%	69.0%
PAST IDEATION	2.0	12.0	10.9
PAST ATTEMPT	0.0	2.5	2.3
CURRENT IDEATION OR PLAN	0.0	0.8	0.7
CURRENT INTENT	0.0	0.0	0.0
IDEATION SINCE HIV+	10.2	15.5	14.9
ATTEMPT SINCE HIV+	10.2	3.1	3.9
IMMINENT RISK	0.0	0.3	0.2
TOTAL PAST:	2.0%	14.5%	13.2%
TOTAL SUICIDALITY SINCE HIV+:	18.4%	16.8%	17.0%

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TABLE 8. METHODS USED IN SUICIDE ATTEMPTS (N=24)*

METHOD	NUMBER	PERCENTAGE
Medication Overdose	12	52.1%
Cutting Wrists	4	17.4%
Firearms	2	8.7%
Jumping	2	8.7%
Attempted Drowning	1	4.3%
Carbon Monoxide	1	4.3%
Attempted Hanging	1	4.3%
Set Self on Fire	1	4.3%

* = One person used two methods

TABLE 9. COMPARISONS BETWEEN HIV-SEROPOSITIVE SUICIDE ATTEMPTERS AND NON-ATTEMPTERS

PARAMETER	(N=15) ATTEMPTERS	(N=15) NON-ATTEMPTERS	p=
PSYCHIATRIC DIAGNOSIS:			
Adjustment disorder	10	2	0.003 *
Alcohol abuse	8	2	0.02 *
Personality disorder	8	2	0.02 *
Major depression	5	1	0.08
Panic disorder	2	0	0.24
Organic mental disorder	1	1	1.00
PAST HISTORY:			
Major Depression	4	0	0.05 *
Alcohol Abuse	6	2	0.11
Suicide Attempt	3	0	0.11
FAMILY HISTORY:			
Completed Suicide	2	0	0.24
Major Depression	3	1	0.30
Alcohol Abuse	5	8	0.23
STRESSORS:			
HIV-Related Interpersonal Problems	10	4	0.03 *
HIV-Related Occupational Problems	9	3	0.03 *
> 2 Nonhealth Stressors	12	9	0.21
POOR SOC. SUPPORT PERCEP.(6):	12	3	0.001 *

* = significant at $p \leq 0.05$

represents the proportion of patients who expressed suicidal ideation, intent, or attempt since notification of seropositive status.

In a study preliminary to the TSBS a chart review of all Air Force HIV-infected personnel was performed to identify individuals who had made suicide attempts. Fifteen individuals were discovered whose medical records confirmed suicide attempts of sufficient seriousness to warrant medical or psychiatric intervention. There were 23 suicide attempts recorded in these individuals. Twenty-four methods were used in the 23 attempts. Table 8 summarizes those methods. Medication overdose was by far the most common attempt method, followed by cutting wrists, firearms, and jumping. Violent suicide attempt means were not uncommon in this group. Eight of the attempters were known to have used alcohol during at least one attempt.

We compared medical and psychiatric findings in the fifteen suicide attempters with those of fifteen HIV-infected non-attempters matched by sex, race, age, and for which annual medical assessment each was present. There were no significant differences between the two groups either in previously uncontrolled demographic factors or in any medical status measures.

In contrast, comparisons of psychiatric diagnoses (Table 9) between military HIV-seropositive suicide attempters and non-attempters reveal several statistically significant differences. Alcohol abuse, adjustment disorder, and personality disorder were each significantly more common ($p = .02$) in the attempters. Though major depression was present more frequently in attempters (33.3%) than in non-attempters (6.7%), the difference was not statistically significant at the $P \leq .05$ level. Past history of major depression was significantly more common in attempters ($p < .05$). Past history of alcohol abuse and history of one or more suicide attempts prior to knowledge of HIV-seropositivity were also more common in attempters, but these differences failed to reach defined statistical significance.

There are notable findings in the number and nature of psychosocial stressors in the suicidal and nonsuicidal HIV-infected groups (Table 9). Occupational and interpersonal problems felt by the examiners to be directly related to knowledge of HIV infection were significantly more frequent in the suicide attempters ($p = .03$). In contrast, there was no statistically significant difference in the absolute number of reported psychosocial stressors not felt to be specifically HIV-related. Examples of HIV-related stressors include abandonment by important others and job change due to being HIV-seropositive. Examples of stressors defined as unrelated to HIV infection are routine changes of military duty station and marital problems predating knowledge of HIV infection. Perception of poor social supports (6) was present much more commonly ($p = 0.003$) in attempters (12 or 80.0%) than in non-attempters (3 or 20.0%) ($p = .003$).

IV. DISCUSSION

Data presented in this paper highlight the complexity of determining cause-effect relationships between military occupational performance, neuropsychiatric status, and psychosocial status. Compared to disabling medical diagnoses, the prevalence of major psychiatric diagnoses in active duty and retired military personnel in early stages of HIV Disease is relatively high. The military HIV research and clinical programs have practiced aggressive primary, secondary, and tertiary prevention with regard to these conditions in an attempt to minimize their potential impact on military duty. Periodic psychiatric screening, routine clinical consultation, aggressive mental health treatment, and workplace interventions are all utilized to prevent loss of HIV-infected military personnel from the military workplace due to neuropsychiatric morbidity.

One measure of success of this approach is the low discharge rate for psychiatric diagnoses through the military medical system: only about 1% of HIV-infected Air Force personnel are discharged for psychiatric reasons prior to medical discharge. Nevertheless, data from Behavioral Medicine HIV Research Program studies to date suggest substantial remaining areas of morbidity which are potentially amenable to more specific intervention.

Global Assessment of Functioning scores (Table 1) are the most direct indicators in this study of general occupational and social functioning. Subjects' scores indicate a wide range of overall levels of functioning since notification of serostatus. Twenty-three points on the GAF scale separate the group mean highest and lowest GAF scores since notification. A number of subjects have scored in the highly dysfunctional range (GAF score below 55). The mean lowest GAF score since notification of HIV status ($GAF = 63$) indicates that at least once after notification of seropositivity the average HIV-infected serviceman experiences at least transient occupational and social difficulty, such as missing work. Fortunately, however, overall group mean current GAF scores indicate at least adequate occupational and social functioning for the majority of subjects most of the time.

A sizable subset of seropositive military medical beneficiaries are found to have depression and anxiety scale scores generally considered to be in the clinical range (≥ 16) and a larger group score in the subclinical, but distressed (≥ 11), range (Tables 2 and 3). These depression and anxiety scores, at levels consistent with the potential need for mental health care intervention, were found in 13.8% ($N = 57$ of 414 completed standard interviews) for anxiety and 16.5% ($N = 57$ of 310) for depressive symptoms.

The ability in the future to maintain and improve military workplace contributions by HIV-infected military personnel depends on defining areas for potentially effective primary, secondary, and tertiary preventive interventions. Only comprehensive, highly sophisticated scientific research can identify such areas. Inappropriate cause-effect attributions may lead to invalid assumptions underlying otherwise well-intentioned interventions. For example, the hypothetical intervention of immunotherapeutic agents for CNS psychomotor slowness/apathetic mood syndrome will fail if the target mood syndrome is due to either psychosocial factors inherent in the individual's occupational and social environment or to pre-existing psychiatric predisposition to primary major depression instead of an HIV-related CNS disorder.

Interventions derived from TSBS research data hold the promise of identifying areas of specific military concern (e.g. function in cognitively demanding jobs such as navigation and flying) and developing means to effect maximal longevity and quality of contributions made by HIV-infected servicemen and servicewomen. These interventions are dependent, however, on the comprehensive study of all inter-related domains of potential determinants of neuropsychiatric and psychosocial morbidity.

A number of biopsychosocial studies are currently being conducted by the Military Medical Consortium for Applied Retroviral Research's Behavioral Medicine HIV Research Program. Their goals are to study neuropsychiatric and psychosocial morbidity and associated factors in a large sample of HIV-seropositive military personnel over time in an attempt to define the interrelationships among neurologic, psychiatric, neuropsychological, psychosocial, genetic, developmental, immunological, medical, and behavioral determinants of that morbidity. Identification of these determinants will lead to interventions with maximum chance of being effective.

A number of specific findings relevant to primary, secondary, and tertiary prevention are already suggested by study findings to date. For example, the defined rate of lifetime psychiatric disorders indicates that only a portion of the current prevalence of neuropsychiatric morbidity is a function of knowledge of seropositivity and its proximate consequences. Pre-existing psychiatric conditions account for much of the neuropsychiatric morbidity seen in HIV-infected military medical beneficiaries.

Air Force HIV-seropositive men have significantly elevated lifetime prevalences of alcohol use disorders at all ages and elevated lifetime rates of other psychoactive substance use disorders in those currently 25-44 years of age when compared to civilian community samples (ECA data). Interestingly, HIV-seropositive military personnel do not have higher *current* substance use disorder rates than the ECA sample. The apparent decline in relative psychoactive substance use disorder frequency may be an artifact of underreporting, although civilian HIV studies have also demonstrated diminished current reported rates of substance abuse (59). It is our anecdotal observation that many patients believe that discontinuing or decreasing use of alcohol or other drugs will help to preserve immune function and forestall the onset of AIDS (42).

The occurrence of suicidality (ideation, attempts, completions) in various HIV-infected populations has received wide attention. We previously reported that HIV-seropositive Air Force suicide attempters and had a rate of known completed suicides between 179 and 255 per 100,000 person-years, or 15-21 times that of the general population (27,60). These findings are consistent with suicide rates in HIV-infected civilian populations (24-26). Suicide attempt risk factors for military HIV-infected males include social isolation, presence of HIV-related psychosocial stressors, alcohol use disorder, adjustment disorder, personality disorder, and history of depression. A significant proportion of suicidal ideation and behavior in HIV-infected military personnel (Table 9) cannot be explained for by historical patterns of reacting to stressors with suicidal thinking and behavior. The frequency of first-time serious suicidal ideation and behaviors after notification of seropositive status suggests the need for new primary, secondary, and tertiary preventive interventions in this area.

An important limitation of data discussed in this paper is the use of age and gender matched ECA community-derived comparison data. The ECA studies occurred in civilian settings and used a different structured interview. A more valid approach, currently planned for implementation in the TSBS, is to study a comparison group of active duty seronegative personnel.

V. CONCLUSION

Cause and effect relationships between biopsychosocial predisposition, social support, level of current stress, HIV effects in the central nervous system, psychiatric status, and occupational/social function are complex. For example, perception of poor social support may be an etiologic factor in the precipitation of a major depressive episode or may be an artifactual perception produced by a depressed individual's affective and cognitive view of the world. The mood syndrome, in turn, may be a reaction to the news of being HIV-seropositive, a product of HIV-related effects in subcortical and limbic areas of the brain, and/or a pre-existing genetically-determined primary psychiatric disorder.

This large multicenter, comprehensive, tri-service research study has the unique ability to study a large group of HIV-infected individuals at all stages of disease over time who have known approximate times of seroconversion. The extraordinarily high enrollment rates (>95% at WHMC) ensure a representative population from which valid conclusions can be drawn. Identification of military-relevant consequences of HIV infection will lead to interventions designed to minimize the impact of the HIV epidemic on military readiness and function. Interventions suggested by data being collected by the TSBS, because they will be based

on data that simultaneously take into account all domains of potential significant causal contribution, will have a maximal chance of being effective.

Neuropsychiatric and psychosocial consequences of HIV infection are among the most frequently occurring medical complications seen in early disease stages and among the most likely to have an adverse impact on military readiness and function. Current primary, secondary, and tertiary preventive efforts in these areas have been partially successful in keeping as many HIV-infected military personnel functioning maximally for the longest possible time on active duty. Specific military comprehensive biopsychosocial study findings gathered over the next two years will lead to interventions designed to improve those efforts.

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DISCUSSION SESSION II

HIV INFECTION (Papers 12 to 16)

S. PORCU'(IT): A question to dr. Rundell. Don't you think that the neurophysiological approach may give some help in the information regarding detection of early involvement of CNS by HIV infection? In particular, the quantitative EEG, the sensory input investigated by sensory evoked potentials, the cognitive aspects investigated by the event-related potentials. I believe that in many processes involving CNS diffusely, we can obtain, before the changes in the performance, subtle changes in many processes related to information processing of the brain, like during hypoxia, hypoglicemia, ageing brain, and other models of diseases of CNS, such as multiple sclerosis.

J.R. RUNDELL (USA): That's a very good question. The neurophysiological approach can not be fully predictive of early CNS involvement during HIV infection. Some events may be partially predictive of cognitive decline, but the actual role of primary HIV infection alone is difficult to ascertain, because many interfering situations, such as depression, alcohol intake, etc... may have an important role.

G.SANTUCCI (FR): En tant que Président du Panel Médical de l'AGARD. Je voudrais adresser 2 questions au Dr RUNDELL. La première : j'ai été très impressionné par ses résultats sur les temps de réactions et je voudrais savoir s'il avait observé un comportement clinique inhabituel.
La deuxième : en ce qui concerne les études prospectives, je voudrais savoir s'il a envisagé d'utiliser la batterie mise au point par l'AGARD, qu'on appelle la S.T.R.E.S. batterie. Car elle a été destinée à être utilisée par plusieurs équipes, qui ont étudié l'effet de l'alcool, de l'hypoxie etc..... Elle a été faite pour faciliter les échanges entre chercheurs et je pense qu'ils tireraient profit à utiliser cette batterie standardisée.

J.R. RUNDELL (USA): Reaction time is a measure that has been used for some time, but it has little clinical value, because all you are finding out is how long it takes someone to push a button that makes the light turn off. Translating that to a cockpit simulation would be difficult. So, the interpretation of reaction time data must be put into proper context. If you are a clerk or a cook, a tenth of a second isn't going to make much difference, but in a cockpit, a situation like pulling 9 Gs might make a difference. It may depend on what else is going on in the cockpit at the same time. It may be a question difficult to answer, so that measuring reaction time itself has limited value. I really decided to choose to discuss reaction time today because it's the measure that is the most significant out of all the ones we studied. We have 9 other measures that our seropositives did worse on than the seronegatives, but the quantitative difference between the performance was not even as great as a tenth of a second difference.
The answer to the second question is that individuals from AGARD had been working for several years on developing a performance assessment battery for use in military settings. They really set out to and considered what tasks are important to different military specialties, which is everything from driving a tank, to operating a sonar or acting as an air traffic controller. They did it with a number of occupational specialties and recommendations were made to what would be important measures to study for particular military occupational tasks. The problem with that approach has been that we also have a lot of clinical neuropsychological measures that we have a lot of experience with, and we have a lot of experienced people who put together what they think is a list of important military occupational tasks but those too haven't been married up yet. The first attempts to do that, actually translating this into the military occupation environment have been taking place and there is indeed a battery. This battery has been turned into something called "cog-screen", which is being used in studies of civilian aviators and like I said earlier it's already been shown to predict whether or not a military aviator will end up being a bomber pilot or a fighter pilot. So, this is the battery, AGARD people have been working on and is just beginning to be translated in the actual military workplace. Dr. Hain, do you have any other comments to say about that battery?

R.E. HAIN (USA): I am the Director of Acrospace Medicine for the US Navy. I have seen the battery and it's very sophisticated. It is being used now by FAA for a variety of different things in the USA and for commercial pilots that don't quite make it when transitioning from a 737 to a 747, they get to try out this battery. So we attempted to find out what the problem is and interestingly enough it is often related to alcohol. I would like to make a couple of comments without making a whole speech here. The issue of a tenth of a second. I particulary feel that's important. And put it into a context on an American carrier where we fire planes off the bowl, a pilot has from the standing start to the end of the ramp 1.65 to 1.7 seconds to decide if he likes that catch up. If he doesn't like it, he has to get out of the plane before the nose starts going down, so he has 1.65 to 1.7 seconds to make that decision. Once the nose starts going out, the physics of the situation are such that he is out of the envelope and probably won't survive the ejection. So, in that context, I rather parochially feel that a tenth of a second is important.
We also discussed other issues that might impact, such as alcohol and drugs will certainly delay reaction times and, at least in the US, we have record books filled of aircraft accidents of people who took too many anti-hystamines and drank a little too much.
So I think that's all important.

J.R. RUNDELL (USA): We are using these batteries by the way in our study, Sir. I hope that it answers your question.

G. DE HEYN (BE): I have a question for dr. Wolfe. I am very interested in your results, particularly in the immunological response of smokers and drinkers. But you know that many smokers drink and many drinkers smoke. And I didn't see the results of this category.

W. WOLFE (USA): We haven't done three-way correlations as yet, but we'll proceed to do that. And if you give us your address, I will make it available to you. We haven't broken those categories out into that dimension yet, but we intend to do so.

F. AIUTI (IT): I want to make a comment on the progression from HIV infection stage 2 to stage 3 or stage 4 to full-blown AIDS related to a multicenter Italian study coordinated by Rezza from the Istituto Superiore di Sanita'. They found that, after calculating all the social and individual cofactors, the progression from stage 2 to 3 or from stage 4A to 4C1 and 4C2 is strictly related to age, youngsters progressing less than older people. So a 25 year old man is progressing more slowly than a 35 or a 45 year old man. Probably, in addition to lifestyle, such as smoking and drinking, age must also be considered as an important factor

able to condition the immune functions. In fact, in the Italian study, age was even more important than behavior, such as being a drug abuser or ex drug abuser, in influencing progression to AIDS.

E. RODIG (GE): Reflecting on the past presentations, I would like to ask Col. Wolfe if, in addition to demographic and life style variables, other factors, such as psychosocial stress or psychiatric disorders like depression, have been considered for their possible influence on immune laboratory data.

W. WOLFE (USA): At this point we have not done this yet.

A. FATTOROSSI (IT): I have a question for the first 2 speakers. The question is on methodology. Because now the assessing of lymphocyte subsets by monoclonal antibodies is becoming increasingly popular and everyone around here does this. So, we know from literature data and from recently performed European trials for quality control, that there are a lot of variables that profoundly affect the results and in addition to this, let's say, "check variables", such as the time of blood collection, the way of blood collection and all things like that, which can be very easily controlled and standardized. There are other less clearly defined causes that produce very big differences in results and for instance the European trial for quality control in flow cytometry shows that the type of laboratory that performs this kind of analyses, that is a laboratory which is mainly involved in research or in clinical practice, and of course the type of instrument are very important. This, of course, does not apply to the very common CD4 lymphocytes, which luckily have a very clear distinction from the negative ones, but it is the case for the CD8 lymphocytes and of course for the CD4/CD8 ratio, not to speak about the activation antigens, that are very difficult to assess. So, I would like to have a comment on this topic and to know whether there are quality controls normally going on in these kind of laboratories.

M.J. DOLAN (USA): I think I can take that question. In the laboratory at Wilford Hall, in order to minimize some of the variables that you discussed, blood for flow cytometry is drawn at a single time every day to minimize diurnal variation. While there will be day-to-day variations within a given normal patient, that factor cannot be controlled other than by drawing repeated samples. As far as internal controls for the flow cytometry, fluorescent intensity gates are set by using monoclonal antibodies of the same isotype and the same fluorochrome as the antibody of question. So, we use an IgG1 monoclonal antibody to label CD4, for a control we use an irrelevant IgG1 myeloma protein, colored with the same fluorochrome and set the gates in such a way that the irrelevant antibody is considered background. This is probably the most reproducible and objective way of setting fluorescent intensity gates, because it does not involve any observer bias. The cell population to be examined is initially gated by forward and perpendicular light scatter and then assessed with anti-CD56 antibody to demonstrate whether the lymphocyte pool has been contaminated with natural killer cells. There are a variety of internal controls that are done, as well as performance of flow cytometry on normal subjects and periodical measurement of performance standards.

P.V. CELIO (USA): I am also from the USAF, Aeromedical Consultation Service in Brooks and my question is to dr. Rundell. The prolongation of reaction time is interesting, but it would seem that a more relevant question would be a prolongation of reaction time as the tasks are being performed repetitively. Did you look at that with time?

J.R. RUNDELL (USA): You meant time spent repetitively in one setting or repeating it every 6 months?

P.V. CELIO (USA): Oh, no, performing the test repetitively over several minutes

J.R. RUNDELL(USA): OK, we did it only once with one repetition. We had done it once and one of the slides I had showed what happened when we did that with the seropositives. We did not get any improvement, but asking to repeat the task a few minutes later, with the seronegatives we did get some improvement in their performance on the complicated version of the reaction time measure. So it would be one of the things we want to do, to have them repeat it several times if you can find out how long it takes HIV positive to actually get better at the task, or whether they stay on a straight line and get worse.

P.V. CELIO (USA): Well, we need a little more clarification. Was there a period of rest in between the first and the second trial?

J.R. RUNDELL (USA): All I know is that it was a few minutes.

P.V. CELIO (USA): OK, because what my question really wants to know is something about reaction time against a scale time, because in the cockpit you don't have a couple of minutes between tasks to rest, and if there is any fatigue factor in performing the task continuously. That's all you have to look at.

J.R. RUNDELL (USA): That's a good point. Your hypothesis would be that someone with this disorder would actually get worse with repeated attempts rather than better. That's a good point.

D.S. BURKE (USA): I want to ask another question on the same data. There is some overlap between the responses of the HIV-infected and the normal controls, that is some individuals who are HIV infected are better than average normals and some normals are worse than some HIV- infected individuals. There is overlap here and when we are making decisions about what is the functional significance, I think we have to take it into account.

J.R. RUNDELL (USA): In the figures I sampled we took out the lines that indicate the range score, but there is an overlap. There are 3-4 patients in each group who fall below or above the top line of the range limits.

W.H. STIGELMANN (USA): I just want to add one comment about the policy statement that dr. Warner made earlier, that currently all of our US seropositive aviators are grounded. That's true. But, as dr. Burke mentioned, these results are also obtained by case by case evaluation and those evaluations continue. I'm willing to speculate this time whether any of those people based on submission to a battery of tests, which should note their ability of performing at least on the ground as well as in the air, whether decisions might be reversed. At this moment it would be very unlikely, but we can not rule it out as a possibility.

J.R. RUNDELL (USA): The very first question was relevant to what was asked. Even if we found no differences between seropositive and seronegative aviators, even if the seropositive aviators did better, we still may not have a predictor and the question is how often would you have to do all these tests to make sure that you could feel safe with an aviator in the cockpit. So we need a data predictor to have faith in our measures.

J. FIRTH (UK) : Coming back to a strict practical note. If we haven't got easily administered, reliable psychological batteries to use out of the aircraft, can we trust simulators? If we can, one way forward is that if, in HIV+ aircrew, we can pick up subtle, progressive incapacitation by simulation, then the only other area we have to address is the potential for acute incapacitation. If one is prepared to accept that, then one has a theoretical basis for considering the return of aircrew to flight status in a multicrew situation, if not as single crew. Would the panel accept this a proposition?

J.R. RUNDELL (USA): That's a good question and it opens up the whole discussion to an issue wider than HIV disease. And I think one of the things dr. Burke was trying to get at, is that we should be careful in not applying double and triple standards, because HIV is often handled on an emotional basis. Sometimes we pay more attention to it than we do to other conditions. If a HIV-infected pilot was flying a plane that crashed, it would be blamed on HIV infection, no matter what the cause was. So, I think we have to be careful again in putting so much emphasis on HIV-infected people in the cockpit. Somebody somewhere is going to raise their hand and ask why we shouldn't also do the same thing with alcohol and prohibit pilots from drinking the day before they fly? So, we have to be very careful how we handle our information on HIV infected flyers and on pilots, on what they can and can't do. We need to treat them like people with any other disease. People with seizure disorders can't fly and for a good reason, because we can not predict when they are going to have a seizure. And, if that's a good reason, it's also a good reason that HIV pilots shouldn't fly. But, if we are emotionally afraid of HIV disease, that's the wrong reason why they shouldn't fly. So, we have to be careful how we put our spin on new policies. I hope that I answered your question, perhaps a partial answer.

M.D. PARKINSON (USA): I think that hopefully the next generation tests, as we move beyond the "see a light and push a button" model of response, will more closely mimic the true operational concerns we have in the cockpit. The alcohol model would suggest that we don't have that. Very recently, about one year ago, in a general article in "Aviation, Space and Environmental Medicine", 10 or 12 P3 Navy pilots were described during a test consisting of a measure of performance after intake of measured doses of alcohol. For the first time subtle decrements of performance were shown long after the traditional 12 hour "bottle to throttle" period elapsed. I think that's the more appropriate, operational, sophisticated type of testing we should be performing to evaluate flight safety.

J.R. RUNDELL (USA): Another problem we ran into when looking at simulators is that we need quantitative computer readouts. Just to see how well they are doing.

J. FIRTH (UK): To answer that: if you can use a 747/400 simulator and you have an enthusiastic hard working 24 hour day simulator skipper, then that is exactly what you can do. This is where we now have an advantage. The point about double standards is an important one. We should be using the same models in all diseases. In a way AIDS could be a God-sent opportunity because it so stirs us emotionally that this could, should stimulate us to develop assessment models for all the other neurological conditions.

R.E. SPIER (UK): I have just a very short point to add. Surely people have been using these association tests, putting in a number into a car at a given time and when presented with a random number. It's a slightly more complicated reaction test. I wouldn't let a pilot get into a plane if he couldn't do at least six or seven simple tests like that

F. AIUTI (IT): Does prof. D'Amelio want to make a comment, after this discussion, on mandatory screening?

R D'AMELIO (IT): I think that another major problem is not only the mandatory screening or not, for pilots or for all recruits, but the medicolegal behavior after a documented seropositivity. After having listened to the contribution of dr. Rundell, it's possible to think that our medicolegal behaviour should be the grounding for seropositive pilots, not only for military but also for civilian pilots. In this field, as documented during this meeting, there is no agreement among the NATO countries, including USA.

COMMUNICABLE DISEASES: A MAJOR BURDEN OF MORBIDITY AND MORTALITY

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Communicable diseases, complicated by malnutrition and other adverse socioeconomic factors, continue in the present decade to contribute greatly to the unacceptably high levels of morbidity, mortality and disability, particularly in the under-five age group, in all developing countries. It is estimated that five million deaths occur per year from diseases which can be prevented by vaccines available today, and that another five million people are being crippled, blinded or mentally retarded as a result of the same diseases. Some of the most important communicable diseases, with their mortality rate, are reported in Table 1.

Apart from the diseases listed in Table 1, other diseases such as hemorrhagic fevers and, in particular, Dengue are making a major impact in a growing number of countries (Figure 1). In Thailand this disease accounted in 1987 for the largest number of deaths. As can be seen in Figure 2, Dengue, a disease that in the past was practically limited to South-East Asia, is now spreading to other continents. Yellow fever, in spite of a very effective vaccine, represents a risk in several countries of Africa and South America (Figure 3).

Sexually transmitted diseases are everywhere on the increase with a general shift towards the teenage group. The rising incidence of the associated complications has high social and economic costs. Protozoa and helminths cause a broad spectrum of diseases of major socioeconomic importance. The number of persons at risk from these diseases is enormous, as shown in Table 2. There are still real threats of epidemics and pandemics of viral and bacterial origin. Of increasing concern are acquired microbial resistance to chemotherapeutic agents and vector resistance to chemical pesticides that impede progress in disease reduction and increase costs of control operations. Rapid urbanization and the expansion of travel and population movement and of trade in human and animal foods within and between countries have all increased the risk of introduction of diseases from one country or region to another.

Environmental management, such as the provision of a safe water supply, the disposal of refuse, waste water and excreta, the securing of adequate housing, the safeguarding of the environment from chemical pollution etc., would undoubtedly reduce the burden of communicable diseases. However, the development of these control measures is, of necessity, a slow process. In urban centres they represent a large capital investment while, in the vast peri-urban and rural areas of the third world, environmental management is a part of overall development.

Related to environment is the control of vectors transmitting disease. Chemical control has been used for many years with excellent results and will continue to be used in the future. Owing to the development of resistance to insecticides, vector control is being oriented towards integrated control; involving the use of chemical, biological and environmental measures in optimal combination that can be implemented by the community itself, again as a part of overall development.

New drugs and antibiotics for prophylaxis and therapy will, of course, continue to be developed, but many will lose their efficacy as the infecting organisms become resistant.

Vaccines are amongst the most potent means ever devised against communicable diseases and provide the greatest hope for a substantial reduction in the toll of these diseases. It is clear therefore that we should endeavour to ensure that the most effective vaccines possible be developed and made available at a price affordable by the poorest countries.

Dr Lambert will review the field of new and improved vaccines later in the Programme.

TABLE 1

Number of deaths per year for the diseases listed below:

Diseases	Number of deaths
Diarrhoeal Diseases	3-5 million
Acute Respiratory Infections	2.2 million
Malaria	1 million children in Africa
Tuberculosis	3 million
Neonatal tetanus	700,000
Hepatitis B	1-2 million

TABLE 2

Number of people affected by some parasitic diseases

Diseases	Number of people affected
Ascariasis	1 000 million
Trichuriasis	500 million
Hookworm Infection	900 million
Schistosomiasis	200 million
Onchocerciasis	18 million

Fig. 1

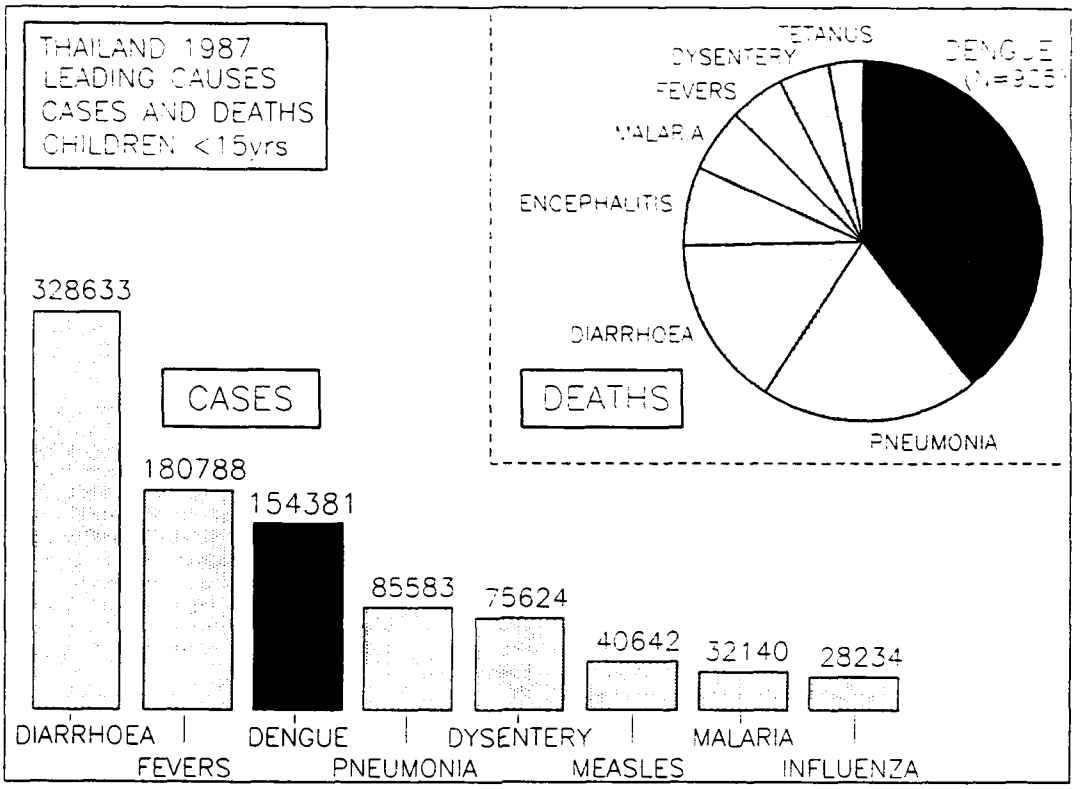
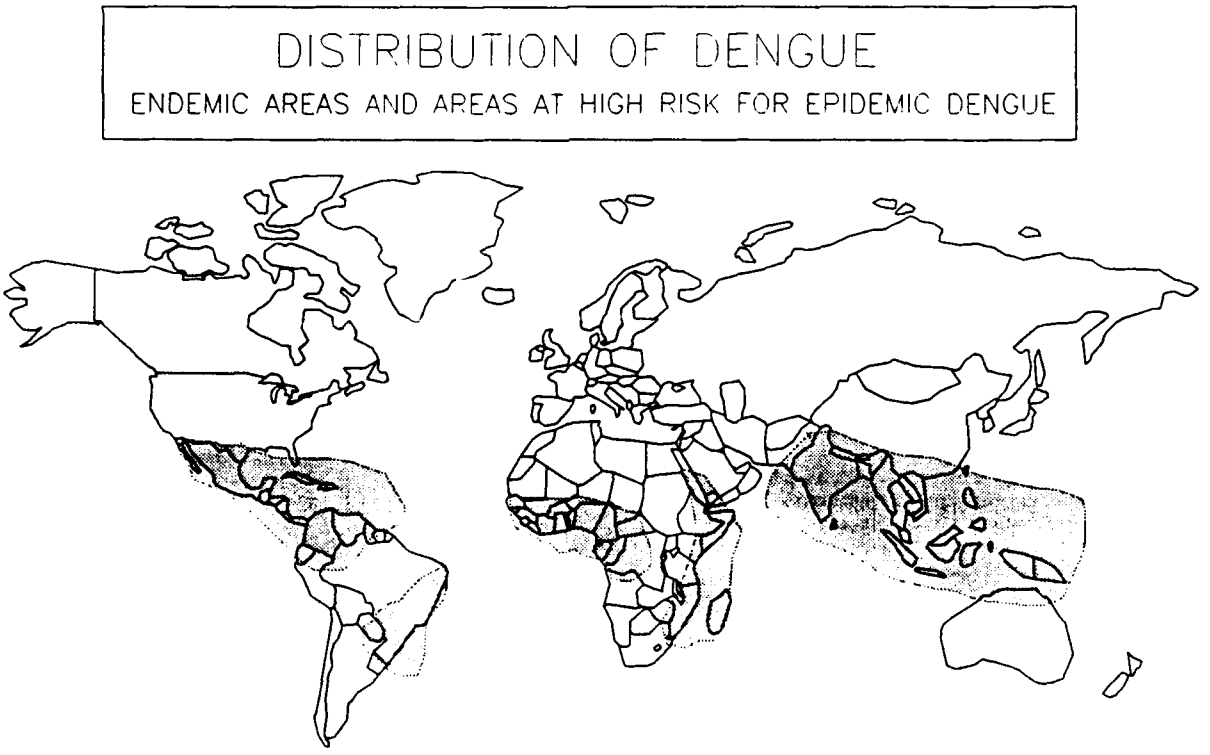


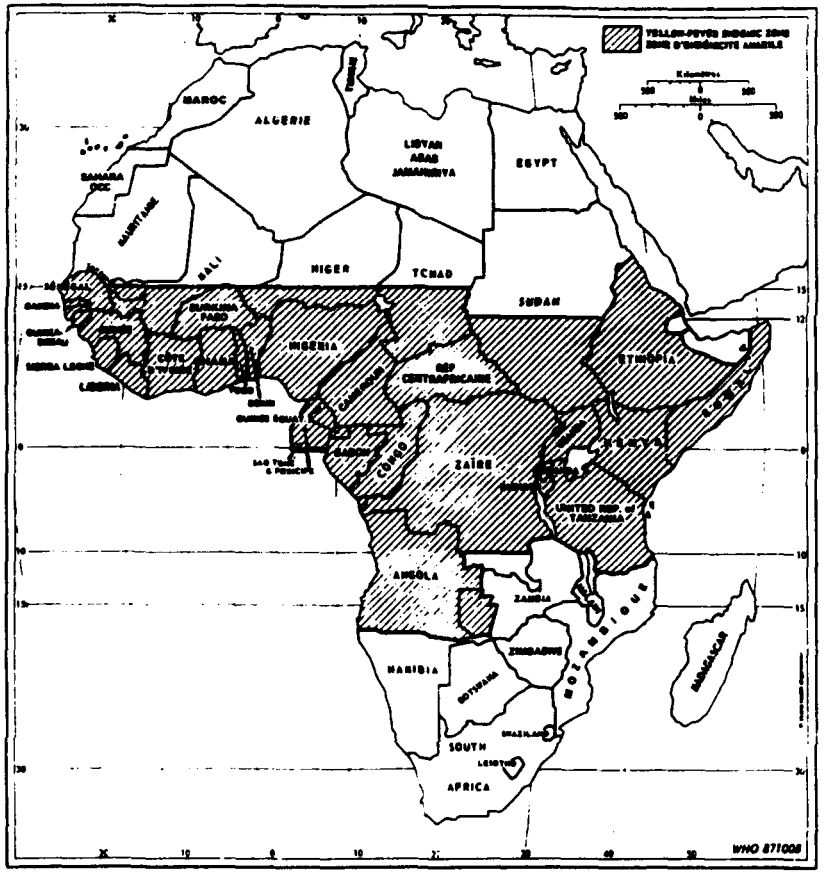
Fig. 2



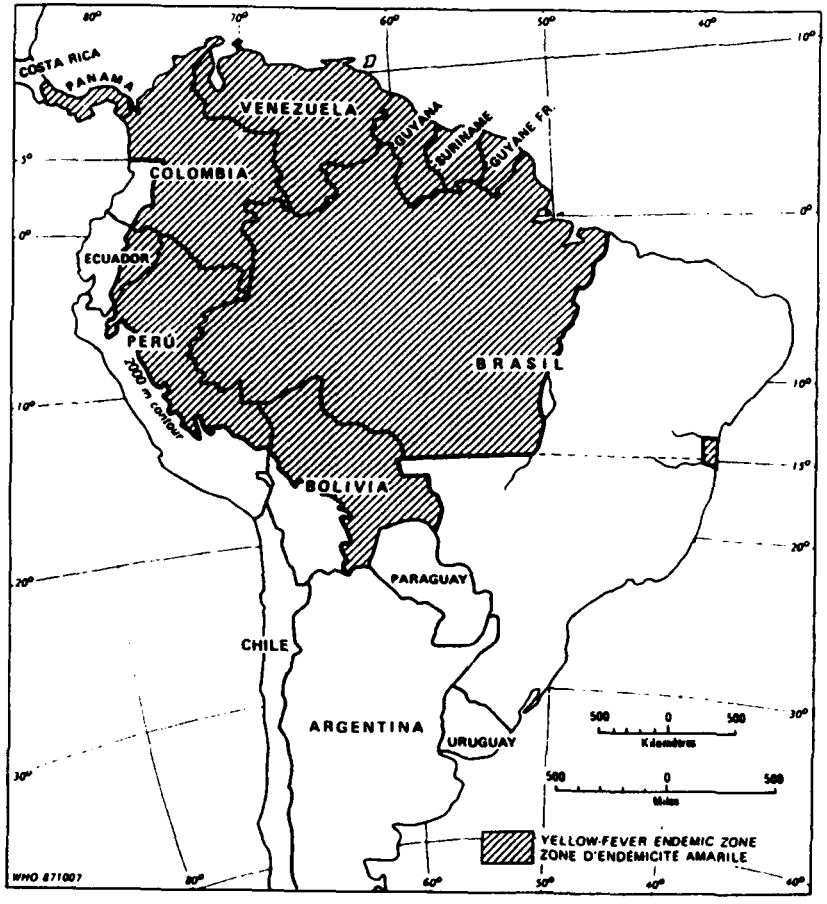
NOTE. This form of presentation does not imply official endorsement or acceptance by the WHO of the status or boundaries of described areas.

Fig.3

MAP 1. YELLOW FEVER ENDEMIC ZONE IN AFRICA



MAP 2. YELLOW FEVER ENDEMIC ZONE IN THE AMERICAS



SUSCEPTIBILITY IN USAF RECRUITS TO VACCINE PREVENTABLE DISEASES

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Due to exigencies, including military moves of the planned presenters the following presentation is made by William F. Clardy, MD, MPH. The work described was performed by Alan H. Mumm, MD, MPH; Cynthia Smith, BSN, MPH, and Daniel R. Mahon, MS. This report is, in large part, a paraphrase of their unpublished works: MEASLES, MUMPS AND RUBELLA SUSCEPTIBILITY AND SEROLOGICAL RESPONSE TO IMMUNIZATION IN U.S. AIR FORCE RECRUITS[1] and MUMPS IN THE US AIR FORCE, 1984-1988: A RETROSPECTIVE AND SEROLOGICAL STUDY TO EXAMINE IMMUNIZATION PRACTICES (with Cynthia Salinas)[2].

SUMMARY

Because of increasing incidence of mumps in a cohort of under immunized young adults and the concern about the impact of this disease on the USAF recruit population, two studies were undertaken. These studies took a retrospective look at mumps in the active duty population, a cost analysis of immunizing all or only susceptible individuals, the actual antibody response in a group of two hundred and seventy-six recruits in basic training, the demographic patterns of susceptibility and the types of previous immunization documentation. The conclusions were that the numbers of new mumps cases per year did not justify immunizing all recruits or screening for mumps antibodies and only immunizing known susceptibles.

Due to concern about the increased incidence of mumps in the United States in populations of young adults (colleges, the workplace and the military)[3,4,5,6,7,8] the authors undertook a study to evaluate the current recruit immunization practices and to examine the occurrence of mumps in the USAF.

The USAF follows the plan listed below in immunizing the recruit population at Lackland Air Force Base, San Antonio, Texas. All non-officer recruits come through this base therefore all recruits are treated identically.

Training Day 2
Serum Pregnancy test on all females
Tuberculosis Monovac skin test
Rubella and Rubeola titers drawn

Training Day 4
Monovac skin test is read
Influenza vaccine given
Meningococcal vaccine given

Training Day 8
Tetanus-diphtheria immunization given
Oral polio vaccine given
Pregnancy test results reviewed
(+'s are discharged)
Rubella-Rubeola vaccine given to susceptibles

In April, 1989 six flights of basic military trainees (BMTs) comprised of a total of 240 males and 41 females were selected to participate in the study. Four males and one female left basic training and were excluded from the final analysis of the data.

A questionnaire to collect personal, demographic, medical, and self reported vaccination status was administered to the groups on their third training day.

Serum specimens were collected from all participants on the fifth training day. These specimens were tested in duplicate for susceptibility to measles, mumps, and rubella. These specimens were first tested by the Whitaker enzyme immunoassay technique. If this test indicated inadequate protective antibody, they were also tested by an alternate (Electro-Nucleonics, Inc.) method. If either method was discordant or equivocal, the specimen was then tested by an indirect fluorescent antibody technique (Electro-Nucleonics, Inc.) and read as negative, equivocal, or positive.

On the eighth training day, recruits found to be susceptible were immunized with single antigen vaccine(s). Letters requesting immunization records were then mailed to next-of-kin.

In order to classify the antibody response as primary or secondary, follow-up serum specimens were collected on days 3, 7, 14, and 28 days after immunization. Antibody responses were classified as secondary (evidence of an anamnestic response) with a rapid rise of three-fold or more to protective levels on day 14. Other responses were classified as primary.

Ninety-five percent confidence intervals for point estimates of rates were calculated and tests of statistical significance for proportions were by the Chi-square with Yates correction.

State immunizations laws in force at the time of high school graduation were derived from Centers for Disease Control data[9].

Of the original 281 participants, 55 (19.5%) were susceptible to measles, 49 (17.9%) were susceptible to mumps, and 42 were susceptible to rubella. The susceptibilities of the original 281 participants are described in table 1 below.

Susceptibility	Number	Rate (%)
Measles only	20	7.2
Mumps only	15	5.4
Rubella only	10	3.6
Measles and Rubella	14	5.1
Rubella and Mumps	13	4.7
Measles and Mumps	16	5.8
Measles, Mumps, & Rubella	5	1.8

Table 1. Susceptibility to measles, mumps, and rubella in USAF recruits: Lackland AFB, Texas, April 1989 (n=281).

The demographic distribution and susceptibility is demonstrated in table 2 below. This table represents the data on those 276 participants who remained in the study. The racial distributions in males and females did not differ significantly. Further, all subjects were high school graduates. This table demonstrates a decrease in susceptibility with increasing age for mumps and rubella. Susceptibility was increased for whites over nonwhites and in males compared to females.

Feature/n	Measles n/%	Mumps n/%	Rubella n/%
Overall Susceptibility	54/19.6	47/17.0	40/14.5
Age (Years)/n			
<20/145	29/20.0	28/19.3	26/17.9
20-24/114	24/21.1	19/16.7	13/11.4
≥25/17	1/5.9	0/0.0	1/5.9
Race			
White/220	52/23.6	39/17.7	34/15.5
Black/34	1/2.9	1/2.9	3/8.8
Other/22	1/4.5	7/31.8	3/13.6
Sex			
Male/236	46/19.5	42/17.8	36/15.3
Female/40	8/20.0	5/12.5	4/10.0

Table 2. Susceptibility to measles, mumps, and rubella in USAF recruits by demographic category: Lackland AFB, Texas, April 1989

The documentation of prior immunization and the state of high school graduation was analyzed. The recruits from states with comprehensive immunizations laws demonstrated slightly higher susceptibility rates for measles and rubella. Overall, as is demonstrated in table three, the stringency of state immunization laws in effect at the time of high school graduation was not a good predictor of immunity.

Law Type/n	Measles n/%	Mumps n/%	Rubella n/%
Comprehensive/117	25/21.4	19/16.2	19/16.2
Partial/108	20/18.5	23/21.3	14/13.0
None/49	9/18.4	5/10.2	7/14.3
Other/2	0/0.0	0/0.0	0/0.0

*Comprehensive law for all grades; partial law does not include all children.

Table 3. Susceptibility to measles, mumps, and rubella in USAF recruits by state immunization law at graduation*: Lackland AFB, Texas, April 1989

51% of the 276 requests for immunization generated written responses. In the analysis of responders vs non responders overall susceptibility rates were lower in non respondents. In the respondent group, the type of immunization record was not related to the susceptibility rates of the three diseases. Table 4 summarizes these points.

	Measles %	Mumps %	Rubella %
Non respondents	22/135 16.3	21/135 15.6	18/135 13.3
Respondents:			
Physician/Health Dept. Records	18/76 23.7	11/56 19.6	13/69 18.8
School Record	12/51 23.5	6/40 15.0	9/48 18.8
Parental Record /Statement	1/13 7.7	7/33 21.2	0/23 0.0
Incomplete Record	1/6 16.7	2/17 11.8	0/6 0.0

Table 4. Susceptibility to measles, mumps, and rubella in USAF recruits by history of immunization: Lackland AFB, Texas, April 1989

There were no statistically significant differences for secondary antibody responses when the types of immunization records were compared. In those participants with records from a physician or health department secondary antibody responses were found in 12 of 18 (61.5%) for measles, 8 of 11 (72.7%) for mumps, and 8 of 13 (61.5%) for rubella. In those with other types of records, secondary antibody responses were found in 7 of 14 (50.0%) for measles, 5 of 15 (33.3%) for mumps, and 6 of 9 (66.7%) for rubella.

In the analysis of the antibody response of those considered susceptible, primary responses were found in 20 of 54 (37.0%) for measles, 26 of 47 (55.3%) for mumps and 17 of 40 (42.5%) for rubella. If those individuals who responded with a primary pattern are considered to be "true susceptibles" then the corresponding susceptibility rates would be 7.2% for measles, 9.4% for mumps and 6.2% for rubella.

Due to concern about the persistent measles transmission in the United States, the Advisory Committee on Immunization Practices has added a recommendation for a second dose of measles vaccine prior to school entry[10]. Concern over increased incidence rates for mumps has generated the suggestion by some authorities[5,11,12] for the immunization of young adults thought to be susceptible to mumps.

Recent studies by the US Army and US Air Force have demonstrated the negative cost benefit ratio for both immunizing all recruits and immunizing only those found to be susceptible to mumps[6,2]. In the cost analysis by Mumm, et al[2] the estimated cost of one case of mumps in an active duty person was approximately $1,700. Under a program to immunize all susceptible recruits the cost to prevent one case of mumps was estimated to be just over $10,000. The number prevented cases of mumps per year which would make such a program cost effective was estimated to be 187. They further demonstrated that the least cost effective option would be to immunize all recruits regardless of their immune status.

This study did not demonstrate a relationship between the source of immunization records and susceptibility rates. The higher rates of secondary antibody response to measles and mumps were not significantly different when compared to the rates for rubella.

The analysis of the serial antibody responses may indicate that a single enzyme immunoassay may be overly sensitive and classify some non-susceptibles as susceptible. The other option, that of increasing the specificity by setting higher cutoff values for positive, would yield the unsuitable result of increasing false negative screening tests. This situation is obviously undesirable because increased numbers of susceptibles would not be immunized.

The recent changes in civilian immunization recommendations will possibly affect the susceptibility of military recruits. This expected decrease in susceptibility would be further enhanced if the trivalent MMR (Measles-Mumps-Rubella) vaccine is given in place of single or dual antigen vaccines at time of school entry.

Finally, the aging of this under immunized cohort should also reduce the number of mumps susceptible recruit accessions over the next several years.

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AD-P006573 Words/Phrases(4 words max) that match Thesaurus Entries

TEXT	THESAURUS
ADULTS	Adults
ARMY	Army
ATTENTION	Attention
DISEASES	Diseases
IMMUNITY	Immunity
MICE	Mice
NEUTRALIZATION	Neutralization
RABBITS	Rabbits
RECRUITS	Recruits
TETANUS	Tetanus
VACCINATION	Immunization
VALUES	Value

@1@ AD-P006573 92-19
@20@ X
@23@ Adults, Army, Attention, Diseases, Immunity, Immunization, Mice, Neutralization, Rabbits, Recruits, Tetanus, Value.
@24@ u
@27@ The schedule of vaccination which is recommended worldwide for diphtheria, tetanus and other diseases, provides good immunity during childhood. However, little attention has been paid to keep an effective immunity in adults. We have collected sera from 334 recruits of the Italian Army and tested them for the presence of protective immunity against diphtheria and tetanus. In vivo neutralization assays were performed on rabbits and mice -and the values below 1/100 IU/mi were considered negative. 22.9% of the recruits were negative for diphtheria, while only 5.3% of them did not have protective immunity against tetanus.
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ABSENCE OF PROTECTIVE IMMUNITY AGAINST DIPHTHERIA IN A LARGE PROPORTION OF YOUNG ADULTS.

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SUMMARY

The schedule of vaccination which is recommended worldwide for diphtheria, tetanus and other diseases, provides good immunity during childhood. However, little attention has been paid to keep an effective immunity in adults. We have collected sera from 334 recruits of the Italian Army and tested them for the presence of protective immunity against diphtheria and tetanus. In vivo neutralization assays were performed on rabbits and mice and the values below 1/100 IU/ml were considered negative. 22.9% of the recruits were negative for diphtheria, while only 5.3% of them did not have protective immunity against tetanus.
This finding shows that a large proportion of the Italian young adults are susceptible to diphtheria, and this could be dangerous if they travel to sites were this disease is still endemic, or if they come into contact with people coming from such areas. A booster vaccination of young adults against diphtheria should enter in the common practice in order to avoid this risk. In order to reduce the side effects which are often associated with diphtheria vaccination in adults, we have developed a vaccine wich contains a highly purified non toxic mutant of diphtheria toxin. This vaccine is combined with tetanus toxoid and can be routinely used as a booster in adults.

INTRODUCTION

Diphtheria and Tetanus are toxin-mediated infectious diseases that can be prevented by the presence of toxin neutralizing antibodies passively transferred or actively induced by vaccination. (1)
Widespread immunization programs have substantially eradicated both diseases in developed countries. In the case of diphtheria, vaccination drastically decreased also the circulation of toxigenic strains of *C. diphtheriae* (2)
In spite of this, outbreaks of Diphtheria have been reported in countries with high rates of immunization. (3-4)
This is the consequence of the presence of large proportion of adult people, having less than 0.01 IU/ml of antitoxin, that is generally considered the minimum protective level. (5-6)
The aim of the study described here was to evaluate the proportion of unprotected people for tetanus and diphtheria in a population of young adults coming from different area of Italy where diphtheria immunization has been compulsory since 1939 and tetanus immunization since 1968.

MATERIALS AND METHODS

A serum sample was obtained from 334 at the Cadet NCOs School of the Army, Viterbo, Italy.
The volunteers were aged 17 to 22 years and came from all Italian geographical areas. The sera, stored at -20°C until tested, were evaluated, by in vivo neutralization assay, to define the presence of at least 0.01 IU/ml of both diphtheria and tetanus antitoxin, that is considered the lowest protective level.

Diphtheria antitoxin evaluation.

The in vivo neutralization assay to evaluate diphtheria antitoxin was performed by the rabbit intradermic test according to the European Pharmacopoeia. Briefly, a mixture composed of 0.1 ml of serum and 0.1 ml of toxin (batch HDM 49, Sclavo S.p.A. , Siena Italy) and a similar mixture with 0.1 ml of standard Diphtheria Antitoxin (office of Biologics, Bethesda, USA) were prepared. After incubation for 60 min at room temperature, the two mixtures were inoculated intradermally in adult white rabbits.
After 48 hrs erythematous areas were measured: the serum is considered positive if the correspondent reaction has a size equal or smaller to that of the standard.

Tetanus antitoxin evaluation.

The in vivo neutralization assay to evaluate tetanus antitoxin was performed by the mouse protection test according to the European Pharmacopoeia. Briefly, a mixture composed of 0.1 ml of serum, 0.1 ml of tetanus toxin (batch 1124 SF, Sclavo S.p.A., Siena), and 0.3 ml of saline solution and a similar mixture with 0.1 ml of standard Tetanus Antitoxin containing 0.1 IU/ml (1/100 Lr) were prepared.
After incubation for 60 min at the room temperature the two mixtures were inoculated subcutaneously into mice of 18-20 g of wheight.
After 96 hrs letality in the two groups of mice was calculated: the serum is considered positive if the survivors are the same or more with respect to the group inoculated with the standard.

RESULTS

As summarized in table 1, the proportion of unprotected subjects for the two diseases is different: 76 out ot 332 volunteers (22.9%) for diphtheria and 18 out of 334 (5.3%) for the tetanus had specific antibodies under the protective level.
Stratifying by the geographic origin of the volunteers (North-Centre versus South-Islands), the proportions of seronegative people were quite different for both diseases: 15 out of 132 (11.4%) for diphtheria and 3 out of 132 (2.3) for tetanus in the North-Centre group and 61 out of 200 (30.5%) for diphtheria and 15 out of 202 for tetanus in the South-Islands group were unprotected. Twelve out of 334 volunteers (3.6%) were negative for both diphtheria and tetanus.

DISCUSSION

The results of our study confirm that also in Italy, where combined immunization against tetanus and diphtheria is compulsory for all newborns, a large

proportion of adults is devoid of protective levels of diphtheria antitoxin. As a consequence, also in this country, outbreaks of clinical disease, caused by toxigenic strains of *C diphtheriae* imported from regions where diphtheria is endemic, can occur. In particular it seems important to note that military personnel, more and more often requested to operate in developing countries, represent a high risk population likely to contract clinical disease during their service abroad. As far as tetanus is concerned, the phenomenon is much more limited. This discrepancy can only be attributed to the different policies of revaccination for the two diseases; in fact booster injections of tetanus toxoid are often administered, generally in coincidence with a wound injury, and conversely diphtheria revaccination is practically neglected. As a matter of fact adults present, after injection of diphtheria toxoid, a number of adverse reactions that do not occur during children vaccination and are responsible of the refusal to perform booster injections, in spite of the availability of an adult-type diphtheria vaccine containing a smaller amount (2 Lf) of antigen. The above reported reactions are considered strictly related to the presence of high amounts of contaminants in the conventional vaccine (1:8) that for practical reasons is generally purified only after formaldehyde treatments, when many contaminants cannot be eliminated any more.

The use, as diphtheria vaccine, of CRM 197, a nontoxic protein immunologically identical to diphtheria toxin, could represent a good chance to promote diphtheria revaccination of adults. With respect to the conventional vaccine, CRM 197 presents a number of advantages such as the natural absence of toxicity that makes the chemical detoxification not necessary, thus allowing an optimal purification and the absence of risk of any reversion to toxicity.

To confirm such a hypothesis we have prepared a vaccine for adult use containing CRM 197 and tetanus toxoid and we have organized, in collaboration with the Office of the Surgeon General of the Italian Army, a case control, controlled versus conventional vaccine, clinical study that is actually in progress.

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Table 1: Number and proportion of seronegative subjects for tetanus and Diphteria.
Geographic Areas Tetanus Diphteria Tet.+Diph.

Geographic Areas		Tet.	Diph.	Tet.+ Diph.
North-Centre	Total N°	132	132	132
	Seroneg.N°	3	15	2
	Seroneg.%	2.3	11.4	1.5
South-Islands	Total N°	202	200	202
	Seroneg.N°	15	61	10
	Seroneg.%	7.4	30.5	4.5
Global values	Total N°	334	332	334
	Seroneg.N°	18	76	12
	Seroneg.%	5.3	22.9	3.6

DRAMATIC REDUCTION OF MENINGOCOCCAL MENINGITIS AMONG MILITARY RECRUITS IN ITALY AFTER INTRODUCTION OF SPECIFIC VACCINATION

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INTRODUCTION

Before the advent of chemotherapy, mortality from meningococcal meningitis was 50%-80% during epidemics and 30%-45% among sporadic cases; among infants, mortality was close to 100% (1). The discovery of the sulfonamides lowered mortality dramatically, and the situation has since remained unchanged. In fact, the overall mortality from meningococcal meningitis varies at present from 2% to 12% (Table I). However, in spite of the considerable success in the development of drugs in this field, the problem of meningococcal meningitis is far from being solved.

It is not clear whether the disease caused by different serogroups differs in severity. The traditional view in the USA is that the infection caused by group C is more severe than that caused by group A (1). This view has been supported by the data from a classical endemic area such as the meningitis belt in Africa, where mortality in group C was 22% against only 12% among patients with group A disease (2).

An important advance against meningococcal meningitis has been the production of suitable vaccination. The major development of the meningococcal polysaccharide (PS) vaccines occurred during the late 1960s. At present, vaccine preparations containing groups A plus C, or A, C, Y and W135, respectively, are marketed (3). Unfortunately, there is no vaccine for prevention of group B meningococcal disease, because group B PS, for still unclear reasons, does not induce bactericidal antibodies in humans (4, 5). This presents a major problem, because group B is the most common cause of meningococcal meningitis in non-epidemic conditions, and sometimes even causes epidemics. Moreover, it accounts for 50% to 89% of disease cases in many countries (Table II) (1, 6, 7).

Meningococcal disease is also considered a military disease, and indeed its incidence among recruits is at least 4 to 10 times higher than in the general population (1) (Fig. 1). The reasons are much the same as in any closed population. A close contact may be defined " the individual who frequently sleeps and eats in the same dwelling with the index case" (8). In military recruits, conditions for the spread of virulent meningococci are optimal and the risk of disease is high during the first several weeks of service, but not later. Permanent military personnel do not seem to be at any special risk (1).

In Italy the results of the National Meningitis Surveillance Programme showed a high attack rate of the disease among recruits in 1985 as well as in 1986, with respectively 92% and 95% of the cases caused by serogroup C, and thus preventable (9)(Fig. 2).

These findings constituted the motivating factors leading to the authorities' decision to make vaccination against meningococcal disease mandatory for military recruits starting from January 1987.

However, after almost 5 years from the introduction of meningococcal vaccination we can sum up the epidemiological and immunological effects of the vaccination.

For the epidemiological situation, we focused on data collected by National Institute of Health, Italy, during the period 1985 - 90 (9-11).

For the immunological data, we investigated the efficacy of the immune response to this vaccine in normal conditions and during exposure to a particular environmental factor such as hypobaric hypoxia, using Enzime-linked immunosorbent assay (ELISA) and Isoelectric focusing (IEF) for quantitative and qualitative analysis, respectively.

MATERIALS AND METHODS

Subjects and immunization in normal conditions

One-hundred military recruits of Italian Air Force (IAF), aged 18-25 years, were vaccinated subcutaneously with a single dose of Menpovax A + C (Sclavo, Siena, Italy), containing 50 μg of each polysaccharide.

Serum samples were obtained from each subject both before and 18 days after vaccination (12).

Subjects and immunization during exposure to hypobaric hypoxia

This study was performed on volunteers participating in the Italian National Research Council (CNR) Expedition to mount Poumori at 4,930 m/16,174 ft as apart of the scientific project "EV-K2-CNR". It was a collaborative study between IAF and CNR.

Eighteen healthy male subjects aged 18-40 years, were vaccinated subcutaneously with a single dose of Menpovax A + C (Sclavo). Vaccine administration was performed 5 days after the arrival at mount Poumori, where the subjects lived throghout the entire period of the study protocol. As a control, a group of 18 age-matched male subjects received the vaccine at sea level, where they lived during the entire period of the study (13).

ELISA for specific anti -PSA and PSC IgG

Antibody quantity determination was carried out using an ELISA as previously reported by Le Moli S. et al. (12).

IEF analysis

IEF was carried out as previously described (13). Antibodies to PSA and PSC were separated by IEF in agarose gel and visualized by autoradiography after labeling with ^{125}I-PSA or ^{125}I-PSC.

RESULTS AND DISCUSSION

Epidemiological data

From the epidemiological point of view we have

observed a progressive decrease in the number of disease cases, until almost total disappearance (9-11) (Table III).
Nearly 300,000 Italian recruits were vaccinated every year from January 1987. Since the vaccine was given only to the new recruits joining the military, full coverage was achieved only in January 1988, and this explain the apparently high number of cases in 1987 (Table III). Only one of the 27 observed cases since the beginning of the vaccination campaign was attributed to vaccine failure. This case belonged to serogroup C (in 1988) and occurred nearly 2 months after immunization (Table III). Regarding the other cases, 12 (11 due to serogroup C and 1 due to serogroup A) occurred in unvaccinated recruits, because they had entered the military in 1986; 1 case in 1987 occurred after 5 days from vaccination and thus no attributable to vaccine failure; 2 cases in 1988 (1 due to serogroup C and 1 culture negative) and 1 case in 1989 (serogroup C) occurred in recruits not vaccinated for medical reasons (allergic diathesis) and for accidental reasons, respectively. The remaining sporadic cases belonged to serogroups other than A or C, consequently were not preventable by the vaccine (10) (Table III).
The cumulative incidence of meningococcal serogroup C in the 600,000 vaccinated recruits during the period 1988-9 was 0.2/100,000 (1 case out of 600,000 recruits), while the corresponding figure in the 600,000 unvaccinated recruits during the period 1985-6 was 11.3/100,000 (68 cases out of 600,000 recruits). ($p<0.001$).
In addition, 1987 was the year in which we had 150,000 unvaccinated and 150,000 vaccinated recruits. The protective efficacy of the vaccine was 91.2% (12 cases of meningococcal serogroup C and A from unvaccinated cases, and 1 case of serogroup C from the corresponding average of vaccinated cases) (Table IV). Of course, in 1988 and 1989 vaccine efficacy was not assessable as all recruits were vaccinated (10).
In addition, there were no reports of any adverse side effects to the vaccine (10).
It has been suggested that vaccination with this group-specific vaccine could result in epidemics caused by other groups of meningococci. Such fears are unfounded: in fact vaccination with group A and C specific vaccines greatly reduced the incidence of meningococcal disease in the military in USA and in Finland, but nowhere has vaccination resulted in an increase in disease caused by other serogroups (9). Also in Italy in the last 5 years we did not observe any increase of serogroup B after the introduction of compulsory vaccination for A and C.

Immunological data

From the immunological point of view, vaccination is highly effective as to seroconversion
In fact, we performed a study on 100 military recruits of the Italian Air Force vaccinated according to this schedule, using ELISA and analytical IEF for quantitative and qualitative specific antibody analysis, respectively.
This analysis showed that, 18 days after immunization, 84% and 91% had a seroconversion against PSA and PSC, respectively (12).
A nearly total overlapping between epidemiological (more than 90% of protective efficacy) and immunological (84% and 91% of seroconversion against PSA and PSC, respectively) data was observed.
Qualitative analysis was performed by IEF, that is a method with a sensitivity level that easily copes with a single clone product. The spectrotypic analysis of anti-PSA and PSC antibodies before and after vaccination showed that the response is mainly oligoclonal (12). In addition, the spectrotype of natural antibodies, that is the antibodies already present before the vaccination, is normally similar to that of antibodies induced by vaccination. Natural antibodies are likely to be induced by whole bacteria therefore with a T-dependent form of polysaccharide. On the other side, the PS by itself, as used in vaccine immunization, stimulates a T-independent pathway. The observation of the same spectrotypic pattern suggests that both natural (T-dependent pathway) and vaccine (T-independent pathway) immunization induce the expression of the same antibody repertoire, for both meningococcal PSA and PSC (12).
In addition, in order to get a qualitative evaluation of long lasting anti-PSA and anti-PSC antibody response, IEF was performed in 20 sera 8 months after immunization. Almost all sera showed a marked increase in band intensity, but the number and the distribution of bands kept basically unchanged with respect to the 18th day (12). The presence of an unchanged antibody pattern after 8 months is fundamental regarding protection in recruits from serogroup A and C meningococci during military service, that is twelve months long.
In addition, in order to assess whether the efficacy of the immune response to this vaccine is modified by environmental factors, we performed a study during prolonged exposure to hypobaric hypoxia.
Eighteen men who participated in a scientific project in Mount Poumori, Nepal, were immunized according to this schedule. Antibody titers versus both polysaccharides were determined by ELISA before and 18 days after vaccination. All subjects developed a good antibody response without statistically significant differences from the control group of 18 men of comparable age vaccinated at sea level (13). Spectrotypic analysis of antibody response to PSC was performed by means of IEF. The spectrotypic pattern of these subjects is oligoclonal, like in the control group, showing that environmental factors do not bring about any decrease in efficacy (13).

CONCLUSIONS

On the basis of all the above-mentioned data, the anti-meningococcal PSA and PSC vaccine can be looked upon as a very safe and effective method to control the spread of the disease in the military recruits since:
1) its efficacy is very high, since approximately 90% of the subjects develop a protective antibody response;
2) the vaccine is safe: no untoward reactions were recorded;
3) it has proven to provide satisfactory immunological response even under unfavourable environmental conditions, like hypobaric hypoxia.
An ideal meningococcal vaccine should protect from group B too. In fact, effective group B vaccines would be particularly welcome in those countries where these organisms currently predominate and where nationwide outbreaks are in progress.
In conclusion personal experience with the presently used meningococcal vaccine has been favourable from the epidemiological and immunological points of view. The epidemiological situation in Italy before the introduction of vaccination, with a prevalence of meningococcal meningitis C, allowed us to reach such a success. Prospects for research are the preparation of vaccine able to protect against all the serogroups together, mainly the serogroup B, which is prevalent now in North America and Northern Europe.
The ongoing clinical trial in Norway with a PSB vaccine conjugated with type-specific outer membrane is very promising to this respect (14).

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SUMMARY

Meningococcal meningitis still represents a serious infectious disease with a mortality rate that can be as high as 10% even in developed countries.

Military recruits are generally a high risk group for meningococcal disease, with a reported incidence of 4 to 10 times greater than that of the general population.

In Italy the results of the National Meningitis Surveillance Programme showed a high attack rate of the disease among recruits in 1985 as well as in 1986, with 92% and 95% of the cases, respectively, caused by serogroup C and thus preventable. These findings constituted the motivating factors leading to the authorities' decision to make vaccination against meningococcal disease mandatory for recruits starting from January 1987. After almost 5 years from the introduction of meningococcal vaccination, we here present a summing up of the epidemiological and immunological effects of the vaccination.

From the epidemiological point of view we have observed a dramatic reduction of the prevalence of the disease. In 1987, that is the year in which we had 150,000 unvaccinated and 150,000 vaccinated recruits, the protective efficacy was 91.2%.

From the immunological point of view, vaccination is highly effective, as seroconversion against polysaccharide (PS) A and C is 84% and 91%, respectively. The spectrotypic analysis of the sera before and after vaccination shows that the type of response is mainly oligoclonal, like the majority of the responses to PSs, and the antibodies induced by sole PS are not qualitatively different from the antibodies induced by natural immunization.

In addition, the efficacy is not modified by environmental factors like hypoxia, as demonstrated *during permanence* at 16,174 feet for 20 days.

In conclusion, the anti-meningococcal PSA and PSC vaccine can be looked at as a very safe and effective method in controlling the spread of the disease in military recruits since: 1) its efficacy is very high, considering approximately 90% of the subjects develop a protective response; 2) it is safe; 3) it has proven to provide satisfactory immunological response even under unfavourable conditions, like hypobaric hypoxia.

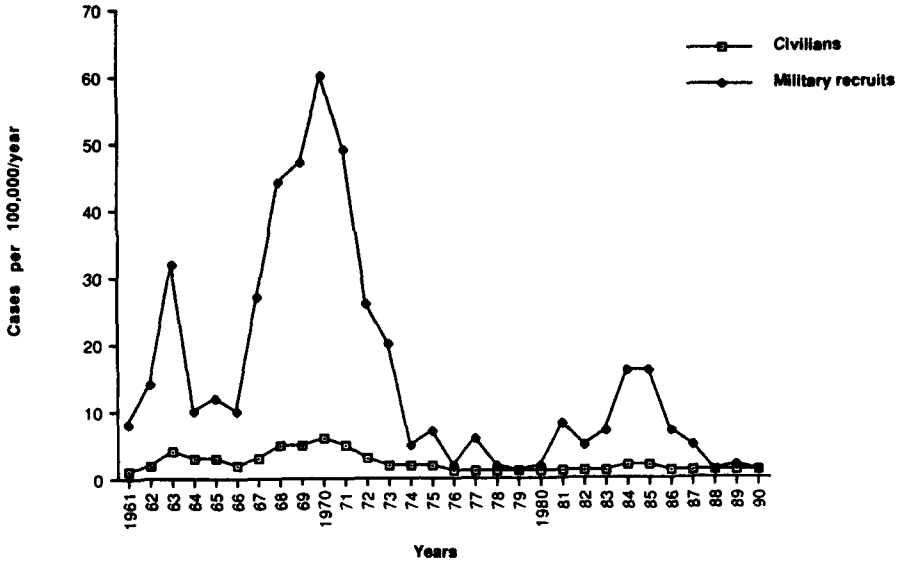
Table I. OVERALL MORTALITY (%) FROM MENINGOCOCCAL MENINGITIS

Conditions, serogroup.	Country (region)	Mortality (%)	Years
EPIDEMIC			
A	Finland (Helsinki area)	3	1973-74
A	Nigeria (Zaria)	5 - 6	1970s
B	Belgium	6	1971-72
B	Norway (Northern)	4	1975-78
C	Brazil (San Paulo)	8	1970s
C	Vietnam (South)	2 - 10	1970s
?	Spain	4	1970s
ENDEMIC			
B	Norway (South)	7	1975-79
?	Scotland	6	1970s
B - C	USA (CA MO NJ OK TN WA)	12	1986

Table II. Predominance of serogroup B versus the other serogroups in some european countries and in USA

Countries	Percent	Year
Belgium	80%	1975
United Kingdom	61%	1977
Netherlands	69%	1978
Austria	57%	1979/81
Spain	89%	1980
Hungary	76%	1980
Germany (Federal Republic)	70%	1981
Norway	75%	1981
USA (CA MO NJ OK TN WA)	49%	1986
Italy	72%	1990

Fig. 1. Incidence (cases per 100,000 persons per year) of meningococcal disease in military recruits and in civilians in Italy. 1961-90.



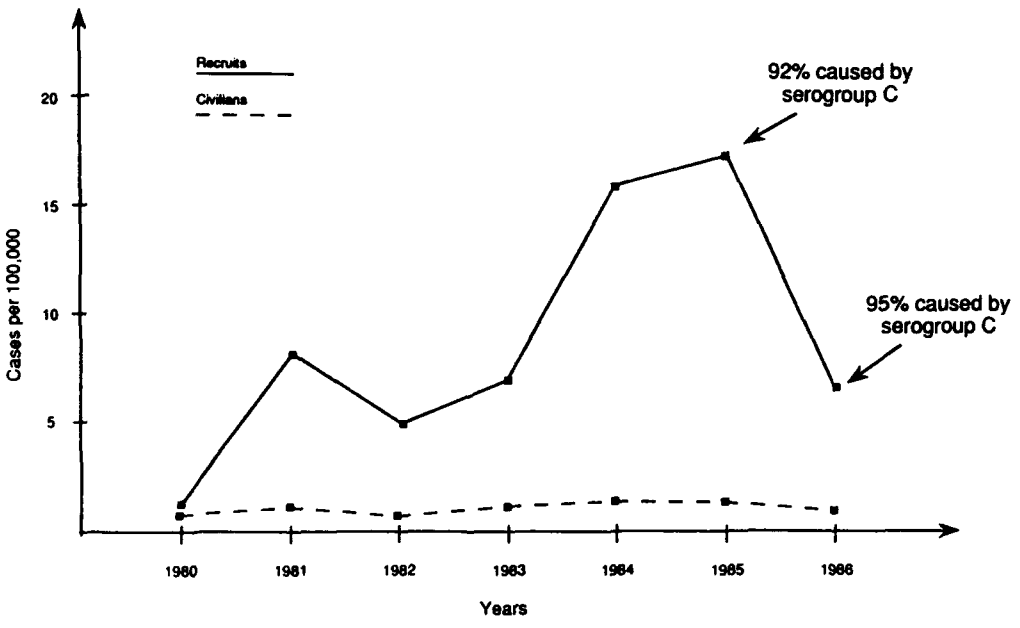


Fig. 2. Incidence (cases per 100,000 persons per year) of meningococcal disease in Italian recruits and in civilians. 1980-86.

Table III. Serogroups isolated from patients with N.Meningitidis among Italian recruits (300,000 per year). 1985-91.

Serogroup	1985	1986	Starting of vaccination ↓ 1987	1988	1989	1990	1991
A	0	0	1*	0	0	0	0
B	2	1	2	1	2	2	1
C	48	20	12+	2**	1*	0	0
Other	2	0	0	1	1	0	0
No grouped	0	0	0	1*	0	0	0
Total	52	21	15	5	4	2	1

* Not vaccinated: 1987, because entered the army before the start of vaccination campaign; 1988, because of medical controindication; 1989, for accidental reason.
+ 11 not vaccinated, because entered the army before the start of vaccination campaign.
** 1 not vaccinated, for medical controindication.

Table IV. Protective efficacy of meningococcal (A + C) vaccine in Italian recruits in 1987.

Vaccination status	Recruits	No. of cases (A and C serogroups)	Incidence/ 100,000	Protective efficacy
Vaccinated	150,000	1	0.7	$\frac{8 - 0.7}{8} \times 100 = 91.2\%$
Unvaccinated	150,000	12	8	

IMMUNISATION DU PERSONNEL NAVIGANT A DESTINATION DES PAYS TROPICAUX POSITION FRANCAISE

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1. SOMMAIRE

Le rappel des risques infectieux encourus lors d'un séjour en zone tropicale, justifie une prophylaxie préalable. A côté des mesures obligatoires, il convient de discerner des actions recommandables et d'autres souhaitables.

Les mesures obligatoires concernent essentiellement la vaccination anti-amarile pour les personnels navigants se rendant dans les zones inter-tropicales d'Afrique et d'Amérique. L'efficacité partielle et limitée de la vaccination anti-cholérique a justifié pratiquement son abandon.

Les mesures recommandables concernent le tétanos, la diphtérie, la poliomyélite, le BCG et la typhoïde. Obligatoires en France, pour tous les enfants, à l'exception de la dernière, ces vaccinations doivent donner lieu à des rappels, chez tous les adultes. Bien toléré, le nouveau vaccin contre la typhoïde (polyosidique, antigène Vi purifié) mérite d'être employé systématiquement.

D'autres immunisations sont souhaitables: vaccinations contre la méningite méningococcique (sérogroupes A et C) chez l'adulte jeune et contre l'hépatite virale B, injection de gammaglobulines pour la prévention des hépatites à virus A. Enfin, la bonne tolérance du vaccin anti-rabique conduit à conseiller la généralisation de son emploi, en raison du risque possible de défaut d'approvisionnement au moment opportun.

Les contre-indications sont rappelées et un calendrier vaccinal est proposé.

A côté des formes orales des vaccins contre le choléra et contre la typhoïde, les perspectives d'avenir concernent les vaccins contre l'hépatite virale A et contre l'encéphalite japonaise. Ceux dirigés contre les risques majeurs, tels le paludisme et le SIDA paraissent malheureusement, encore actuellement, aléatoires.

2. INTRODUCTION

2.1 Evaluation des risques infectieux

Qu'il y fasse des séjours de durée variable ou des escales réitérées, le personnel navigant est exposé aux risques infectieux qui persistent dans la majorité des pays tropicaux. Certaines de ces maladies sont cosmopolites, d'autres sont en relation avec des conditions particulières.

Les infections cosmopolites qui ont beaucoup reculé dans les pays tempérés, restent fréquentes dans les régions chaudes où elles sont bien plus liées à la pauvreté, au manque d'hygiène et à l'insuffisance du développement sanitaire, qu'au climat. Certaines, relevant du péril fécal, sont secondaires à une contamination orale (typhoïde, choléra, poliomyélite, hépatite virale A). D'autres connaissent une transmission respiratoire (tuberculose, méningite cérébro-spinale). D'autres enfin, sont des conséquences de relations sexuelles (hépatite virale B, SIDA). A cette liste s'ajoutent les infections ubiquitaires que sont le tétanos et la rage.

Les maladies propres à certaines zones géographiques sont transmises par des insectes vecteurs (paludisme, fièvre jaune, dengue, trypanosomiase, filariose, leishmaniose) ou encore contractées lors d'un contact cutané avec l'eau douce (bilharziose, anguillulose) et/ou la terre humide (ankylostomiase).

2.2 Moyens prophylactiques

Si certaines de ces affections peuvent être évitées grâce au respect de règles élémentaires, la majorité d'entre elles exigent une prophylaxie active par les vaccinations ou plus rarement, une immunisation passive par les gamma-globulines.

Il est logique de classer les immunisations selon leur caractère :
- obligatoire : vaccination anti-amarile ;
- recommandable : vaccinations contre le tétanos, la diphtérie, la poliomyélite, la tuberculose et la typhoïde ;
- souhaitable : vaccinations contre les méningites cérébro-spinales A et C, l'hépatite virale B, la rage et la grippe.

C'est dans cet ordre que nous les envisagerons après avoir déploré l'absence actuelle de toute immunisation contre les grands fléaux que sont le paludisme à plasmodium falciparum, le SIDA et à un moindre degré, les bilharzioses.

3. VACCINATIONS OBLIGATOIRES

3.1 Vaccination anti-amarile, obligation unique

Depuis l'éradication de la variole, l'immunisation contre la fièvre jaune est la seule vaccination rendue obligatoire par le règlement sanitaire international pour les régions d'endémie qui correspondent aux zones intertropicales d'Afrique et d'Amérique situées entre les quinzièmes degrés de latitude nord et sud. Elle est également exigée pour certains pays d'Asie, indemnes de la maladie, mais présentant toutes les conditions épidémiologiques propices à son développement (1). Cette vaccination est indispensable car le réservoir du virus animal est impossible à maîtriser. Plus de 150 cas sont notifiés en moyenne, en Afrique, chaque année, avec un taux de létalité de 60 % (2).

L'immunisation fait appel à un virus vivant atténué préparé sur embryon de poulet. D'une parfaite innocuité, l'injection unique de 0,5 ml, valide dès le dixième jour, protège pendant dix ans. Elle n'est contre-indiquée que chez les immuno-déprimés et chez les femmes au cours du premier trimestre de

la grossesse. Elle ne doit pas être associée aux vaccins bactériens. Enfin, rappelons qu'elle ne peut être réalisée que dans un centre de vaccination agréé.

3.2 Vaccination anti-cholérique

Autrefois obligatoire, l'immunisation contre le choléra a été abandonnée par le règlement sanitaire international en 1973, en raison de son efficacité partielle et limitée. Elle peut cependant être exigée par certains pays de façon temporaire ou permanente, notamment pour les personnes provenant de zones d'endémie. Seuls 50% environ des sujets vaccinés sont protégés pendant six mois, après une injection unique. Un nouveau vaccin administré per os, sera bientôt disponible; constitué de bactéries entières inactivées et de la sous-unité B de la toxine cholérique, il aura l'intérêt de protéger également contre les Escherichia Coli entéro-toxiques. Deux prises orales à 15 jours d'intervalle, associées chacune à l'absorption d'un comprimé de citrate et de bicarbonate de soude pour neutraliser l'acidité gastrique, confèrent une protection d'un an.

4. VACCINATIONS RECOMMANDABLES

4.1 Immunisation anti-tétanique-diphtérique-poliomyélitique

Parmi les nombreux vaccins recommandables, l'association de l'immunisation contre le tétanos, la diphtérie et la poliomyélite est obligatoire en France avant l'âge de 18 mois. Elle demande trois injections espacées d'un mois, suivies de rappels un an après, puis tous les cinq ans, quoique des intervalles de dix ans semblent suffisants. Cependant chez l'adulte non antérieurement vacciné, une protection satisfaisante contre la poliomyélite et le tétanos, est obtenue par deux injections espacées d'un mois, suivies d'un rappel un an plus tard. En ce qui concerne l'immunisation contre la poliomyélite, la présentation injectable doit être préférée en raison du risque de maladie chez le vacciné et dans son entourage après l'utilisation de la forme orale (3). L'association vaccinale diphtérie-tétanos-poliomyélite est très bien tolérée. Elle n'est contre-indiquée que par une immuno-dépression, une maladie infectieuse évolutive et, seulement pour la diphtérie, par l'existence d'une néphropathie.

4.2 Le BCG

Egalement obligatoire en France dès l'enfance, le BCG, d'une excellente tolérance générale, peut être rarement, responsable d'adénites. Ce risque est minime par rapport aux dangers de la tuberculose. Ainsi est-il indispensable de vérifier chez l'adulte jeune, la persistance de l'anergie tuberculinique et le cas échéant, de pratiquer une nouvelle vaccination par scarification (1 cm par année d'âge, sans dépasser 20 cm) ou de préférence par injection intra-dermique (0,1 ml). La seule contre-indication est l'immuno-dépression.

4.3 Vaccination anti-typhoïdique

La thyphoïde sévit sur le mode endémique dans toutes les régions où les équipements sanitaires sont insuffisants : Asie du sud, Moyen-Orient, Afrique, Amérique centrale et du sud. Elle connaît un mode endémo-épidémique en Asie du sud-est et dans le pourtour méditerranéen, en particulier au Maghreb. Son danger est moins présent en Amérique du nord et dans la plupart des pays européens où le mode est sporadique. Plus de la moitié des typhoïdes observées actuellement en France, ont été contractées en zone tropicale (2). Ainsi l'immunisation du personnel navigant est-elle justifiée surtout depuis la mise à notre disposition d'une nouvelle modalité. Le vaccin classique TAB à germes entiers inactivés, qui exigeait trois injections à un mois d'intervalle, et qui entraînait des réactions secondaires importantes, a fait place au vaccin polyosidique d'antigène Vi purifié, très bien toléré, qui confère une immunité durable dès le septième jour après une seule injection. Son association à la vaccination contre la diphtérie, le tétanos et la poliomyélite est possible. Enfin, la perspective d'une immunisation orale contre la typhoïde semble prochaine.

5. VACCINATIONS SOUHAITABLES

5.1 Vaccination contre l'hépatite B

Parmi les vaccinations souhaitables, celle dirigée contre l'hépatite B occupe une place prédominante. Cette affection constitue un problème majeur de santé publique en Chine, en Asie du sud-est et en Afrique intertropicale où 10 à 20 % de la population sont porteurs chroniques de l'antigène HbS (1). Son incidence atteint 21 pour mille outre-mer alors qu'elle n'est que de 1,5 pour mille en métropole. L'immunisation est obligatoire en France, dans les armées pour tous les personnels affectés outre-mer depuis le 1er juillet 1991. Dénuées d'effets secondaires, trois injections intra-musculaires dans la région deltoïdienne de 0,5 ml, espacées d'un mois, sont efficaces chez 95 % des vaccinés, un mois après la troisième dose. Les rappels sont nécessaires un an plus tard, puis tous les cinq ans. Une administration concomitante avec les autres vaccins, en deux sites différents, est possible. Il est inutile de rechercher au préalable les marqueurs sérologiques de l'affection, car le vaccin n'aggrave pas une maladie latente et n'a aucun inconvénient chez le sujet déjà immunisé. La vaccination protège contre les différents sous-types du virus B et indirectement contre l'agent Delta qui ne peut se répliquer qu'en présence du virus B.

5.2 Immunisation contre l'hépatite A

Contre l'hépatite A, un virus inactivé a pu être réalisé aux Etats Unis d'Amérique, mais son emploi se heurte à l'obstacle du faible rendement des cultures de virus sur cellules d'hépatocarcinome. Ainsi la prévention de la maladie dont l'épidémiologie est très comparable à celle de la typhoïde, ne peut-elle faire appel qu'à l'injection intra-musculaire de gamma-globulines standard, à la dose de 0,02 ml/kg, efficace pendant trois mois seulement. Cette modalité immunologique est obligatoire pour les militaires français servant en zone endémique.

5.3 Vaccination anti-méningococcique

L'immunisation contre la méningite cérébro-spinale est intéressante en zone sahélienne, surtout pendant la saison sèche et fraîche de décembre à mars, ainsi qu'en Inde, au Népal, en Mongolie et au Brésil. Le vaccin polysaccharidique bivalent ne protège que contre les séro-groupes A et C pour une injection unique de 0,5 ml. Elle est efficace pendant trois ans.

5.4 Vaccination anti-rabique

La disponibilité actuelle d'un vaccin de culture cellulaire, d'une totale innocuité et d'une parfaite efficacité, rend souhaitable l'immunisation contre la rage en raison du risque possible de défaut d'approvisionnement en Afrique, zone importante de circulation du virus rabique. Deux injections de 0,5 ml, espacées d'un mois, sont nécessaires. Les rappels sont utiles après un an et ensuite tous les trois ans.

5.5 Vaccination anti-grippale

Enfin, en raison de l'indisponibilité qu'elle entraîne, l'immunisation contre la grippe est d'autant plus intéressante que la connaissance des virus qui sévissent dans le monde permet la préparation d'un vaccin parfaitement adapté au contexte épidémiologique.

6. TOLERANCE DES VACCINS

D'une façon générale, à l'exclusion des circonstances d'immuno-dépression, les immunisations actuelles sont très bien tolérées. Il convient en particulier de souligner l'extrême rareté des néphropathies post-vaccinales (4). Les glomérulonéphrites interdisent la pratique des vaccinations contre la diphtérie et contre la typhoïde. Les protéinuries orthostatiques ne sont pas une contre-indication. Il en est de même pour le diabète, quelque soit son type, quand il est bien équilibré (5). Enfin, depuis la purification des préparations, les réactions allergiques sont devenues exceptionnelles (6). Elles peuvent être induites par les vaccins obtenus par culture sur oeufs embryonnés (grippe, fièvre jaune), ou encore par la présence d'antibiotiques à l'état de traces (poliomyélite, rage). Les réactions urticariennes observées après l'injection d'immuno-globulines standard, sont provoquées par le mertiolate, agent conservateur. Enfin, il est intéressant de savoir que plusieurs immunisations sont réalisables le même jour, en des sites différents (7).

7. CALENDRIER VACCINAL

Quel calendrier vaccinal proposer à une population qui a le plus souvent été immunisée dès l'enfance et chez laquelle les obligations de rappel ont été respectées de façon très irrégulière?

Il convient de s'adapter à chaque cas particulier à partir du programme complet suivant :

- première vaccination anti-hépatitique B et anti - diphtérique - tétanique - poliomyélitique, en deux sites différents;
- le troisième jour, BCG en l'absence d'anergie tuberculinique, vérifiée par une intradermo-réaction préalable;
- le trentième jour, vaccinations anti-typhoïdique Typhim Vi, anti-amarile et deuxième injection de l'anti-hépatitique B ;
- le soixantième jour, seconde administration anti - diphtérique - tétanique - poliomyélitique, et troisième d'anti-hépatitique B, ainsi qu'une injection de gamma-globulines.

Ce calendrier tient compte des vaccinations de l'enfance qui rendent suffisante l'administration de deux doses anti - diphtérique - tétanique et poliomyélitique.

8. CONCLUSION

Il est certain que les progrès dans le domaine du génie génétique conduiront à la production de vaccins encore plus efficaces, satisfaisant parfaitement aux trois critères d'efficacité, d'innocuité et de moindre coût. Souhaitons surtout que ces recherches permettent un jour, l'immunisation contre le SIDA et contre le paludisme à Plasmodium falciparum.

Les propositions précédentes, qui correspondent aux propositions françaises, ne sauraient bien sûr être satisfaisantes pour tous les autres pays. Il convient en effet, dans tous les cas, de tenir compte des obligations vaccinales appliquées dès l'enfance à l'ensemble de la population, des circonstances épidémiologiques et des missions du personnel navigant de l'aéronautique.

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DISCUSSION SESSION III

INFECTIOUS DISEASES AND VACCINATIONS (Papers 17 to 22)

F. AIUTI (IT): I have a question for dr Biselli. The question is related to meningococcal meningitis, found in subjects with primary complement deficiency. The question is: is there any study on these sera or other sera of patients with meningococcal meningitis dealing with possible complement deficiency or Ig deficiency?

R. BISELLI (IT): These patients are at high risk for meningococcal disease and must be protected. So, we vaccinated patients with complement defect and the response to the meningococcal vaccine in these patients seems to be similar to that of normal subjects, either in ELISA or in IEF and reverse blotting. It is consequently important to vaccinate these subjects who are at high risk.

F. AIUTI (IT): The question was not only related to vaccination, but mainly related to possible complement defect found in patients with meningococcal meningitis.

R. BISELLI (IT): Patients with meningococcal meningitis and complement deficiency, present, in our experience, a more severe disease and many recurrences. Consequently vaccination is crucial for the prevention of recurrences.

G. GRAY (CA): My question is for dr Biselli. You said that the response to meningococcal vaccine was also good at altitude. We know that acute mountain sickness can depress the immune reactions. I would like to know at what time the recruits were vaccinated after the stress of exposure to altitude.

R. BISELLI (IT): We studied these subjects after 10 days. We also performed studies also in mice, in a hypobaric chamber. Also in animals the situation is the same. The rise to hypobaric hypoxia depress the immune system, but in our experience, both in humans on mountains, and in mice in a hypobaric chamber, there was no alteration in the B-cell response to T-independent antigens.

G. GRAY (CA): The question is: how many days did the subjects remain at high altitude before vaccination?

R. BISELLI (IT): Five days of permanence at high altitude after nearly one week of progressive ascension, vaccination, then another 20 days of permanence at high altitude.

G. GRAY (CA): So, probably there were no more effects of acute mountain sickness, because there was acclimatization. A second question is related to the incidence of meningococcal meningitis C in the 3 years before the introduction of the vaccine. This incidence shows a continuous decline of the cases from 1985, in which there were 48 cases, to 1986, in which there were 24 cases, until 1987, with only 12 cases. I wonder what's your explanation for that.

R. BISELLI (IT): Actually we introduced our vaccine during the decline of incidence of the disease. Vaccination has accelerated such a trend.

W. WOLFE (USA): Just a comment on meningococcal B disease. During the Gulf War, all the US Forces vaccinated for at least 5 years were reimmunized with quadrivalent meningococcal vaccine. We had 2 cases of meningococcal disease both type B, in 2 men who shared a tent, one was a fatal case and the other recovered.

J. A. BELLANTI (USA): I would like to make some comments on dr Biselli's paper. He made reference to the change in the reduction in disease resulting from chemotherapy with sulfonamides. I think it is important to recall, particularly at a Meeting for the military, the importance of changing the macro-eco-systems, that we do either by chemotherapy or by immunization. I think we need to recall that sulfonamides have both a beneficial as well as detrimental effect. They were responsible, as you recall, for the emergence of sulfonamide-resistant strains of meningococci that occurred in the '60s, when vigorous attempts of chemoprophylaxis were made and the emergence of resistant organisms were seen. The same is true with immunization. When you use vaccine to repress or suppress one eco system or one organism in an eco system, I think that we have to be conscious that other strains may emerge. We haven't seen that with meningococcus, but the possibility of suppressing A and C and of emergence of B is real.

The third point is related to anti-B vaccine. It has been recently reported in the "Proceedings of the National Academy of Sciences (USA)" that there is now a new vaccine against meningococcus B, probably it is a conjugate protein- polysaccharide, constructed in such a way to render the polysaccharide immunogenic.

Another comment to Col. Clardy's paper. I think it is also important to point out in the very fine study that your group did, that the absence of detection of antibodies is not necessarily associated with lack of protection. Another question that I think you alluded to in your discussion related to the sensitivity and specificity of the detection system. We have to be careful in saying that those individuals who lack detectable antibodies are not protected. And in fact the studies that you reported with the primary and secondary response were very informative. You made some extrapolations, I didn't quite understand, related to the percentages of individuals who are susceptible, based upon the primary antibody response, and I think, if I judged the numbers correctly, we can extrapolate something like 2% for measles, 9.4% for mumps and 6.2% for rubella. Could you explain how you did this extrapolation from primary immune response and how these figures were calculated?

W.F. CLARDY (USA): That was 7.2% for measles, 9.4% for mumps and 6.2% for rubella.

J. A. BELLANTI (USA): That was the group that was susceptible.

W.F. CLARDY (USA): Those were classed as susceptible when they did not demonstrate a primary antibody response.

J. A. BELLANTI (USA): And no detectable antibodies.

W.F. CLARDY (USA): They were considered to be true susceptibles, because of their antibody response.

J. A. BELLANTI (USA): These figures very nicely show and are remarkably close to the protective efficacy that we see with measles immunization, which is in the order of 93-95%. And the last point that I would like to make is the current recommendation of giving 2 immunizations for children; I think it can be attacked in the following way. The problems we have

in the USA, and I imagine in developing countries, is that we have a group in the 0-4 year old children's group, who don't get immunized. When children are 5 years old or older, of course, they get immunized and need to be documented before the entry into school. What we have seen in our country, as you know, is that children in the inner cities are underserved, don't get immunized in their age group and they form a reservoir of infection, and when the virus is introduced in that susceptible population, there are these epidemics that emerge and if we could immunize that group, there would be no need for a second immunization. The second group we see in college students and presumably in recruits, because the fact that the vaccine isn't 100% effective, would never be a problem if we can get to the primary group and eliminate this reservoir of infection and this is the point I wish to make. And I would recommend that, and I know that the American Academy of Pediatrics and US Public Health everywhere are trying to get to that group. This is a very difficult problem, as you know. But that seems to me to be the primary reason for this second peak we see in the older, at least in the recruit group.

W.F. CLARDY (USA): These recruits seem to be not a unique group, but certainly a group of people who are to be immunized for more than one reason, rather than the population you described. There was a period of time when vaccination was not in vogue and people were not immunized for several reasons. Measles and mumps have become epidemic now in the same group. As this cohort ages, you won't expect the recruit population of susceptibles to diminish. I have no problem in the reimmunization of someone, because it is not dangerous. Not on a routine basis.

J. A. BELLANTI (USA): Even the best immunization never reaches 100% of protection. The factor is that we will have an acceptable proportion of adult population, even at best.

R. D'AMELIO (IT): I have a few words to add to the reply of dr Biselli to prof. Aiuti, about complement deficiency and meningococcal meningitis. This is a great problem all over the world, because, it is now known that all subjects with late complement factor deficiency are at high risk for meningococcal meningitis. So, during the last months, we made a retrospective survey of 59 cases of meningococcal meningitis occurred during the last 5 years in Italy. Among these cases we found 10 cases with terminal complement factor deficiency (C6, C7 and C8) (17%). So, also in Italy it is confirmed the association between late complement factor deficiency and meningococcal meningitis. The new thing is that this association has been described, until now, for complement deficiencies and uncommon meningococcal serogroups. In our experience this association is beween complement deficiency and common meningococcal serogroups (A, B and C). I believe this is important information, because it is an additional reason for the introduction of meningococcal vaccination among Italian recruits, because these people are, also in the Italian population, at high risk of infection and they can be protected by vaccination. These people, in fact, as dr Biselli replied to prof. Aiuti, seem to respond to meningococcal polysaccharides with a protective response as normal people.
Regarding the observation by Prof. Bellanti, on the scare of the possible emergence of new meningococcal serogroups, this was also our scare. Until now in Italy there isn't such an emergence, and since the introduction, 5 years ago, of vaccination as a compulsory in the schedule of military recruits, we could not observe an increase of serogroup B.
And lastly to dr Gray. I think your question was a very important one. About the moment of immunization, if related to acute mountain sickness or during acclimatization, our rationale was the study during acclimatization, because many troops are forced to live at high altitude for relatively long periods.

M.D. PARKINSON (USA): A question I had. All the services in the USA in the last few years have been involved in problems linked to streptococcal infection, such as rheumatic fever. I am curious if there is a similar problem in Europe.

S. FARRACE (IT): I'd like to give some more explanations about your question on altitude problems regarding meningococcal vaccination. We climbed up to 16000 feet in about 5 and half days and we administered the vaccine in the very first days after we reached 16000 feet. Some subjects were still undergoing symptoms of acute mountain sickness. So I think that this further information confirms that there was no effect of hypoxia in the response to vaccine in those cases.

D.S. BURKE (USA): I have one comment and one question. When we started the screening for HIV in all incoming recruits into the USA military, we obtained serum specimens for testing a centralized serum bank. So, in the last 2 years, we collected all these sera and stored them. Prospectively, in a data bank, and in a frozen serum bank, it would be possible to test for antibodies prospectively. So in the question like - where are protective levels of antibodies against several illnesses? - having a prospective pre-illness sample may be very useful in answering those questions.
That's the reason we did this and we decided to maintain an expensive sera bank for long time. This may be worth investing in terms of research finding for acute disease, like this, and also for chronic diseases, perhaps there will be other agents that may be discovered in the future. That's the comment, and it will be available to the Air Force in the future.
The question is for dr Rappuoli. Could you explain to me the epidemiology of diphtheria in developed countries? How common are carriers of diphtheria and if you do a surveillance for presence of the organism in populations today, how common is it encountered? Does the immunization have any effect on the epidemiology of bacteria itself?

R. RAPPUOLI (IT): There are no recent studies on the epidemiology of diphtheria. The last experience has been in Roumania, where diphtheria vaccination was introduced in the '50s. There, the introduction of the vaccination which was supposed to be only against the toxin and not against the bacterium, surprisingly decreased also the circulation of the bacterium. So diphtheria vaccination not only eliminates the disease, but also the bacterium.

D.S. BURKE (USA): Is there any attempt in tryng to culture diphtheria from individuals who have been vaccinated to determine whether or not it affects the carrier rate?

R. RAPPUOLI (IT): If I remember correctly, in Roumania they cultured vaccinated people and concluded that vaccination decreases the circulation of toxinogenic *Corynebacterium* of diphtheria. We have done a study in Italy, and we have not been able to find any toxinogenic strain in normal population, while we found a lot of normal commensal *Corynebacteria*. Nevertheless, we have the disease. This is because the bacterium can be imported very easily by travellers.

R. STEFFEN (CH): You mentioned repeatedly that diphtheria has been imported repeatedly by travellers unaware of any published case or any sistemic diphtheria which was imported. On the other hand, there are rather many cases of imported cutaneous diphtheria, partly also in immunized subjects. Am I

right in assuming that immunization against diphtheria is giving at most a marginal protection for the form which is often imported?

R. RAPPUOLI (IT): You are correct. Most of the reported cases of imported diphtheria are cases of cutaneous diphtheria and it is difficult to answer because that isn't the most common phenomenon I remember well one case of systemic diphtheria in England, which was published in "The Lancet" a few years ago by Pappenheimer and Murphy. A young girl went to a school and imported a phage. This phage (she wasn't sick, because she was protected) colonized the commensal *Corynebacteria* of the children of the same school and those got sick. So, a viral transmission of a bacterial disease was demonstrated, because in diphtheria the genes are carried by the toxinogenic phage.
There are studies in Sweden where they had an outbreak of diphtheria. The strain which caused the epidemics in Sweden had been isolated in Denmark a few years before. That's not a long travelling distance.

R. D'AMELIO (IT): Is there any evidence of specific cell-mediated immunity against your diphtheria vaccine, in addition to specific humoral antibodies?

R. RAPPUOLI (IT): We have not tested that yet. That will be tested.

J. A. BELLANTI (USA): A question to Dr Rappuoli. I found the hypothesis that you put forth of the cross linking by peptons is very attractive. I haven't heard that before. We know that the reactions are only observed in adults, not in children, so the amount of Ld50 of toxoid is greater in the infant preparation than in the adult, it cannot be considered responsible for the appearance of these reactions. If you could just look at the hypothesis you put forth, that if there is cross linking of peptons in the preparation by the formaldheyde treatment, and if you look at the fact that children don't get reactions after 4 or 5 immunizations, then there must be something different in the children than in the adults. I suppose it's possible that adults eat beef and they may have allergic antibodies to peptons, that are not seen in children. But I wonder if you want to comment on the other possibility, that reactogenicity may be related to the greater Ld50 toxoid content in the infant preparation.
And the last question I would like to direct to you is: a few years ago, as you know, we saw some non-sterile abscesses with some of the preparations of diphtheria/tetanus that we used and I believe that this was related to the amount of adjuvant.

R. RAPPUOLI (IT): Obviously when we talk about side effects, there are many players. So, it is difficult to dissect one side effect from the other. The amount of adjuvant, aluminium hydroxide or phosphate, is, of course, important. And I guess the story of abscesses that you observed was due to an excessive amount of adjuvant. In the case of diphtheria, I can't claim that 100% of reactions were due to cross linking of peptons. But I think that we have been discussing this with Pappenheimer and Murphy on people with side effects by the vaccine from the '50s and there is a common consensus that impurities, especially from bovine origin, may contribute a lot to the reactogenicity of diphtheria antigens in the adults. That doesn't mean that there are no side effects which are only due to the antigen. This hypothesis, I think, may be strengthened by the fact that in Denmark, at the State Serum Institute, they produce a diphtheria toxoid first purifying the toxin and then detoxifying that by formaldehyde. So, they don't get peptons and they use the children dose in adults, without observing any adverse reaction. There is only one paper which has been published, which I think substantiates what I said.

R.E. SPIER (UK): A question to dr Torrigiani. Does WHO put enough effort effort and money into fertility vaccines, in order to give people the option of choice as to where and when they have a child and bearing in mind that, whether the children they are going to have, are going to survive?

G. TORRIGIANI (CH): It is not easy for me to answer, because I am not responsible for this Programme. I used to be very close to it in the past when I was Chief of Immunology. I do not feel that you should take what I say as the WHO position. WHO has been supporting research on fertility vaccines for a long time. The Programme, in the past, was covering anti-sperm vaccine and vaccines against several antigens of the female and male reproductive tract. Recently the emphasis has been concentrated on vaccine against chorionic gonadotrophin. The idea was to have a vaccine against an antigen present only during pregnancy which would not cause anti-immunity. To assure that this did not occur, WHO decided to use only a specific peptide of HCG and not the all ß-subunit. Of course, this is not very immunogenic - it needs to be conjugated with different proteins and given together with an adjuvant.
The Programme has also given the conjugated peptide in microcapsules for prolonged release. I believe that field trials (phase 1) have commenced but at the present time I do not know the results. I believe that the Programme will also consider other aspects.

D.S. BURKE (USA): I think your question is an optimistic one, because as far as I know the projections from many countries now, where this might have been an issue a decade ago, the HIV epidemics are changing that equation, so that the net population growth would be negative rather than positive.

R.E. SPIER (UK): I think this is true for a very small portion of total world population.

G. TORRIGIANI (CH): I am sure that in Africa you are 100% correct. I think that in other countries, like China, they have an enormous population growth and so far, I don't know the statistics, HIV is not so serious as in Africa.

CLINICAL AND IMMUNOLOGICAL RESPONSE TO VACCINATION WITH PARENTERAL OR ORAL VACCINES IN TWO GROUPS OF 30 RECRUITS

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INTRODUCTION

Typhoid fever is a distinctive acute systemic febrile infection of the mononuclear phagocytes caused by several Salmonella species, mainly *S. typhi, S. paratyphi A and B* and occasionally S. typhimurium. Men can be infected by contaminated water or food contaminated by a human carrier.With improvements of environmental sanitation in developed countries the incidence of typhoid has gradually dropped. In Italy the incidence of typhoid fever is rapidly declining, but areas with endemic typhoid still persist. Prevention of typhoid fever relays primarily on prevention of exposure, but immunization is indicated in endemic areas, in travellers to these areas, in household contacts of typhoid carriers, in laboratory or sewer workers and in military personnel (1). The use of parenteral vaccines (TAB) has shown to be effective (2-4), but associated with a high incidence of local and systemic adverse reactions (5). The availability of a galactose epimerase mutant *S. typhi* (Ty21a) (6), that invade mononuclear cells, stimulate immunity but neither survive within the cells and, therefore, is avirulent, gave the possibility to test a vaccine whose administration route parallels that of natural infection (7, 8). Before the introduction of the mandatory vaccination with oral vaccine in Italian Air Force recruits, we performed a comparative in vitro study on the clinical and immunological responses to typhoid vaccination with TAB and oral vaccine in two groups of 30 adult male subjects in order to establish in vitro parameters of its efficacy.

MATERIALS and METHODS

After obtainment of an informed consent, the first of two groups of 30 healthy male volunteers, aged between 16 and 22 with negative fecal coltures of *S. typhi* and with irrelevant amount of serum anti *S. typhi* lipopolysaccharide antibodies (anti-LPS), were vaccinated subcutaneusly with TAB (ISI, Milano, Italy) on day 0 and 28. The second group was treated with Ty2la (Neotif, Sclavo, Italy) on days 0, 2 and 4.
Blood specimens were drawn on days 0, 15 , 30 and 240. Fecal samples were obtained on days 0, 30 and 240. On day 30, fecal coltures for *S. typhi* were repeated in the orally vaccinated group. Aliquots of blood specimens were allowed to coagulate, centrifuged, then the sera were stored at -70°C until use. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood aliquots after centrifugation at 900 g X 20 min. on a density gradient (Lymphoprep). For total and anti-LPS fecal IgA determination, feces (10 g) from each subject were centrifuged and supernatants diluted 1 : 10 with phosphate buffer saline (PBS).
Serum IgG and IgA or fecal IgA concentration was determined in a nephelometric assay. Standard enzyme linked immunosorbent assays (ELISA) were used for anti-LPS IgG, IgA and IgM determination as well as for rheumatod factor (RF) with minor modifications. Briefly, RF were determined by using 96 well polystirene plates coated with rabbit IgG (10 μg/ml) as antigen. Serum samples were diluted 1: 50 in PBS and, after incubation, IgA and IgM RF were detected with alkaline phosphatase conjugated $F(ab)_2$ goat anti human (AP-anti-H) IgA or IgM. IgG RF was determined by pretreating sera with reducing and alkylating agents (0.4 M 2-mercaptoethanol and 0.4 M iodacetamide) and using an AP-anti-H IgG prepared in our laboratory immunizing the same rabbit from which the IgG used as antigen were previously obtained, in order to minimize the background. Internal positive standard reference sera were included in each plate of all tests, together with negative sera. Results were expressed in ug/ml.
To determine the relative percentage of lymphocytes of the CD4 and CD8 subset, PBMC were labelled with monoclonal antibodies OKT3-4-8 (Orto Pharmaceutical Corp. Raritan, N.J.) and then with fluorescein isothiocyanate-labelled goat anti-mouse IgG (Melory Labs., Mississuaga, Ontario, Canada). The percentage of fluorescence positive cells was determined by the means of a Leitz UV microscope equipped with vertical illumination.
An antibacterial assay was performed as previously described (9) to determine the antibody dependent leukocyte inhibition of bacterial growth. The results were expressed as percentage of antibacterial activity (% aBA) calculated as follows: % aBA = 100-[100 x (number of colonies in experimental tubes/number of colonies in control tubes)]. F(ab)2 goat anti human IgG, IgA or IgM were used in specific antibacterial cellular inhibition.
Statistical analyses were carried out by one-way analysis of variance modified for randomized groups or Student's paired t test.

RESULTS

An increase in serum concentration of IgG anti LPS was observed in both groups of vaccinated subjects 30 days after immunization, whereas a slight increase of serum IgA was observed only after Ty21a immunization. At day 240, serum concentration of specific antibodies returned to prevaccination titers, with the exception of IgG in the TAB vaccinated subjects. A significative increase of IgM RF was observed only in the TAB vaccinated group at days 15 and 30 (fig. 1). At days 30 and 240, an increase of fecal IgA and an even more significative increase of specific IgA anti LPS in orally vaccinated subjects was observed (fig. 2). Specific IgA were still detectable after 8 month from the immunization.
No significant variations were observed either in the percent or in the absolute number of CD4 and CD8-positive PBMC, so that the T4/T8 ratio remained virtually unchanged on both days 15 and 30 versus day 0.
At day 30, a significative increase of direct antibacterial activity was observed in Ty21a-vaccinated subjects against *S. Typhi*, cross-reacting S. paratyphi A and B but not against antigenicaly unrelated C. This activity was still present against *S. Typhi* at day 240 (tab1). Arming activity of vaccinated sera on PBL from non-vaccinated subjects (tab 2) was increased in Ty21a vaccinated subjects only.
Inhibition experiments demonstrated that F(ab)2 anti-human IgA completely blocked the natural as well as the Ty21a-induced antibacterial activity. Pretreatment with F(ab)2 anti-human IgG, on the contrary, abolished the low natural antibacterial activity of sera from TAB vaccinees.
None of the subjects treated with live attenuated oral vaccine reported noteworthy side effects, whereas 65% of TAB-vaccinated subjects reported local tenderness at the site of injection, general malaise, fever or headache.

DISCUSSION

In the industrialized world, the incidence of typhoid fever declined rapidly during the 20th century as sewage disposal and water supplies improved, but recurrencies have been reported during natural catastrophes and in war scenarios (1). In developing countries, on the contrary, typhoid fever is still a problem, with millions of reported cases occurring every year.
International travel to areas with endemic typhoid has accounted for an increasing proportion of all cases. Military personnel is at risk for typhoid because of inadequate hygienic supplies that might be realized in temporarily utilized camps, in combat conditions or because rapid mobilization to war scenarios in endemic areas.
In Italy the incidence of typhoid fever is rapidly declining, but areas with endemy sill persist. The decline of typhoid in the general population parallels the decline observed in the number of cases gathered in the centre for disease control of the Italian Armed Forces (Direzione Generale di Sanità Militare)(fig. 3). The

traditional heat-phenole inactivated vaccine TAB has shown to be effective in enhancing human resistence to typhoid but determines side and occasionally severe effects. When a new live attenuated oral vaccine, prepared with enzyme deficient microorganisms, Ty21a (6), has been licensed and has resulted in long lasting (nearly 3 years) and effective protective immunity (8, 10,11), it seemed an ideal candidate for compulsory vaccination of military recruits. In fact, experiences with oral vaccine Ty21a in Egypt and Chile showed a general good efficacy with few minor adverse reactions, and its overall safety has been proven.

Before the introduction of the mandatory vaccination with oral vaccine in Italian Air Force recruits, we performed a comparative in vitro study on the clinical and immunological responses to typhoid vaccination with parenteral TAB and oral vaccine in two groups of 30 adult male subjects in order to establish in vitro parameters of its efficacy.

In accordance with literature data concerning clinical tolerance, in this study Ty21a has proved to be completely safe, whereas TAB induced local marked local phenomena and systemic symptomatology in over 65% of vaccinated subjects. manly after the second injection.

Immunological monitoring was done by evaluating cellular and humoral specific and nonspecific alterations. Specific cell-mediated immunity, assessed in PBMC by a simply short-term in vitro assay, involved the appearance of a strong specific immune response against *S. typhi* on day 30 and also against *S. paratyphi A and B* in subjects immunized with Ty21a, whereas a complete absence of response was observed in TAB-vaccinated subjects in this test (tab.1). Cell-mediated activity against *S. typhi* was still present on day 240 in Ty21a vaccinees (but did not appear in subjects taking TAB). Thus, these results confirm our previous experience (10,11) and clearly indicate that the immune responses induced by oral and parenteral vaccines differ greatly. Taking into account the presence of fecal IgA which was observed after Ty21a vaccination in this and a previous study (12), it might be suggested that the induction of specific antibodies of IgA isotype against *S. typhi* and perhaps cross-reacting bacterial species is the primary event of vaccine-induced immunity. This may then lead to a mucosal response mediated by secretory IgA and/or local and systemic IgA-driven cellular immunity.

Since lymphocytes of the CD4 subset have been shown to be the cellular arm of IgA-dependent antibacterial activity (11,13), the observation reported here that CD4 cell numbers are not increased in Ty21a vaccinees could further indicate that the vaccine action resides mainly in a potentiation of the humoral arm of lymphocyte antibacterial activity. A study by Levine and coworkers (14) concerning two attenuated auxotrophic mutant strains of *S. typhi* tested as vaccine candidates has also shown the presence of antibody-driven cellular immunity. All these results taken together suggest that this is a general immune mechanism evoked by oral vaccines.

As far as the parenteral vaccine is concerned, it was shown here that TAB is not able to induce cellular immunity beyond natural values (14), nor is it capable of generating arming antibodies, as was observed with Ty21a. It is, however, of great interest that blocking experiments showed that the low cellular immunity measurable after TAB vaccination is mediated by IgG rather than by IgA, as is the case in natural immunity (14) and oral vaccine-induced immunity (11). A major role of IgG after parenteral vaccination is widely expected, and the animal models for salmonellosis have demonstrated the role of complement and/or phagocytic cells (15, 16,17). Indeed, TAB vaccine has shown efficacy for decades in millions of people, even though the extent of its action may be debatable. The preliminary observations reported, that the administration of a parenteral vaccine would substitute an IgG-driven response for IgA-dependent antibacterial activity, stimulate further investigation.

A first, rather evident effect of qualitative change in antityphoid immunity by TAB vaccine might be the appearance of RF. In fact, IgM-RF was observed in subjects vaccinated with TAB on days 15 and 30, whereas no increase could be detected in IgG-, IgA-,or IgM-RF in Ty21a vaccinated subjects. This increase was transient, however, and was no longer present on day 240, similar to results in a case observed by us of subjects vaccinated against hepatitis B with a vaccine of French manufacture (18). The significance of such an increase is uncertain, in the light of recent results by Nemazee (19) on the induction of RF in mice after the secondary response and the enhancing effect of such RF in antigen-antibody reaction, as stated by the same author. This might be either a physiological response of the immune system to the formation of circulating immune-complexes, with a regulatory role, or a sign of B-cell polyclonal activation. We conclude that the oral vaccine is safe and immunologically efficacious.

We have shown here, in fact, that in addition to the lack of strong side effects with Ty21a, contrary to what occurs with TAB, marked differences exist between the two vaccines in evoking a specific immune response. It is important to further elucidate advantages and disadvantages of the two types of immunization in consideration of the continuous effort to provide new, and hopefully more effective, vaccines against *S. typhi*, such as the Aro mutant (14) or the parenteral anti-Vi vaccines (20) and considering that little experience still exists in using the oral vaccine in certain populations subgroups, as pregnant or lactating women, infant or young children and acutely ill persons nor to evaluate the effect of simultaneous use of Ty21a with other vaccines. In addiction, the vaccine's efficacy and duration of protection in overseas travelers from developing countries has not yet been firmly established. Finally mass vaccination programs may be difficult: the Ty21a vaccination schedule is distributed during a period of five days which relies strongly on patient behavior rather than of physician direct control.

SUMMARY

The clinical and immunological responses to typhoid vaccination with parenteral and oral vaccines in two groups of 30 adult male subjects were studied. Specific anti-*Salmonella typhi* cell-mediated immunity and total or specific anti-lipopolysaccharide fecal Immunoglobulin (Ig) A titers in vaccinated subjects were monitored. Cellular antibacterial activity was significantly increased only in orally vaccinated subjects. Serum arming activity and inhibition experiments suggested an IgA-dependent cellular cytotoxicity in those orally vaccinated. In these subjects, a total and anti-lipopolysaccharide fecal IgA increase was observed lasting up to 8 months after completion of vaccination schedule. In parenteral vaccinated subjects, an early onset transitory increase of IgM rheumatoid factor was observed. Oral vaccine was well tolerated and free of side effects, whereas 65% of parenterally vaccinated subjects reported side effects such as fever, headache, malaise and local tenderness in the injection site.

Table 1: Cell-mediated immunity against Salmonella Infection.

Vaccine	Time (day)	E/T [a]	% Antibacterial activity against: S.Typhi	S.Paratyphi A	S.Paratyphi B	S.Paratyphi C
Ty21a	0	6	-25	-20	-12	-3
		12	-8	-13	-3	-10
		25	-0	-2	-3	-3
		50	-8	1	-1	7
	30	6	8	12	6	-16
		12	25	22	16	2
		25	37	37	13	-8
		50	53[b]	42[b]	18[b]	-9
	240	6	19	nd[c]	nd	nd
		12	19	nd	nd	nd
		25	23	nd	nd	nd
		50	43[b]	nd	nd	nd
TAB	0	6	-5	-5	-7	-15
		12	-8	3	1	-15
		25	-1	4	3	-15
		50	15	12	2	11
	30	6	-3	-8	-11	-7
		12	1	8	9	-10
		25	9	-9	-13	-19
		50	17	16	8	8
	240	6	1	nd	nd	nd
		12	6	nd	nd	nd
		25	10	nd	nd	nd
		50	15	nd	nd	nd

[a] E/T= effector/target ratio; [b] P<0.01 versus values at day 0; [c] nd= not done

Table 2: Arming activity of serum samples from volunteers before and after vaccination with oral or parenteral vaccine

Vaccine	Time	% Antibacterial activity at E/T : 12	25	50
none		12	5	13
Ty21a	0	13	10	17
Ty21a	30	43	41	49
TAB	0	12	4	4
TAB	30	8	12	14

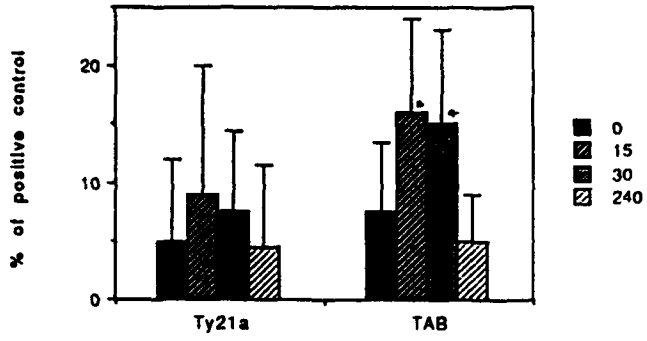

Fig. 1: IgM-RF at O, 15, 30 and 240 days from the first administration of TAB or Ty21a in two groups of 30 subjects. An asterisk indicates P<0.01 versus value at day 0.

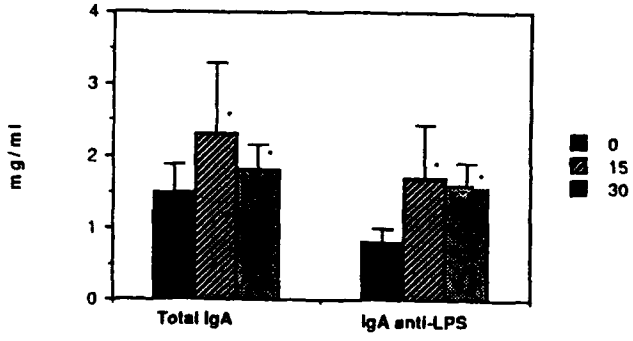

Fig. 2: Total and anti LPS fecal IgA at 0, 30 and 240 days from oral administration of Ty21a. P<0.01 (•) and P<0.05 (*) versus valua at day 0 are indicated.

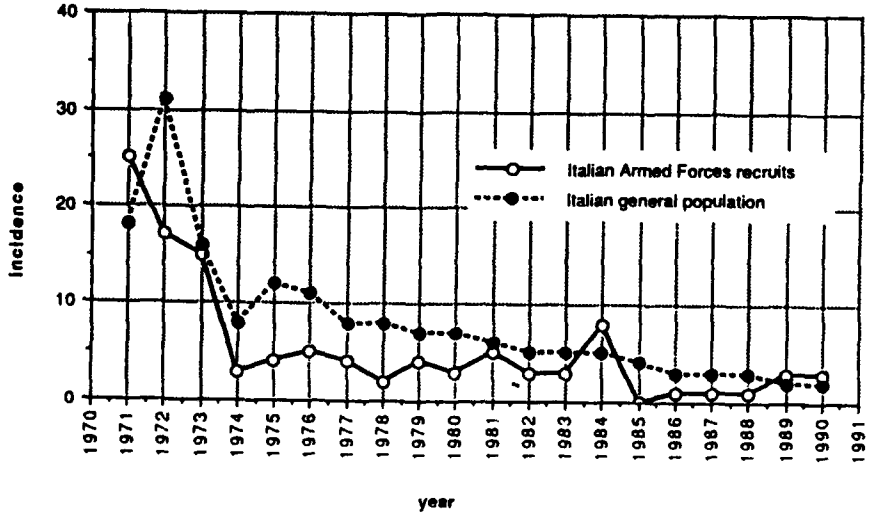

Fig.3: Incidence of typhoid fever in the Italian population and in the Italian Armed Forces recruits in a period of 20 years (1970-1990)

STUDIES OF SAFETY, INFECTIVITY AND IMMUNOGENICITY OF A NEW TEMPERATURE SENSITIVE (ts) 51-1 STRAIN OF S. TYPHI AS A NEW LIVE ORAL TYPHOID FEVER VACCINE CANDIDATE

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SUMMARY
This report describes the results of a phase 1 study evaluating the safety, infectivity, and immunogenicity of a new live oral **S. typhi** ts 51-1 typhoid fever vaccine in the human. Three normal male subjects ranging in age from 20-40 years received 3 oral doses of **S. typhi** ts 51-1, each dose containing 10^9 organisms. Prior to and following immunization each subject was carefully monitored by clinical and laboratory parameters over a two week period during which serial specimens of blood and stool were analyzed for the presence of the organism. Blood specimens were also obtained for the determination of serum antibody and cell-mediated immune responses and stool filtrates were analyzed for the development of coproantibody. The results of these studies indicate that: 1) the vaccine is well tolerated with no clinical or laboratory evidence of adverse reactions; 2) ts 51-1 was detected in only one stool specimen from one volunteer; the organism recovered displayed characteristics of the ts 51-1 vaccine strain; 3) although no significant humoral or cell-mediated lymphocytotoxic immune responses were detected in the blood, coproantibody was detected in stool specimens from all of the 3 immunized subjects and IgA-armed ADCC activity was detected in 2 of 3 subjects.

These studies indicate that S. typhi ts 51-1 may be a suitable strain for the development of an improved oral typhoid fever vaccine. Studies are in progress to determine optimal methods of vaccine delivery preparatory to larger phase 2 studies of efficacy.

1 INTRODUCTION

Typhoid fever continues to be a major health problem, particularly in the developing countries of the world where primitive conditions of sewage disposal and contamination of water contribute to the spread of the disease.
Over the years a wide variety of vaccines have been used for the prevention of typhoid fever. Until recently the only typhoid fever vaccine available for clinical use was the phenol heat-killed vaccine which has a protective efficacy of approximately 50-70% (Ref 1). In recent years, a live, oral **attenuated gal epimerase** deficient strain, **S. typhi** Ty21a was licensed for human use (Ref 2,3). Although the Ty21a vaccine has been shown to offer greater efficacy than the killed vaccine in selected field trials, problems of stability following oral administration have contributed to suboptimal levels of protective immunity in subsequent field studies (Ref 4-6). A need clearly exists, therefore, to develop an improved live attenuated oral typhoid vaccine with greater stability, improved immunogenicity and enhanced protective immunity.

In response to this need, our laboratory has been engaged over the past several years in the development of a genetically engineered strain of **S. thypi** (ts 51-1) containing three independent temperature-sensitive (ts) lesions (Ref 7). Because of the nature of the attenuation, strain 51-1 grows optimally at a permissive temperature (29°) and ceases replication at the non-permissive temperature (37°) and therefore has the dual advantage(s) of greater stability and immunogenicity and of minimal reactogenicity with an estimated reversion frequency of 10^{-21}. This report describes the first clinical studies in the human to assess the safety, infectivity, immunogenicity and genetic stability of the ts 51-1 strain of **S. typhi** as a potential new, live oral typhoid fever vaccine candidate.

2.0 METHODS

2.1 Vaccine

The vaccine used in this phase 1 study was a liquid preparation of the ts mutant 51-1 (ref 7). Single harvest cultures of ts mutant 51-1 maintained at -70°C were thawed and used directly and an aliquot was plated on trypticase soy agar (TSA) on the day of vaccination to verify dosage size and other identifying characteristics. The identity of the organism was confirmed by thermal restriction, growth characteristics on selective media and by serologic identification using **S. typhi** *O, H and Vi antisera.*

2.2 Subjects

Three healthy young male subjects (1, 2, 3) ages 32, 26 and 23 years, respectively, were entered into the clinical study unit of Georgetown University Hospital for a 15 day period after entry criteria were met and pre-immunization testing performed. The entry criteria consisted of the following: the health of the participants before immunization was assessed by medical history, physical examination, electrocardiogram, chest radiograph, and laboratory tests including a complete blood count (CBC), platelet count, blood chemistry analysis (Chem 20), liver function tests, serologic tests for syphilis, hepatitis B surface antigen, and human immune deficiency virus (HIV) antibody.

The study was explained in detail and witnessed and written consent was obtained and each subject was hospitalized for a total of 15 days.

2.3 Dosing plan

Each of the three subjects received a single oral dose of the ts mutant 51-1 containing 2-3 x 10^9 viable organisms on each of three successive alternate days (i.e. days 1, 3, and 5). Each subject ingested 120 ml of a 1.3% NaHC03 solution to neutralize gastric hydrochloric acid in order to enhance vaccine survival during passage through the stomach. Approximately 1-5 minutes later each subject ingested an additional 30 ml of the 1.3% NaHC03 solution to which 1 ml of a single harvest vaccine stock had been added.

2.4 Experimental design

The experimental design of the study is shown in Table 1. Before vaccination, in addition to the hematologic and biochemical studies performed as part of entry criteria, stool specimens were examined for the presence of bacterial enteropathogens and ova and parasites. Pre-immunization blood specimens were obtained for the measurement of serum antibody to **S. typhi** O, H and Vi antigens and for the determination of cell-mediated immunity studies using a mononuclear leukocyte mediated bactericidal assay (Ref 8). These assays were performed at intervals during this 15 day period of hospitalization and at 90 day

intervals for 1 year after immunization as shown in Table 1.

During the 15 day period of hospitalization all stools passed by the volunteers following immunization were collected and immediately transported to the laboratory for determination of consistency, weight and bacterial culture as wvell as for determination of coproantibody (Table 1).

2.5 Immunologic Studies

Specific methods for the measurement of serum antibody to **S. typhi O**, H and Vi antigens consisted of an ELISA method. Heat inactivated wild Ty2 **S. typhi** served as a source of 0 antigen; a formalin fixed motile strain of wild Ty2 S. typhi served as a source of H antigen and live ts 51-1 as a source of the Vi antigen. In addition the presence of antibody to Vi antigen was measured by radioimmunoassay kindly performed in the laboratory of Dr. J.B. Robbins.

Similarly the measurement of IgA associated coproantibody was performed by an ELISA method using antigens prepared as described above but modified to detect secretory IgA antibody by the use of a secretory IgA specific antiserum.

Lymphocytotoxicity assays using patients'lymphocytes and serum were performed to detect cell-mediated bactericidal activity during the first week of the study (Ref 8).

Specialized competitive-inhibition studies of antibody-dependent cell cytotoxicity (ADCC) (Ref 9,10) using normal donor effector lymphocytes were kindly performed by Dr. L. Nencioni on 1:50 and 1:100 dilutions of preimmune sera and sera obtained at 1 and 2 months following immunization. The identification of the specific immunoglobulin isotype responsible for the ADCC was determined by competitive-inhibition assay using rabbit affinity purified unrelated IgG and sIgA antibody against Shigella X16.

2.6 Bacteriologic studies

Blood specimens were obtained on alternate days beginning on day 2, to determine any evidence of bacteremia. Stools were resuspended in sufficient media to achieve a final concentration of 10%. In the initial phases of these studies the suspending media consisted of the selective media, tetrathionate broth (TTB); in later phases, 0.15M phosphate buffered saline (PBS), pH 7.9, was found optimal in preserving IgA coproantibody and was used in all subsequent studies. Stool suspensions were titrated and plated on bismuth sulfite media to determine colony forming units (cfu). All specimens of blood and stool were cultured at both the permissive temperature (29°C) and the non-permissive temperatures (37°C).

3.0 RESULTS

3.1 Clinical and general laboratory parameters

The vaccine was well tolerated by each of the three subjects and no adverse reactions were observed (Table 2). All of the hematologic and biochemical parameters performed after administration of three doses of live, oral ts 51-5 remained within normal limits and there were no significant changes in these values from those obtained prior to immunization.

3.2 Bacteriologic studies

All of the blood cultures performed in each of the three subjects were negative for any bacterial organism including Salmonella. There was no change in weight, consistency or normal flora in the stools of any of the three immunized subjects. All of the stool cultures remained negative for **Salmonella** with the exception of one stool specimen from subject 1 obtained 12 hours after the second oral immunization (Table 3) which was positive for **S. typhi** ts 51-1. The quantity of organism shed in this specimen was 1 $x10^5$ organisms per gram of stool (total 3 x 10^7 organisms shed) and the organism maintained its thermal restrictive characteristics with no evidence of reversion to a virulent parental **S. typhi** Ty2 strain. No other pathogenic bacterial organisms of any strain were detected.

3.3 Immunologic studies

Serum ELISA antibody determinations to Salmonella O and Vi antigens were performed on preimmunization sera and on sera obtained at 8, 15, 30 and at 90 day intervals after immunization. No significant rises in serum antibody were detected between pre- and post-immunization sera. Specialized studies of serum Vi antibody were also performed in the laboratory of Dr. John B. Robbins which also showed no rise in specific Vi antibody following immunization (Table 4).

Cell-mediated immune bactericidal responses to **S. typhi** were also performed using purified suspensions of peripheral blood lymphocytes from immunized subjects and an autologous serum enhanced and unenhanced methodology. No significant differences in bactericidal activity were observed between pre and post-immunization specimens.

3.4 Coproantibody studies

A series of specific studies were performed to examine for the presence of local secretory antibody in stool (i.e. coproantibody) which could have been stimulated as a result of oral immunization. Centrifuge-clarified and filter-sterilized suspensions of stool were prepared and IgG and sIgA coproantibody determined by ELISA. The results of these studies revealed that all 3 of 3 immunized subjects had detectable rises in sIgA-associated coproantibody to Vi and O antigens and 2 of 3 (subject 1,3) to H antigen of **S. typhi** (Table 5).

3.5 ADCC antibody

The results of ADCC assays are shown in Tables 1 and 2. Subjects 1 and 2 demonstrated significant arming activity of donor lymphocytes at dilution of 1:50 and 1:100 (data not shown). The ADCC activity of subjects 1 and 2 was completely blocked by the pretreatment of effector cells with unrelated sIgA but not by IgG indicating that the ts 51-1 vaccine was capable of stimulating sIgA mucosal immunity. It was not possible to demonstrate such activity in subject 3 because of the high natural serum antibacterial activity.

4.0 DISCUSSION

Until recently the only available typhoid fever vaccine was the phenol-heat killed vaccine which has a protective efficacy of 50-70% but, which, because of vaccine-associated adverse reactions, has had limited applicability. The search for new live oral vaccines with good immunogenicity and minimal reactogenicity led to the development of the Ty21a strain of **S. typhi** which has become the first licensed prototype live oral typhoid fever vaccine (Ref 2). Although shown to have an efficacy of 69% (Ref 1) with no reactogenicity, because of the nature of its genetic attenuation, i.e. gal epimerase deficiency, Ty21a suffers from a lability of the strain which presents difficulty with lyophilization, storage, and administration of the vaccine. Because of these problems several other strains of **S. typhi**, attenuated by a variety of techniques, have been developed.

For the past 8 years, a major investigative effort of our laboratory has been directed to the development and testing of a triple temperature-sensitive mutant of **S. typhi** as a new candidate vaccine strain (Ref 7). Following a series of controlled animal experiments (Ref 3,11) which demonstrated both safety and immunogenicity, the present studies were conducted to evaluate safety, infectivity and immunogenicity in the human. The results suggest that the vaccine is well-tolerated and unassociated with any adverse effects.

No significant gastrointestinal shedding of the organisms was seen; following 3 oral immunizations, only one stool specimen from a single subject (after second oral dose) revealed **S. typhi** which had the characteristics of the ts 51-1. No significant rise in serum antibody or lymphocyte-mediated bactericidal activity was observed, which is the usual response seen following immunization with live oral **S. typhi** vaccines. Of particular interest, however, was the development of sIgA-associated coproantibody in all 3 and the demonstration of sIgA-armed ADCC activity in 2 of the 3 immunized subjects. These findings are consistent with the induction of local mucosal-immune responses which may be important predictive markers of protective immunity to **S. typhi**. Based upon the results of these studies a larger clinical investigation is planned in which a comparative study will be performed to determine the effectiveness of vaccine delivered either in enteric-coated capsules or as a liquid preparation. In a recent comparative study of Ty21a in children (Ref 12) the liquid preparation was found to be preferable to enteric capsules. Thus,

S. typhi ts 51-1 appears to hold promise as a suitable candidate strain for a new live oral typhoid fever vaccine which offers additional advantages of safety, immunogenicity and stability.

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Table 1
Experimental Design

Specific hematologic, biochemical, microbiologic and immunologic parameters tested following immunization with ts 51-1

	Day of study																		
	Pre	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	30	q 3 mos	for 1 yr
Oral immunization		x		x		x													
Hematologic & biochemical studies	x															x			
Stool culture & coproantibody	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Blood culture			x		x		x		x		x		x		x				
Serology (O,H & Vi)	x							x								x	x		x
Cell-mediated immunity	x							x									x		x

Table 2
Record of adverse reactions during the first 15 days following immunization with ts 51-1

Symptom	Subject 1	2	3
Fever (≥37.8°C)	0	0	0
Malaise	0	0	0
Nausea	0	0	0
Vomiting	0	0	0
Diarrhea	0	0	0
Change in appetite	0	0	0
Abdominal discomfort	0	0	0
Headache	0	0	0
Feverishness	0	0	0
Other symptoms	0	0	0

Table 3
Results of stool cultures for vaccine or revertant strain(s) from subjects immunized with ts 51-1

Subject	Day 1 (1st)*	2	3 (2nd)*	4	5 (3rd)*
1	0	0	+**	0	0
2	0	0	0	0	0
3	0	0	0	0	0

* Immunization schedule

** 3×10^7 ts 51-1 were isolated from a single stool 12 hrs after 2nd immunization.

Table 4

Results of RIA determinations of serum antibodies to Vi antigen of S. typhi of 3 volunteers following immunization with ts 51-1

Subject	Pre ug Vi Ab/ml	Post • ug Vi Ab/ml
1	<0.030	<0.030
2	0.083	0.074
3	2.50	2.36

• Post-immunization specimens were obtained approx. 3 weeks after immunization

Table 5

IgA coproantibodies to Vi, O & H antigens of S. typhi in stool filtrates of 3 volunteers prior to & after immunization with ts 51-1

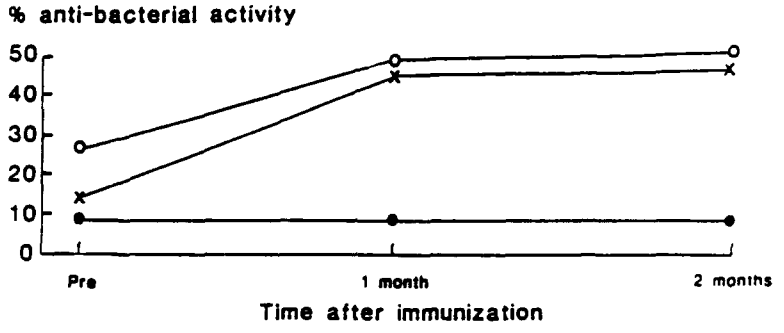
	Anti-Vi			Anti-O		
Subject	Pre	Post-1	Post-2	Pre	Post-1	Post-2
1	15	2 X	4 X	19	4 X	8 X
2	6	3 X	5 X	6	2 X	4 X
3	103	1.6 X	0	36	2 X	1.5 X

	Anti-H		
	Pre	Post-1	Post-2
1	5	1.6 X	3 X
2	1	0	0
3	12	1.6 X	0

Results are reported as ELISA values for pre-immunization specimens and X-fold rises for 2 specimens at 1 & 4 months post immunization.

Figure 1

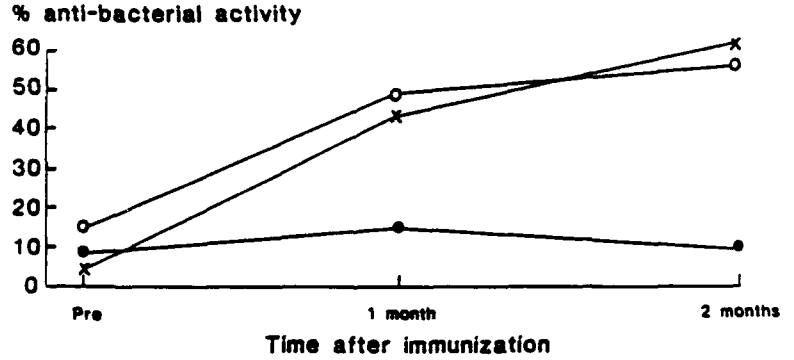
Results of antibody dependent cell cytoxicity (ADCC) assay using a 1: 50 dilution of serum of subject 1 prior to & after immunization with ts 51-1



Effector cells were either untreated (O) or treated with unrelated IgG (X) or sIgA (●)

Figure 2

Results of antibody dependent cell cytoxicity (ADCC) assay using a 1:50 dilution of serum of subject 2 prior to & after immunization with ts 51-1



Effector cells were either untreated (O) or treated with unrelated IgG (X) or sIgA (●)

RECENT LESSONS ON THE SAFETY AND EFFECTIVENESS OF MALARIA CHEMOPROPHYLAXIS IN A NON-IMMUNE POPULATION

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SUMMARY

To assess adverse events (AE) and the effectiveness of malaria chemoprophylaxis in short term travelers to East Africa, two similar follow-up studies were conducted, the second of which is ongoing. Pooled data of both studies are presented, in which all passengers returning from Kenya by British, German or Swiss charter flights were distributed a questionnaire aboard and a second one three months later. Any report of documented malaria or of a hospitalization for adverse events was investigated with the physician.

So far, 98,650 travelers have completed at least one questionnaire. AE were reported by 22.3% of 30,871 having used mefloquine (MQ), by 19.9% of 5,342 having used chloroquine (CQ) 300 mg base/week, by 22.2% of 7,930 having used CQ 600 mg base/week, by 29.4% of 1,114 having used amodiaquine (AQ), by 16.3% of 24,532 having used sulfadoxine/ pyrimethamine (SP), and by 26.1% of 6,851 having used CQ plus proguanil (PG). Dizzyness was more frequent with MQ than with other agents. Twenty hospitalizations were attributed to AE: seven occurred after prophylaxis with CQ (including 2 cases of psychosis), four were attributed to SP (2 fatal), and five to AQ (2 fatal). Just two were attributed to MQ: one each for psychosis and one for seizures in a known epileptic, one additional case with seizures was not hospitalized. — Prophylactic effectiveness was 94% (95% C.I. 85-96) for MQ, 84% (77-90) for SP, 74% (48-88) for CQ+PG, and 30-54% for CQ in various dosages.

In conclusion, mefloquine appears to be highly efficacious for malaria prophylaxis in areas with widely distributed chloroquine resistant P. falciparum. Tolerance seems to be comparable to the one of chloroquine, and thus the contraindication of the use of mefloquine in pilots will have to be reconsidered.

1 INTRODUCTION

Malaria chemoprophylaxis has become a concern for those having to advise persons planning to stay in an endemic area. This is due primarily to an increasing chloroquine resistance of P. falciparum and of other serotypes (1, 2), secondarily to severe adverse events attributed to chemoprophylactic drugs (3, 4), and thirdly to a lack of data which would allow to compare the risks and benefits of various regimens for a given destination. In prophylactic measures it is particularly important not to cause harm to a previously healthy population.

The recently introduced agent mefloquine has been anecdotically associated with a variety of adverse events, of which the neuro-psychiatric ones were the most severe (5, 6). They occurred primarily after a therapeutic usage (attack rate about 1%), but some cases were also reported after chemoprophylaxis (WHO, in preparation). As some cases occurred several weeks after the last dose of mefloquine, the World Health Organization was worried that persons having to fulfill tasks with fine coordination might be incapacitated and thus advised the International Air Transport Association that pilots should not fly during a period of three weeks after the use of this agent.

To be able to evaluate and to compare the rates of adverse events and the effectiveness of the drugs used for malaria prophylaxis, particularly the newly introduced agent mefloquine, a follow-up study has been initiated. This is a preliminary report of this still ongoing study.

2 METHODS

Similarly to a previous study (7), all passengers aboard British, German and Swiss charter flights returning from Kenya are asked to complete a questionnaire during their flight home. These questionnaires are distributed and collected by the cabin crews, the response rate aboard the airliners is approximately 80%. As malaria and adverse events may occur after returning home, a second questionnaire is mailed 3

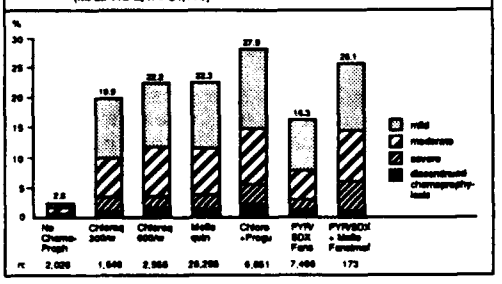
months later to all those who responded to the first questionnaire. Non-responders are contacted by mail, later by telephone. We finally achieve a response rate of 92% in this second step. Any report of malaria documented by a blood smear or of hospitalization for adverse events is investigated by contacting the doctors in charge of these patients and by inviting them to send a detailed report.

In comparison to the recently published similar study "Malpro 1" with 44,472 travelers which focused on Fansidar adverse events (3) we have in "Malpro 2" with so far 54,178 travelers added more questions on mild to moderate neuro-psychiatric adverse events. Thus, we base the analysis of mild and moderate adverse events only on Malpro 2 data, whereas we use the combined Malpro 1 plus 2 dataset (n=98,650) to compare the serious (defined here as hospitalized or any seizure) adverse events of various chemoprophylactic agents. The combined dataset will also be used to analyse the effectiveness of chemoprophylaxis.

3 RESULTS

Adverse events:
Travelers frequently report adverse events which they attribute to chemoprophylaxis; 16-28% report some ailment. Half of the side effects are subjectively mild, 38% of the travelers describe the side effects as moderate and 14% as severe. Only 1.7% discontinue chemoprophylaxis because of adverse events, and just 0.4% of the travelers are confined to bed for assumed side effects of chemoprophylaxis. Clearly, the combination of chloroquine plus proguanil was least well tolerated (figure 1).

Figure 1: Adverse events of malaria chemoprophylaxis reported by the travelers (MALPRO 2, n = 54,178)



The rate of bed confinement was the highest with 0.8%, twice the mean rate and also twice the rate as compared to mefloquine users. Similarly, the rates of discontinuation of chemoprophylaxis and of subjectively severe adverse events were higher in chloroquine plus proguanil users than with any other medication. Mefloquine was comparatively well tolerated, similar rates of adverse events were noted when compared to chloroquine users. Surprisingly, many travelers still continue to use Fansidar although it is not recommended by any expert group because of possible severe cutaneous adverse reactions.

The most frequently reported adverse event with all chemoprophylactic agents is nausea, noted most frequently in chloroquine plus proguanil users. In view of anecdotical reports of neuro-psychiatric adverse events after the use of mefloquine and chloroquine, we are of course particularly interested to know the rates of adverse events, which may be prodromal to such neuro-psychiatric impairment. Dizziness is observed significantly more often after the use of mefloquine, whereas no significant difference is noted with respect to headaches, depression, or insomnia. Visual problems tend to be more frequent after the use of chloroquine, whereas mouth ulcers were reported almost only after the use of proguanil.

To evaluate the serious health risks we must analyse the hospitalized adverse events. Severe neuro-psychiatric events were reported by users of chloroquine, chloroquine plus proguanil, and of mefloquine. Neuro-psychiatric adverse events were reported in three users of mefloquine (only two of which were hospitalized), which results in a rate of 1 in 10,000 prophylactic users. The rates just for neuro-psychiatric adverse reactions for chloroquine monoprophylaxis or for chloroquine plus proguanil were 1 in 7,500 and 1 in 7,000 respectively, which is very similar to the one in mefloquine users (table 1).

Table 1: Hospitalized probable (pr) or possible (ps) adverse events MALPRO 1 + 2 - April 1991

DRUG	USERS n	ADVERSE EVENTS pr	ps	fatal	TYPE OF ADVERSE EVENTS
Amodiaquine	1,114	5	-	2	Agranulocytosis (4), toxic hepatitis (1)
Chloroquine	15,379	7	-	-	Psychosis (2), gastrointestinal (3) skin (1), thrombocytopenia (1)
Chlo+Proguanil	7,082	2	-	-	Duodenal ulcer (1), psychosis (1)
Mefloquine	30,284	1(+1)	1	-	Seizures (2a), psychosis (1)
PYR/SDX	24,810	2	3	2	Toxic hepatitis (2), skin (2), gastrointestinal (1)

* patients additionally took other antimicrobials a: one patient not hospitalized

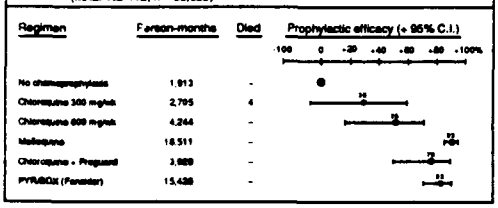
It is worthwhile briefly to describe some details in these three patients we observed: one patient with seizures was a known epileptic using continuous anti-convulsive medication. Use of mefloquine was clearly contraindicated in this traveler. The second patient with seizures both in Africa and back in Switzerland had no previous history of epilepsy, but continued to experience occasional seizures for many months after discontinuing the use of mefloquine. The patient with psychosis reported anxiety reactions without any previous similar history. The overall hospitalization rates

attributed to adverse events of chemoprophylaxis were 0.5 and 0.3/1000 in chloroquine and chloroquine plus proguanil users respectively and 0.07 in mefloquine users ($p<0.05$).

Effectiveness:
In the examined population, consisting predominantly of tourists staying on the coast of Kenya at least for one week, the monthly incidence rate of malaria was 17/1000 if they used no chemoprophylaxis. P. falciparum infections were dominant. This rate was reduced by use of appropriate chemoprophylaxis.

Mefloquine with a effectiveness rate of 93% was most effective, but there is so far only a borderline significance as compared to chloroquine plus proguanil or to Fansidar (figure 2).

Figure 2: Prophylactic effectiveness of various regimens of chemoprophylaxis (MALPRO 1+2, n = 98,850)



Chloroquine in the dosage of 600 mg base per week may be superior to the dosage of 300 mg base per week which did not offer an significant protection. It was in this group that four fatalities due to malaria were recorded, whereas none occurred in any other group.

4 DISCUSSION AND CONCLUSIONS

Such follow-up studies are prone of course to various biases. Above all, the drugs were not distributed along with the rules of a controlled, double-blind study and as the travelers were not questioned daily by a physician, there might be some recall bias. Nevertheless, the data generated in this study appear to be valid, particularly in view of the fact that travelers from different nations using the identical drug reported very similar results.

Those using mefloquine for chemoprophylaxis report a similar rate of adverse events as chloroquine users. They report significantly less adverse events then those using chloroquine plus proguanil. Only the rate of dizziness is slightly higher than with other agents. Surprisingly, the rate of neuro-psychiatric adverse reactions tends to be rather lower in mefloquine users as compared to chloroquine users. Both psychosis and seizures have been previously described after the use of chloroquine (8-10), but this was never considered as a relevant problem. As compared to other agents, mefloquine has a significantly lower hospitalization rate.

Additionally, mefloquine probably offers the best protection against malaria due to P. falciparum.We therefore conclude that for stays in areas with high transmission of chloroquine resistant P. falciparum, such as in East Africa, mefloquine is presently the drug of choice for malaria chemoprophylaxis, unless if there is a contraindication. In accordance with this conclusion the World Health Organization recommends mefloquine as first choice for travelers visiting areas with high transmission of chloroquine resistant P. falciparum (11).

With respect to the concern about mefloquine use by cockpit crews this study illustrates that in prophylactic dosage no more neuro-psychiatric adverse events are attributable to mefloquine than to chloroquine, which has previously widely been used by pilots and other flying personnel without any published case of flight incapacitation. If clearly indicated, it therefore seems warranted to prescribe mefloquine for chemoprophylaxis to cockpit crews, particularly considering the fact that this population usually is known to have had normal EEGs and to be non-psychotic. It

Table 2: Health risks in 100,000 travelers East Africa and in low endemicity area (South America)

Duration of stay:	1 month		1 night		1 week	
Regimen:	Chemoprophylaxis with mefloquine	Stand-by therapy only	Chemoprophylaxis with mefloquine	Stand-by therapy only	Chemoprophylaxis with mefloquine	Stand-by therapy only
Malaria infection	1500	1500	50	50	12	12
- symptomatic infection (PER 95%)	75	1500	2.5	50	0.5	12
- hospitalized infection (50%/10%)	33	150	1	5	0.25	1.2
- mortality (CFR 2%)	1.5	30	0.02	1	0.0002	0.01
Adverse events						
- hospitalization	8	malaria cases only	8	malaria cases only	8	malaria cases only
Total						
- hospitalization	41	151	8	5	8	1.2
- mortality	1.5	30	0.02	1	0.0002	0.01
Conclusion	Chemoprophylaxis has advantage		No certain advantage of chemoprophylaxis --> use standby reserve medication		Advantage for standby therapy	

PER = protective effectiveness rate, CFR = case fatality rate

should be stressed, however that for very brief stays in an area of high plasmodial transmission or for any stays in areas with low plasmodial transmission the benefit of chemoprophylaxis may not significantly outweigh the risks associated with such medication, as illustrated in table 2, and as recently reviewed (12).

Higher doses of mefloquine are used in the therapy of malaria or in the self-treatment of presumed malarial attacks (11, 12). As mentioned, these are associated with a very considerable risk (approximately 1%) of neuro-psychiatric adverse events which to some extent may be due to infection. A thorough review of the data by the World Health Organization has shown that 94% of these neuro-psychiatric adverse events attributed to mefloquine occur within one week after medication (WHO, in preparation), the majority of such adverse events occurring later were found to have had a positive family or a previous history of such neuro-psychiatric disease. After the use of mefloquine in a therapeutic dosage, it therefore seems sufficient to ground pilots for a minimum of one week instead of three weeks as previously suggested.

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USE OF NOVEL ADJUVANTS AND DELIVERY SYSTEMS TO IMPROVE THE HUMORAL AND CELLULAR IMMUNE RESPONSE TO MALARIA VACCINE CANDIDATE ANTIGENS.

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SUMMARY

The immune effector mechanisms responsible for the solid protection against malaria, as demonstrated by immunization with radiation attenuated sporozoites, are poorly understood. An effective malaria vaccine must induce a well orchestrated combination of humoral and cellular immune responses directed against critical parasite antigens / epitopes expressed during different stages of the parasites complicated life-cycle. Currently licensed human vaccine adjuvants, such as alum, may improve antibody production but are poor stimulators of cellular effector mechanisms, while potent cellular stimulants such as Freund's adjuvant are too reactogenic for human use. Over the last five years we have systematically evaluated several methods of antigen presentation to include chemical conjugation to bacterial carrier proteins, emulsification in "Freund's-like" preparations, and incorporation into liposomes. This work has resulted in the production of safe, potent vaccine delivery systems capable of targeting multiple antigenic determinants to the host's immune system. Further advances in malaria vaccine development now depends on the identification of appropriate parasite epitopes for inclusion in a multicomponent-multistage vaccine.

LIST OF ABBREVIATIONS

CS	Circumsporozoite
CWS	Cell wall cytoskeleton of *Mycobacterium phlei*
ELISA	Enzyme linked Immunosorbent Assay
GM	Geometric Mean
IgG	Immunoglobulin G
MPL	Monophosphoryl lipid A
$NS1_{81}$	81 amino acids derived from the nonstructural protein 1 of Influenza A
R32	32 tetrapeptide repeats derived from the *P. falciparum* CS protein repeat region, amino acid sequence = $[(NANP)_{15}\ (NVDP)]_2$
tet32	32 amino acids from a tetracycline resistance gene read out of frame
TA	*Pseudomonas aerugenosa* toxin A

BACKGROUND

Malaria is a protozoan infection caused, in man, by members of the subgenera *Plasmodium* (*P. vivax, P. ovale,* and *P. malariae*) or *Laverania* (*P. falciparum*). The life-cycle of this parasite is complex and includes an extrinsic, sexual phase which occurs in Anopheline mosquitoes, and an intrinsic, asexual phase that occurs in man. The parasite is normally transmitted to man when the sporozoite stage is introduced by the bite of infected female mosquitoes. These sporozoites travel through the blood stream and invade liver cells where they multiply asexually as exoerythrocytic stage parasites. In contrast to *P. falciparum, P. malariae,* and *P. ovale* which have only one type of exoerythrocytic form, *P. vivax* has two types of exoerythrocytic forms, a primary type which matures in 6 to 9 days as well as a secondary type, referred to as hypnozoites, which can remain dormant for months before maturing and releasing tissue merozoites. Once released, tissue merozoites invade erythrocytes and initiate the erythrocytic phase of the infection. During the erythrocytic phase, merozoites invade erythrocytes, undergo asexual maturation, and ultimately rupture the erythrocyte releasing new merozoites. It is this phase of the infection that results in the clinical symptoms of the disease referred to as malaria. A portion of the merozoites which invade erythrocytes do not multiply but differentiate into sexual forms, gametocytes. When ingested by a mosquito, male and female gametocytes unite to form an ookinete which then develops into an oocyst on the gut wall of the mosquito. After undergoing further development, the oocyst ruptures releasing thousands of sporozoites which migrate to the mosquito's salivary glands where they undergo final maturation. The lifecycle is complete when mature sporozoites are injected into new vertebrate host.

Immunity to malaria is even more complicated than the lifecycle, involving both humoral and cellular mechanisms directed against critical parasite epitopes expressed during different stages of the parasites complicated life-cycle.[1] Investigators around the world are working on identifying and characterizing useful immune targets from the various stages of the parasites development for incorporation into a multi-stage, multi-component, vaccine preparation.[2,3,4] One problem that must be addressed, however, is how to effectively present these numerous epitopes to the immune system in order to induce the necessary

humoral and cellular mechanisms responsible for solid protective immunity. The remainder of this presentation will summarize our clinical experience with vaccine adjuvants and delivery systems over the past 5 years.

ADJUVANTS AND DELIVERY SYSTEMS

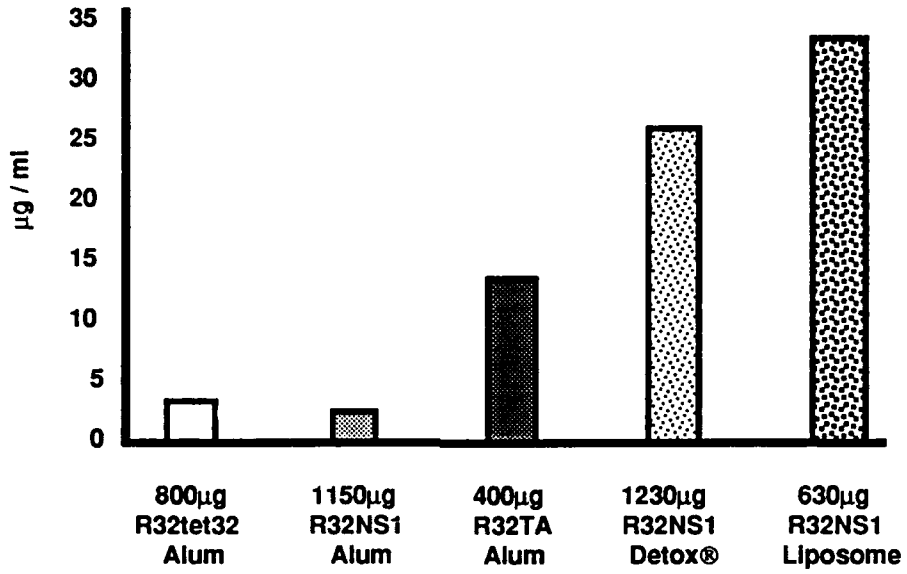
One of the first malaria vaccine candidates to undergo safety, immunogenicity, and efficacy testing consisted of the alum adjuvanted, *E. coli* expressed, recombinant protein R32tet32.[5] This study evaluated five doses ranging from 10 to 800 μg of R32tet32. Although this preparation was safe, it was less immunogenic in human volunteers than expected. Only one volunteer in the 800 μg dose group demonstrated boosting with subsequent doses of vaccine. The GM anti-R32 specific IgG concentration of the three individuals in the 800 μg dose group was only 3.7 μg/ml, as measured by ELISA. For reference the anti-R32 specific IgG antibody level in the one protected individual, at time of challenge, was 9.8 μg/ml. One possible explanation for the generally poor antibody response to R32tet32 may have been an absence of adequate T_{helper}-cell epitopes in the tet32 expression partner of this fusion protein. Subsequently, the expression system was reengineered so that the R32 fragment would be expressed fused to the amino terminal of an 81 amino acid fragment derived from the nonstructural protein 1, ($NS1_{81}$), of Influenza A, a fragment previously shown to contain T_{helper}-cell epitopes and enhance fusion protein expression.[6] $R32NS1_{81}$, at 11.5, 115, or 1150 μg per dose, was also well tolerated when administered as an alum adjuvanted vaccine. Improved immunogenicity was demonstrated by the observation that three doses of $R32NS1_{81}$- alum, administered at 11.5 μg per dose, produced a GM anti-R32 IgG antibody level of comparable to that seen with the highest dose of R32tet32 (2.3 μg/ml vs 3.7 μg/ml). No significant increase in GM antibody levels, however, was observed in the 115 or 1150 μg dose groups.

At this point, two different approaches were taken to try and improve the overall immunogenicity of the R32 fragment of the *P. falciparum* CS protein. One approach involved the systematic evaluation of repeat based synthetic peptides and recombinant proteins chemically conjugated to various bacterial proteins which served as carrier molecules. Initial studies, conducted in volunteers, designed to optimize the carrier protein, peptide to carrier conjugation ratios, and peptide orientation, revealed that the recombinant peptide R32-Leu-Arg, chemically conjugated to detoxified pseudomonas toxin A, (R32TA), resulted in the highest seroconversion rate (21 of 22 volunteers), with the best overall response being observed in the 400 μg dose group (GM anti-R32 antibody levels of 11.0 μg/ml).[7] Subsequently, an expanded Phase 1 / Phase 2a safety, immunogenicity, and efficacy trial of alum adjuvanted R32TA was conducted. This recently completed study confirmed the relative safety of this formulation. The GM anti-R32 antibody level in the 20 volunteers two weeks after the second dose of vaccine was 12.2 μg/ml.[8]

Concurrent with these studies, another series of experiments were conducted evaluating new adjuvant preparations,[9] or combinations of potent immunomodulators and delivery systems.[10] For these studies the recombinant fusion protein $R32NS1_{81}$ was used. Five volunteers were vaccinated with 1230 μg of $R32NS1_{81}$ adjuvanted with the Ribi DETOX® adjuvant consisting of 5 μg MPL, 50 μg CWS, and 0.5 μl of squalane. Similar doses of vaccine were given two and four months after the first. $R32NS1_{81}$-DETOX® was more reactogenic than $R32NS1_{81}$-alum by historically comparison. All five volunteers receiving $R32NS1_{81}$-DETOX® complained of moderate pain at the site of injection one to two days after each dose. Four individuals demonstrated erythema and warmth at the injection site and one volunteer developed enlarged axillary lymph nodes after the third dose of vaccine. None of these signs or symptoms were seen with the alum adjuvanted preparation. $R32NS1_{81}$-DETOX® was more immunogenic than $R32NS1_{81}$-alum based on comparison to archived sera run concurrently in a standard ELISA assay. The GM anti-R32 antibody level two weeks after three doses of $R32NS1_{81}$-DETOX® was 25.4 μg/ml compared to 2.3 μg/ml measured in sera obtained at a comparable time from volunteers immunized with $R32NS1_{81}$-alum, ($p<0.05$, Mann-Whitney U test). Although $R32NS1_{81}$-DETOX® was more reactogenic compared to the alum adjuvanted preparation, none of the volunteers declined further participation in the study. In light of these observations and the significant improvement in antibody levels, current plans include efficacy studies, using a laboratory challenge model, of the $R32NS1_{81}$-DETOX® vaccine formulation.

We have also evaluated the use of liposomes as an antigen / adjuvant delivery vehicle for malarial antigens. The rational for their use has recently been reviewed elsewhere.[11] A Phase 1 safety and immunogenicity study, in humans, again using $R32NS1_{81}$ as the antigen, has recently been completed. A single formulation containing 1,260 μg/ml of $R32NS1_{81}$, and 4,400 μg/ml of MPL encapsulated in liposomes consisting of 25,400 μg (37.5μ moles) of dimyristoyl phosphatidylcholine, 2,900 μg (4.4μ moles) of dimyristoyl phosphatidylglycerol, and 13,000 μg (33.6μ moles) of cholesterol was prepared. Actual vaccine doses were prepared at the time of vaccination by mixing either a 1:100, 1:10, 1:4, 1:2, or an undiluted sample of the original $R32NS1_{81}$,-Liposome formulation with an equal volume of aluminum hydroxide. This approach resulted in vaccine doses with constant ratios of liposome components and an increasing liposome:alum ratio. Thirty adult, malaria

Fig 1. Geometric Mean anti-R32 Antibody Levels



naive volunteers were randomly divided into five groups with each subsequent group received an increasing dose of vaccine. The vaccine was generally well tolerated. Objective local reactions were limited to erythema or induration and were observed after 11 of the 88 administered doses. These reactions were only seen in individuals receiving vaccine doses containing 1100 µg or more of MPL. All vaccine doses were highly immunogenic. The five individuals receiving the most dilute preparation (6.3 µg of R32NS1$_{81}$, and 22 µg of MPL) had a GM anti-R32 antibody level of 22.7 µg/ml (range 6.4 to 287.2 µg/ml). The GM decreased to 12 µg/ml if the extremely high responder is excluded. Increasing the amount of R32NS1$_{81}$ and MPL to 630 µg and 2,200 µg respectively resulted in an increase in the GM anti-R32 antibody level to 33 µg/ml.

Figure 1 summarizes the GM anti-R32 antibody levels from clinical Phase 1 safety and immunogenicity studies evaluating vaccine formulations designed to improve the humoral immune response to the repeat region of the *P. falciparum* CS protein. Although three doses of R32NS1$_{81}$-alum, given at either 11.5 µg, 115 µg or 1150 µg per dose, were able to induce antibody levels comparable to three doses of R32tet32 administered at 800 µg per dose, markedly better antibody levels were observed after only two 400 µg doses of R32TA. Significant improvement in antibody levels were observed with 1230 µg of R32NS1$_{81}$-Detox® compared to either the R32tet32 or R32NS1$_{81}$-alum formulations, however the increased reactogenicity of the Detox® adjuvant may limit its usefulness.

The liposome formulation tested offered several advantages. Limited amounts of antigen (6.3 µg per dose), when combined with the potent immunomodulator MPL, are able to induce a GM anti-R32 level of 22.7 µg/ml (range 6.4 to 287.2 µg/ml, n=5). However, liposomes are capable of incorporating relatively large amounts of "antigen", thus a variety of *different antigens providing target* epitopes from molecules expressed during the various stages of the parasites lifecycle may be incorporated in future preparations. The liposome formulation was significantly less reactogenic than R32-Detox®, particularly if one considers the difference in the amount of MPL used in all but the most dilute liposome dosage. Incorporation of the MPL into liposomes greatly reduces its inherent toxicity.[12] The highest dose of MPL administered, 2200µg per dose, represents a 12-fold increase in the previously reported maximal safe dose of free MPL.[13] The improved humoral immune response observed is, in part, dependant on the use of MPL, however, processing of the liposome delivered material is also crucial. Liposomes are actively cleared from the site of injection by macrophages. Subsequently the antigen is processed both through endosomal mechanisms with presentation to class II restricted T_{helper} cells as well as through cytoplasmic mechanisms and delivered to class I restricted cytolytic T cells.[14] This later route of presentation may facilitate the induction of cytotoxic T cell which have previously been shown to be important in a *P. berghei* rodent model of malaria.[15]

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CYTOKINES AS VACCINE ADJUVANTS: INTERLEUKIN 1 AND ITS SYNTHETIC PEPTIDE 163-171.

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SUMMARY

The possibility of preventing infectious diseases by employing efficacious vaccine is rapidly growing as the consequence of the new technologies in recombinant DNA and protein chemistry. However, the increasing number of synthetic and recombinant antigens further stresses how important is the role of appropriate adjuvants to ensure the maximal vaccine activity and the protection of all vaccinees. Several approaches can be applied to develop safe and effective agents capable of enhancing specific immune responses which can then protect the host from the pathogen. Among others, the direct use as adjuvant of those cytokines which are induced in animals by the classical Freund adjuvants has recently become matter of investigation. In particular, interleukin 1 (IL-1) has been shown to possess adjuvant activity for a variety of infectious and tumor antigens. However, the numerous side effects associated to the proinflammatory action of IL-1 represent a serious disadvantage for its use as a vaccine adjuvant. It was therefore of great interest the observation that a nonapeptide contained in the IL-1β sequence (residues 163-171 corresponding to the sequence VQGEESNDK) is devoid of all the proinflammatory activities but maintains the immunostimulating activity of the whole IL-1β. Thus, 163-171 peptide was successfully employed in animals to potentiate the specific immune response against T helper-dependent cellular antigens, T helper-independent polysaccharidic antigens and recombinant as well as synthetic antigenic preparations derived from human pathogens. Furthermore, IL-1 and 163-171 peptide have been successfully used in tumor vaccines in experimental systems. It can be therefore concluded that 163-171 peptide is potentially a good candidate as vaccine adjuvant for human use.

LIST OF SYMBOLS

BSA	bovine serum albumine
CSF	colony stimulating factor
HBsAg	hepatitis B surface antigen
HGG	deaggregated human gamma-globulins

IL-1 interleukin 1
mab monoclonal antibody
RIA radioimmunoassay
SRBC sheep red blood cells
VQGEESNDK 163-171 peptide of human interleukin 1β

1 INTRODUCTION

In the last few years the vaccine field has been deeply transformed by the revolution of genetic engineering. Suddenly, antigens that were difficult to obtain by purification or previously unknown major antigens of microbial diseases have become available in large quantities, as the consequence of gene cloning. Thus,the need to boost the host immune system against the recombinant protein of the new vaccines to gain protection against infections has become of major relevance. In the past, the term adjuvant has referred to those materials which were mixed and administered with the antigen with the purpose of augmenting the immune response: these were mainly bacterial components mixed with oil, such the complete and incomplete Freund adjuvant, or aluminum hydroxide. In these cases the adjuvant has the role of allowing the slow release of antigen plus potentiating the specific immune response. Indeed this was empirically achieved although the mechanisms beyond the adjuvant effect remained unknown for a long time. More recently, it has become clear that bacterial components are great inducers of those soluble factors, now called cytokines, which are known for their immunostimulant and inflammatory effects. Among the different factors which determine proliferation of immune cells, the one playing a major pivotal role is interleukin 1 (IL-1). In fact, this cytokine acts as the initiator of a cascade of events which eventually brings to the protective immune response against infection. Among other biological effects, IL-1 is capable of inducing receptors for IL-2 and CSFs, which are involved in leukocyte growth, differentiation and activation, and synthesis and release of IL-2, IL-4, IL-6 and IL-8, which sustain the host immune and inflammatory response. This paper therefore deals with the possibility of considering IL-1 and its less toxic derivatives hereafter described as ideal adjuvants for vaccines.

2 MATERIALS AND METHODS

The detailed description of techniques employed and all the animal species, materials and biological specimens used in the studies hereafter reviewed have been reported in the original papers that are quoted extensively in the bibliography section.

3 RESULTS AND DISCUSSION

The first demonstration that IL-1 can act as adjuvant was obtained by Staruch and Wood (1) in 1983 employing purified IL-1 preparations. In

those studies, the simultaneous administration of IL-1 with BSA could increase antibody production against the antigen. After the cloning of IL-1 (2,3), we reevaluated the possibility of exploiting its adjuvant effect in animal models employing recombinant material. In fact, by means of computer analysis it was possible to identify a short peptide of nine residues (VQGEESNDK), in position 163-171 of human IL-1β, which by a variety of criteria could be proposed as the protein domain selectively responsible for immunostimulation (4). At the beginning, T helper-dependent antigens such as SRBC were employed demonstrating that IL-1 and its 163-171 peptide could increase primary as well as secondary immune response in mice (5). Furthermore, positive results were obtained when adjuvanticity was tested for a T helper-independent polysaccharidic antigen such as SIII, which is derived from Streptococcus pneumoniae (5). Adjuvanticity of IL-1 and its synthetic nonapeptide could be observed also in mice immunodeficient for genetic (nude mice), natural (aged mice) or iatrogenic reasons (radiation or cyclophosphamide treatment) (6,7).

A strong dicotomy in biological effects between IL-1 and 163-171 peptide was however observed at the proinflammatory level. In fact, the nonapeptide did not cause in vivo fever or any of several associated metabolic changes inducible by the whole IL-1 β molecule such as hypoferremia, hypoglycemia, hyperinsulinemia, increase in circulating corticosterone, serum amyloid A and fibrinogen, decrease in hepatic drug metabolizing enzymes (6). Furthermore, at variance with IL-1β, the 163-171 fragment did not show the toxic effect causing shock and death in adrenalectomized mice (6). On the other hand, PGE_2 induction in fibroblast, the most sensitive in vitro assay for measuring IL-1 inflammatory effects, was not obtained with maximal concentrations of 163-171 peptide. Thus, these results demonstrate that selective functional domains are identifiable within the pleiotropic cytokine IL-1β.

A further line of evidence in support to this was obtained by testing in biological assays a monoclonal antibody (Vhp20) raised against the synthetic fragment VQGEESNDK which was able to recognize IL-1β in RIA and immunoblotting. In vivo, the mab Vhp20 was able to selectively inhibit the immunostimulatory activity of IL-1β, but it could not affect the fever-inducing capacity of IL-1β (8). Taken all together, these results demonstrate the adjuvant role of IL-1 and, more important for the lack of toxicity, of its synthetic 163-171 peptide. Our studies were therefore directed to more important antigens for human vaccines. It was found in experimental systems that IL-1β and its peptide strongly increase the level of antibodies

against recombinant hepatitis B surface antigen (HBsAg) (9). This observation of ours was elegantly confirmed and extended by Rao and Nayak (10) who showed that a peptide of HBsAg (residues 12-32), when synthesized together peptide VQGEESNDK separeted by a spacer of two glycine residues, can induce a higher antibody production than when employed alone.

Also in experimental tumors it was possible to demonstrate the adjuvanticity of IL-1 and of its 163-171 peptide. In fact studies of Forni et al. (11, 12) showed at first that both the molecules are able to recruit antitumor reactivity in tumor recipient mice expressed by activated lymphocytes. This prompted McCune and Marquis (13) to employ as adjuvant IL-1β or its peptide in antitumor vaccine consisting of irradiated tumor cells. Results showed that 70-100% of the mice treated with vaccine plus adjuvant became tumor free, while mice receiving vaccine alone were only 0-20% tumor free.

Even though these results clearly demonstrate the adjuvant effect of IL-1β and of its peptide, the mechanisms underlying this phenomenon are poorly understood. Therefore some recent studies of Gahring and Weigle (14) can be of particular help to beginning to understand how these molecules act at the cellular level. In fact it was found that tolerance induced in mice by HGG can be prevented by treatment with IL-1 or VQGEESNDK peptide. To explain these observations the interaction of cytokines with subpopulations of helper T cells (namely Th1 and Th2) has been evocated. Of course this is an important issue in immunology these days and a broader understanding of this point will bring to a completely new generation of adjuvants of greater molecular definition and, hopefully, activity in the absence of unpleasant side effects.

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FUTURE APPROACHES TO VACCINE DEVELOPMENT
SINGLE-DOSE VACCINES USING CONTROLLED-RELEASE DELIVERY SYSTEMS

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The development of new vaccines, both more efficacious and easier to deliver, has become an area of research that can certainly benefit from recent technical developments. In particular, the conversion of multiple-dose vaccines into single-dose vaccines may represent an important advance which should lead to improved vaccination coverage, as well as to a reduction in vaccination costs.

In order to be fully immunized against diphtheria, pertussis, tetanus, poliomyelitis and hepatitis B, the successive administration of three doses is needed. In many developing countries the drop-out rates from individuals receiving the first dose, but not successive doses is high and can reach values of up to 70 %. The reasons for these drop-out rates are numerous: unavailability of vaccines, difficulty of access, ignorance on the part of parents of the fact that some vaccines require multiple doses, lack of faith in vaccination, fear of side-effects, sickness at the scheduled time of vaccination. Countries with large number of people living in poverty, of people living in isolated or hard to reach areas, and countries experiencing war or political turmoil, are often the areas were this problem of dropping-out and overall low immunization coverage are the most extreme. Therefore, the ability to fully immunize at the first health-care contact would significantly raise coverage and directly reduce disease incidence.

Taking these considerations into account, one of the main goals of our programme has become the development of a strategy to convert multiple dose vaccines to single-dose vaccines with the same or increased effectiveness.

The initial priority of this project has been to develop a single-dose tetanus vaccine as one of the most urgent needs of the Expanded Programme on Immunization is to control neonatal tetanus. Indeed, close to 565,000 newbornes died of tetanus in 1991, and the prevention of neonatal tetanus would greatly benefit from the development of a vaccine requiring only one injection in pregnant women or women at child-bearing age. However, this technology, if successful, will also be applied to other vaccines, namely DPT and hepatitis B, and possibly inactivated polio vaccine. For practical purposes, tetanus toxoid (TT), because of its characteristics and availability appears as a suitable antigen to begin alternative approaches to the existing vaccines.

The ideal preparation should deliver antigen in such a way that a long-lasting effect is achieved with a single administration and provides protection as good as, or even better than, with the conventional vaccine.

The use of controlled-release systems, already applied to deliver a whole array of drugs and hormones both in cattle and man, appears to be one way of accomplishing our goal. Among these systems, polymeric particles have been widely used for injectables.

The polymers chosen as excipients for parenterally administered particles must meet some requirements, including being biodegradable, safe (tissue compatible, no secondary reactions) drug compatible and permeable, stable *in vitro*, easy to process, allow reproducible formulation and ideally, inexpensive. The copolymers of polylactide/polyglycolid (PL/PG) have already been utilized for surgical suture and are one of the most used vehicles because they are biodegradable.

The most common forms of polymers are microcapsules and microspheres. These are spherical particles ranging from a few μm to around 200 μm in diameter. Their internal structure varies, determined by the microencapsulation process utilized.

They can be classified into two main groups: the reservoir type, with material in solution in the cavity formed by a polymeric membrane, and the monolithic type, with material evenly dispersed throughout the polymeric matrix.

Indeed, the first type is usually known as microcapsules and the second as microspheres

Microspheres seems to be preferred to microcapsules because of the better control of drug or vaccine liberation. The release mechanism is a combination of bulk and/or surface erosion and diffusion through the pore. The release rate depends on drug loading, polymer molecular weight and composition and particle size and porosity. Two alternatives have been proposed: pulsed and continuous delivery systems. The first would mimic the conventional vaccination schedules, e.g., three to four doses released over a year. The second is a continuous delivery of antigen over a similar period of time.

When in 1988, PVD decided to develop a controlled-release TT vaccine using PL/PG microspheres, research was commissioned to four independent groups who prepared both types of TT vaccines.

Continuous release formulations already been provided to WHO to conduct a parallel, independent, *in vivo* testing in mice with the conventional vaccine as control: non-microencapsulated and consisting of three doses of alum-adsorbed TT, conveniently spaced, prepared with the same antigen source . Both immunogenicity (by measuring neutralizing antibody titers) and protection (by means of utilizing a modified potency test) are being assessed. Moreover, a detailed quality control is being conducted as an independent monitoring of the formulation provided, to determine the characteristics, safety and stability of the microsphere. Pulsed released formulations are being preparated that contain a mixture of beads of different sizes and composition. Fig . 1 depicts an illustration of any of these formulations. They are expected to provide a strong optimal immune response (protective antibody) as soon as possible after injection and a boost at a later point. This can be achieved with less than 10 μm microspheres for priming and early boosting (around 2 months) and larger microspheres for late boosting (around 10 months).

In parallel, quite extensive studies have been made by other groups on the evaluation of different microencapsulated antigens both for parenteral and oral administration. These suggest that at least for a certain number of antigens, different PL/PG microspheres can be prepared and mixed in order to obtain the desired vaccine. However extensive testing is still to be conducted to prove protection induced by the administration of such vaccines.

It is hoped that in the very near future, Phase I human trials will be conducted with any of the above discussed candidate vaccines, and the same or similar technology will be applied to other antigens. At present, the pre-clinical results obtained seem to point in the direction of the successful development of single-dose controlled-release vaccines.

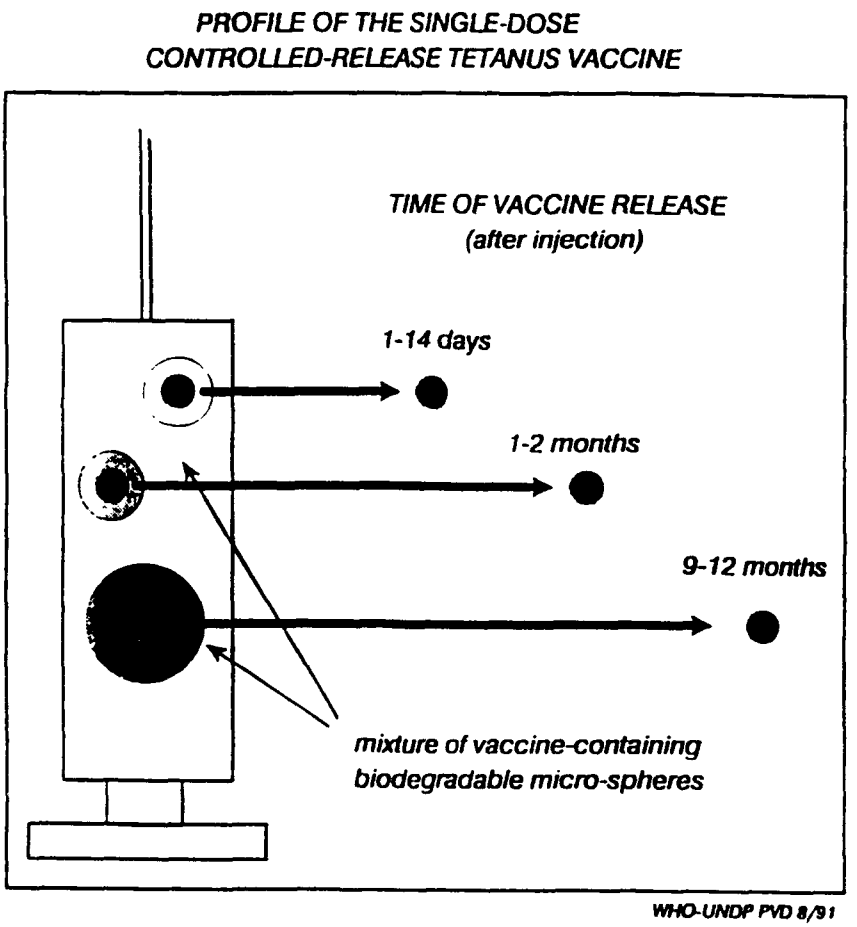
PROFILE OF THE SINGLE-DOSE
CONTROLLED-RELEASE TETANUS VACCINE
TIME OF VACCINE RELEASE
(after injection)
1-14 days
1-2 months
9-12 months
mixture of vaccine-containing
biodegradable micro-spheres
WHO-UNDP PVD 8/91

Figure 1

DISCUSSION SESSION III

INFECTIOUS DISEASES AND VACCINATIONS (papers 23 to 28)

M.D. PARKINSON (USA): I have a question for prof. Steffen. Prof. Steffen in your study concerning mefloquine and dizziness, were you able to garantee, - that wasn't a blinded study - that the knowledge of the problem could be a potential bias? I was curious if you had any opportunity in looking at that in your questionnaire at all, in terms of whether or not a person was briefed concerning the potential side effects before departure on his trip and if you think that there might be other potential confounding factors that may contribute to the perceived increase.

R. STEFFEN (CH): Yes, as you rightly stressed, that wasn't a blinded study. However in those travellers as seen in the control questionnaire in a subset, the vast proportion don't know the details about the potential side effects. Well, we were worried, where there could be a bias, by the fact that travellers of different nationalities often use different prophylactic regimens, but even when we analyzed the substrate by nationality, we got exactly the same picture.

A. FATTOROSSI (IT): Dr. Steffen, I wonder whether there is an increase of adverse side effects if we deal with, let's say, "often travellers", that is travellers who travel twice or three times to the same malarial area and then have to take twice or three times per year the mefloquine. The second question is: all the adverse effects finish after discontinuing the therapy?

R. STEFFEN (CH): Let me take the second question first. Yes, the side effects disappear after discontinuation. Very few continue to report side effects. Obviously, we assume that this is not due to the chemoprophylactic agent.
Regarding the first question, we analyzed that in the first MALPRO study. We asked them: - How often have you been to malaria endemic countries? - We didn't find a significant difference in side effects. Well, a third aspect which is important is the duration of stay and whether the proportion of side effects diminishes in, for instance, long-term residence. We don't have a lot of data on this in our study, but there is another study on peace corps volunteers, which was recently published, at least in part. They show that with increasing duration, particularly mefloquine, you get no increasing rate of side effects, there was rather a diminished proportion of side effects.

R. D'AMELIO (IT): A question to dr. Tagliabue. I'd like to know something about the network of increase of response, if any, against polysaccharide, by nonapeptide 163-171.

A. TAGLIABUE (IT): You mean why peptide 163-171 increases the response to a polysaccharide, a classical T-helper independent antigen? The general idea is that IL-1 is acting mainly by boosting T-cells. Now, there are many lines of evidence showing that there is a receptor for IL-1 on B-cells. Thus, it's possible to suggest a direct effect of the peptide of IL-1 on B-cells. On the other hand, it is now coming out that the response to SRBC, is against a saccharide on that cell. So, also in this case, you could suggest that, while you are thinking about T-cell dependent response, you are actually acting mainly on B-cell.

R. RAPPUOLI (IT): My question is to Dr. Gordon. Just a curiosity. In your first slide you have an interesting note at the end of the slide, "IMMUNODOMINANT EPITOPES - ARE NOT NECESSARY THE BEST ONES". Probably you'd like to comment on that.

D.M. GORDON (USA): That's actually with reference to some of the other regions. Originally it was shown that if you look at individuals who are living in areas endemic for malaria, there is an increase of frequency of antibodies against the central repeat region of the protein. This is associated with a measurement of decreased incidence in malaria with age. However, what we found is that we can only protect perhaps 20% of individuals that are actually responding to the repeat region. You can protect some individuals simply by inducing antibodies to the repeat region. We have shown that there are other B-cell epitopes, particularly in the C-terminal region of the molecule, that are also able to serve as a good target, for example aminoacids 300-312 is a very good B-cell epitope. If you induce antibodies to this region, these antibodies are very effective in preventing the sporozoite formation. So, what we anticipate is incorporated into the next generations of malaria vaccines, the repeat region, part of the C-terminal region as well as other antigens from other stages of malaria life cycle.

R.E. SPIER (UK): Can I comment on the question of immunodominant antigens not necessarily being the best one? This particularly, I think , is the case in rhinovirus, where the immunodominant antigen does not enable you to develop cross-protection in vaccines. You can actually get immunodominant or subimmunodominant epitopes in the virus to which you can get a monoclonal antibody which will cross-neutralize accross a range of rhinovirus strains. So to some extent, if you are now looking for vaccines against situations where you have a lot of different strains, then the immunodominant situation is confusing the issue.
So, we have to go to an immuno subdominant epitope and then, get the cross neutralization, that will give you the kind of protection that you need in a multistrain situation.

D.S. BURKE (USA): I have two questions, the first one to dr. Tagliabue. That is: is it likely that IL-1 is equally important in the first and second immunization, or is it likely that it would only be important in the initial or in the memory response?
Can you say something about the type of cells that are involved in the response against IL-1? Is it likely to make a difference?

A. TAGLIABUE (IT): It is not an easy answer. There are still many things we should learn about IL-1. For instance the understanding of the issue of two receptors, one mainly present on T-cells and the other, that has been recently cloned, on B-cells is likely to provide insights on the mechanisms of immunostimulation by IL-1. I think it will be also very important to understand more why and how IL-1 can break tolerance. In answering your question, IL-1 may be important in the same way, in primary immune response, as well as in memory response, acting on both T-cells and on B-cells. It remains to be elucidated why there is a second receptor on B-cells, which is quite similar at the molecular level to the first one in the extracellular part, but with almost no intracytoplasmatic tail. So it is not clear how the signal can be transduced. This again suggest a completely different mechanism of action of IL-1 on B and T cells.

D.S. BURKE (USA): Thank you. The second question is for dr Aguado. I heard rumors that microspheres can be effective, when given orally. Is that true?

M.T. AGUADO (CH): It is true. In the sense that they are able to stimulate the immune response, although at a different level. The problem is that, given orally, there is a very inefficient uptake, in the order of 1%. That can be improved, but so far a lot of work needs to be done on this point.

D.S. BURKE (USA): Is it likely that technology will evolve to the point where these microcarriers can be administered orally, instead of having to do injections, in terms of logistics?

M.T. AGUADO (CH): I am sure, if enough research is carried-out on this subject. Indeed WHO is already supporting some projects on this area.

P.M. MATRICARDI (IT): I have a question to dr. Aguado. In the case of an adverse reaction to tetanus toxoid with a vaccine which can deliver antigens after one, three or eight months after injection, what would you suggest?

M.T. AGUADO (CH): This is a problem and a limitation of this approach and we are well aware. The only thing we can do is to be extremely cautious in the development of these vaccines, and eventually, if necessary, to use a purified antigen of some sort. I imagine dr Rappuoli could comment a little more on this. If that would be the case, may be that technology could even be applied to any antigen. Another way would be to use an implant or something similar, and to remove it. But this is not practical for mass vaccination.

J. A. BELLANTI (USA): I have a similar question. One of the uses to which antigen-delivery could be used, is in the hyposensitization to allergic diseases. And here, of course, you even have a much more difficult problem, because you want to administer antigen in a slow way. But once it's in, it's in, and we have very serious theoretical problems of adverse reactions, with hypoallergenic treatment. But getting back to the question of antigen delivery for vaccines for infectious diseases, one has to be careful. I think your point is very well taken, that, if there are adverse effects due to the administered antigen, it could be removed quickly, because these could be life-threatening reactions.

A. TAGLIABUE (IT): I would like to ask prof. Bellanti a question about the typhoid vaccine. I was very pleased to see these positive results obtained with *in vitro* tests and at this point it is essential to make a comparison with the existing vaccine that has been tested in very large field trials. So what's your idea of the advantage of your TS mutant compared to the Ty21a? With your strain do you get immune response against the Vi antigen?

J. A. BELLANTI (USA): Yes, I think that's a very fair question. There is no question that the Ty 21A is the flagship strain that has been used now for the oral vaccine for typhoid fever. The problem with the Ty21a, I don't mean to be hypercritical of other strains, but the problem with it, is the nature of the genetic attenuation, as you know, is a galactose-epimerase deficiency, which leads then to a piling up of metabolic products, proximal to the genetic lesion, and when this occurs the organism is extremely labile, it's difficult to stabilize when it's given orally, and also poses problems of storage and particularly in parts of the world where the vaccine must be used, while to be sure it is the flagship strain, there is a need for improvement. A second point is, our vaccine we think is improved, because the nature of the genetic lesions are autosomal and the strain is stable, if considered at room temperature, because this temperature is permessiveness, at 29^C, it doesn't have the problem with the galactose- epymerase lesion, and we feel, there is another advantage in the vaccine, there is something that is developing in culture with the development of pseudorevertants, that is not true reversion, but they are attempts to reverting back, but they retain the non virulence of the strain, so there is a piling up of antigenic mass that this vaccine may offer. But of course your point is well taken, we need to go into the next phase into seeing, the best delivery system liquid preparations, or in capsules, gelatin capsules, we also need comparative studies of the Germanier strain with our strain.
But certainly I think that there are some leads of potential advantages.

R. D'AMELIO (IT): I'd like to make just a general consideration at the end of this session. First of all I'd like to thank the chairmen and all the speakers for their strong engagement, for the exciting discussion in this session. I would like to offer to your consideration, at the end of this session, the present situation of the vaccinations among the NATO countries. I showed this slide (tab. III in the TER) on the first day, during the opening remarks, for a very short time, and I think it is important to offer this slide for a better consideration now at the end of this session, because, on the first day, our Chairman, prof. G. Santucci, said something, I think is very important. Military environment, can be the engine in the fight against infectious diseases, because it is possible to operate in military environments some types of compulsory operations, that are difficult to operate in other situations. These efforts must be coordinated, because, this is the present landscape of the vaccination schedules among NATO countries. The difference does not depend just on the different epidemiological situation of infectious diseases in the country or on the different vaccination schedule in children because the occasions to operate together among different countries worldwide are now increasing continuously. Now, in this session we heard that there are many realities, for example in Italy, the disapperance of meningococcal meningitis in military recruits after the introduction of the vaccination. There are many future approaches now, as we heard from dr. Aguado, dr. Gordon, dr. Rappuoli, dr. Tagliabue, the efforts for new adjuvants for new vaccines, we heard from the beautiful introduction of dr. Torrigiani the epidemiological present situation of infectious and parasitic diseases, the numbers are very worrying. This situation of the NATO countries, - this is a NATO agency, so I think it is the right site to stress this point, - should be one of the parts of the engine to help WHO in the fight against infectious diseases. These efforts, of course, must be coordinated and meditated to be really effective.

G. TORRIGIANI (CH): Thank you very much dr D'Amelio. I think we have had a long morning.

EPIDEMIOLOGIC VIEW OF ALLERGIC DISEASES IN NORTH AMERICA: IMPLICATIONS FOR AEROSPACE MEDICINE

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SUMMARY

The allergic disorders comprise a heterogeneous group of diseases which involve the **respiratory tract** (e.g. **allergic rhinitis and asthma**), the **skin** (e.g. **atopic eczema**), and the **gastrointestinal tract** (e.g. **allergic gastroenteritis, food allergy**) and which afflict 50 million Americans. The respiratory allergic diseases affect major segments of the population (15-17%) who may be recruited as aircraft personnel and therefore these diseases pose particular risk to flying safety of personnel either **directly, or indirectly** as a consequence of their disease or of the adverse effects of medications now used in the treatment of these disorders. Moreover, recent evidence is now accumulating to suggest that other environmental factors encountered in aerospace operations, e.g. gravity, oxygen and stress, may also contribute to the immunologic responses involved in and responsible for clinical manifestations of these allergic disorders. A thorough knowledge of these allergic diseases and reactions associated with therapy are, therefore, essential for proper screening of personnel prior to entry into the aerospace field. Moreover, principles of allergic therapy should be applied to existing trained personnel and combined with a knowledge of careful use of medications which are least likely to be associated with performance decrement. The availability of new medications which have minimal adverse effects, e.g. non-sedating antihistaminic drugs, are addressing some of the problems associated with these issues.

1 INTRODUCTION

The allergic disorders comprise a heterogeneous group of diseases which involve three major target organs: 1) the **respiratory tract**, (e.g. **allergic rhinitis and asthma**); 2) the **skin**, (e.g. **atopic dermatitis**); and 3) the **gastrointestinal tract** (e.g. **allergic gastroenteritis and food allergy**).

Allergic diseases have a significant impact not only in North America but also throughout the world. It is estimated, for example, that approximately 50 million Americans suffer from asthma and the allergic diseases (Ref 1).

The total estimated direct costs for asthma in the U.S. alone in 1985 was 2.5 billion. If one includes indirect costs associated with asthma, including loss of work days and school and reduction of life-time earnings, the total estimated cost exceeds 4.5 billion per year (Ref 2).

Because of their frequency and heterogeneous expression throughout the lifespan of the individual, allergic diseases, particularly asthma, not only present problems in diagnosis and treatment but also have profound implications for aerospace medicine. The seriousness of asthma as a disease has become more apparent in recent years. There has been a disturbing rise in morbidity and mortality throughout the world and in the United States (Ref 2). This scientific session will include a variety of presentations which will address both the management of these disorders in trained aircrews as well as the current status of screening techniques for identification of high risk subjects prior to training. The present report will offer an overview of allergic diseases from the **pathogenetic, clinical, diagnostic** and **therapeutic** perspectives and will emphasize aspects relevant to **aerospace medicine**.

THE MOLECULAR BASIS OF THE ALLERGIC REACTION: THE ROLE OF IgE

The allergic diseases represent a collection of genetically controlled disorders of the immune system each of which is characterized by a hyperresponsiveness to foreign substances in the environment referred to as **allergens** and which are associated with hyperproduction of IgE antibody (Ref 3). This genetic tendency to hyperproduce IgE is referred to as the **atopic condition** or **atopy** and not only provides the molecular basis for the pathogenesis of the allergic reaction but also the basis for diagnostic screening of the allergic state. Once produced, IgE antibody molecules fix to the surface of mast cells in tissue or basophils in the circulation. When these molecules react with allergen there begins a biochemical cascade during which a series of mediators is released including histamine, products of the arachidonic acid pathways and other products that contribute to the smooth muscle constriction, e.g. bronchospasm, mucus secretion, vasodilatation, edema and inflammation that are a part of the allergic response(s). We now recognize that the IgE mechanism really has two pathways (Ref 4). The first is the immediate or **«early phase»** responses of smooth muscle contraction, edema, and mucus secretion which occurs usually within minutes. There is also a **«late phase»** that occurs within hours or days in which is seen an infiltration of the eosinophils, neutrophils and, later on, mononuclear cells including of macrophages, fibroblasts and lymphocytes. A thorough understanding of the immediate and late reactions are both very important with regard to diagnosis and treatment.

Asthma is now characterized by airway hyperresponsiveness in which the airways show exaggerated bronchoconstrictor responses to many physical and environmental agents including allergens. Of paramount importance in the hyperresponsiveness of asthma is the recent recognition of the role of airway inflammation. Therapeutic measures that reduce bronchial inflammation have also been shown to decrease the degree of airway hyperresponsiveness (Ref 5).

CLINICAL PERSPECTIVE: ALLERGIC TRIGGERS, THE HISTORY AND PHISICAL EXAMINATION

Because of the known genetic factors which predispose individuals to allergic diseases, the patient's personal and family history becomes a crucial tool in making the correct diagnosis of allergic disease. If one parent has a history of allergic disorders there will be about a 40% incidence of allergic disease in the offspring (Ref 3). If both parents are involved then the incidence increases to about 80% or twice the amount (Ref 3). It is also important to recognize that in the case of allergic disease it is not the clinical disease that is transmitted but rather the tendency to develop atopic disease. For example, asthma may be seen in a child whose parent(s) manifest only seasonal allergic rhinitis.

A carefully taken history based upon a series of questions can be the most important and most cost effective technique for screening. Such questions as **«when do your allergies occur»**, **«is there a seasonal pattern»** **«are there animals in the house»**, **«are your symptoms aggravated by exposure to smoking»**, **«are your symptoms relieved by the use of antihistamines»** - all are important questions by which we can often identify the allergic state.

Wheezing may not always be a prominent symptom in asthma and cough or tightness of the chest may be the only manifestation. The history should also consider the possibility of hidden allergens. One area of particular interest is the role of food intolerance or allergy and patients should be questioned carefully to determine if food or beverage consumption account for allergic disease. A diligent search for hidden food allergy should be undertaken in any patient who presents with recurrent infections, otitis media or symptoms of allergic rhinitis and asthma. The role of smoking as an exacerbating event in stimulating immunoglobulin E has been well documented (Ref 6). A careful occupational history should always be taken; what the patient does and what types of hobbies he engages in are also extremely important.

Infections are commonly responsible for exacerbating the onset of allergic disease and commonly may trigger asthma. Although any infection can be involved, viral infections continue to be the most important trigger.

Respiratory viruses cause airway inflammation and may sensitize nerve fibers promoting bronchoconstriction and inflammation (Ref 7). Other responses to infection that trigger asthma have been suggested such as beta adrenergic responsiveness (Ref 8), the production of IgE antibody to certain respiratory viruses, e.g. respiratory syncytial virus (RSV) (Ref 9). Since infection is a precipitating factor in allergic disease immunization against common viral diseases, e.g. influenza, should be recommended to prevent this infection.

Another mechanism whereby asthma may be triggered by viral infection is through the production of interferon which can enhance mediator release (Ref 10). In addition to aggravating clinical symptoms of asthma, upper respiratory viral infections are known to increase airway hyperresponsiveness that may persist for several weeks beyond the initial infection (Ref 11).

The history should also record the past history or presence of polyps. The triad of rhinitis and asthma, polyps and aspirin sensitivity is an important clinical clue to allergic disease. The physician should also inquire about the use of aspirin in patients with asthma because of its known exacerbating effects. The precise mechanism(s) of the exacerbation is incompletely understood but appears to be related to metabolism of arachidonic acid. The history of other medications is likewise important since these can sometimes be hidden allergens. Pregnancy can very often exacerbate or change the course of asthmatic disease.

THE PHYSICAL EXAMINATION

The physical examination should not only include a record of the patient's vital signs but a careful assessment of the nasal mucosa and skin for allergic shiners, polyps and other stigmata of allergic disease. Polyps should always be sought in patients with allergy. They are very prominent with a grayish appearance and are easily detected on physical examination.

Careful examination of the chest, the cardiovascular system and the skin are likewise important in the assessment of the allergic patient.

DIAGNOSTIC PROCEDURES

Procedures useful in the diagnosis of allergic disease include a complete blood count and a nasal smear to examine for eosinophilia. Total Immunoglobulin E (IgE) measured by radioimmunoassay should be measured as it is often elevated in patients with allergic disease. Although the measurement of serum total IgE is helpful when elevated, it is important to recognize that total IgE levels can be normal even in the highly allergic patient and therefore tests of specific IgE antibody are commonly performed.

Specific IgE can be detected by both *in vivo* skin testing or by *in vitro* tests such as radioimmunoassays, e.g. RAST. X-rays of the sinuses are appropriate where there is clinical or historic evidence of sinus involvement; chest x-rays are likewise important. Examination of the sputum for bacteria and cellular content may be warranted along with studies of pulmonary function and blood gas analyses. Shown in Table I is a summary of the procedures useful in screening for allergic diseases and which are relevant for aerospace medicine.

MANAGEMENT

There are three main areas of importance in the management of allergic rhinitis. These include avoidance therapy, i.e. the identification and removal of the offending allergens from the environment. The physician should encourage the patient's environment be cleaned and should advise dehumidifiers to be used in the home. In the management of allergic asthma avoidance therapy, i.e., elimination of offending allergens, is extremely important. The second area of treatment is symptomatic use of antihistamines particularly the new non-sedating second generation H1 antagonists. Cromolyn has been useful in symptomatic therapy together with nasal steroids and decongestants. Immunotherapy or hyposensitization is likewise shown to be an important arm of management.

Pharmacologic therapy can be summarized by the acronym, the ABCS -- A, the use of aminophylline or theophylline, B, the beta agonists such as albuterol, C, the use of cromolyn and S, the use of steroids available in inhalant and systemic forms. Immunotherapy while not as effective in allergic rhinitis, has been shown to be useful in some cases of allergic asthma.

The question of whether all asthma is allergic has been debated for several years. Some feel that all asthma is allergic, others feel that there are **intrinsic** and **extrinsic** forms. Extrinsic asthma is the type where an external antigen can be identified and is associated with elevated IgE levels, eosinophilia, a positive family history of allergy, and positive skin tests.

In the intrinsic form, usually seen in adults, IgE levels are normal and the pattern differs from the extrinsic form in that no detectable external antigen is present. Recent studies such as those of Burrows et al. (Ref 12) have shown that even intrinsic forms of asthma are now being associated with elevated levels of IgE. Thus, the belief is growing that more and more asthma is involved with IgE mechanisms and may have an allergic component.

The adult with allergic asthma also deserves special consideration in view of the differences described above. It is important to adequately treat asthma to prevent endstage irreversible chronic lung disease and to manage it carefully particularly in aerospace personnel for optimal performance levels.

Patient education and supportive care are also of prime importance.

Knowledge about what triggers the symptoms, information about the disease process and the development of self-management techniques have recently been shown to be highly effective. Many self-management programs are now available and are strongly recommended. A recent report of the National Heart, Lung and Blood Institute, **Guidelines for the Diagnosis and Management of Asthma** has appeared (Ref 13) and represents a very important expert panel report of the National Asthma Education Program of the Institute.

Appropriate periodic care of allergic patients including the taking of interval histories and physical assessments along with periodic measurements of pulmonary function are important. The peak flow meter has been shown to be a very useful tool in having patients assess the adequacy of their lung function. Consultation and referral to appropriate specialists including the allergist and the pulmonologist are important in the co-management of these patients.

SPECIFIC ASPECTS RELEVANT TO AEROSPACE MEDICINE

From what has been discussed thus far the allergic diseases have important implications for aerospace medicine. For ease of discussion, it is useful to address these implications at three levels of importance: 1) **general management and risk assessment**, 2) **effects of allergic disease on performance, and** 3) **implications of therapy on performance** (Table 2). The implications for general management and risk assessment can be divided between those that relate to trained aircrew in which better management of the disease including immunotherapy and pharmacotherapy are goals. With regard to the screening of personnel prior to training, carefully taken history and diagnostic procedures to identify individuals at risk, e.g. pulmonary function and *in vivo* and *in vitro* tests appear to be warranted.

There are a number of disease entities, e.g. otitis media, sinusitis, serous otitis media, respiratory failure from asthma, which can affect neurosensory and cognitive functions.

The altered central nervous system function as indicated by drowsiness and impaired psychomotor performance is often a consequence of the use of traditional antihistamines. The newer second generation H1 receptor antagonists have now been shown to have potent antiallergic effects without causing sleepiness (Ref 14). There are a number of studies which have evaluated the effects of antihistamines on mental processes related to automobile driving (Ref 15) and also the effects of these agents on aircrew (Ref 16). Recent studies suggest that antihistamines may have more significant behavioral effects than previously realized. For example, the pilot involved in the fatal crash of a fighter airplane on the aircarrier Nimitz (with a cost of 14 lives and $100 million) had a blood level of the antihistamine brompheniramine, 11 times in excess of that produced by the recommended dosage (Ref 17). Recent studies indicate that the newer non-sedating

antihistamines while free of many of these side effects at higher concentrations can have adverse effects and therefore these too should be used cautiously in flight crew.

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TABLE 1

Procedures useful in Diagnosis of Allergic Diseases

	Example
History	Seasonal pattern, smoking, hidden food allergy
Physical Examination	Polyps, allergic shiners, cough
Diagnostic	
in vivo	Skin tests
in vitro	Immunoassays for total IgE, (e.g. PRIST); specific IgE, (e.g. RAST)

TABLE 2

Implications of Allergic Diseases in Aerospace Medicine

Category	**Example**
General management and risk assessment	
Trained aircrew	☐ Better management of allergic disease ☐ Immunotherapy; pharmacotherapy
Screening prior to training	☐ History ☐ Diagnostic procedures to identify individuals at high risk - Pulmonary function studies (methacholine challenge) - In vivo and in vitro tests for IgE
Implication of disease	Effects of disease on neurosensory function, cognition, e.g. serous otiti media, sinusitis, respiratory disease
Implication of therapy	Side effects of medication, e.g. sedation, drowsiness, visual disturbances from antihistamines

ACTIVITIES OF THE EUROPEAN ACADEMY OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY

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SUMMARY

Present activities of the European Academy of Allergology and Clinical Immunology (EAACI) and possible area of collaboration with AGARD are briefly discussed by the EAACI Secretary General and Treasurer, on the basis of programmes and directives of the EAACI Executive Committee.

HISTORY

The idea of a closer European collaboration in the field of allergology and clinical immunology was firstly suggested in Paris, on the occasion of the 1st European Congress of Allergology in 1950.

After a hard preparatory work and due to the generous commitment of several European scientists including E. Bruun, Z. Eriksson Lihr, J.J. Farrerons-Co, B. Halpern, K. Hansen, C. Jimenez Diaz, P. Vallery Radot, V. Spoujitch, U. Serafini, W. Quarles van Ufford and D.A. Williams, the European Academy of Allergology was constituted in Florence in 1956. In 1971 the name of the Academy was changed to the present one because of the strict relationships between allergology and clinical immunology outlined by the tremendous progress of knowledges occurred in these areas during the 50s and 60s. Following the Florence 3rd European Congress regular triannual Congresses and Annual Meetings were organized by the EAACI in different European countries up to the most recent 14th EAACI Congress held in Berlin in 1989 and the 1990 and 1991 Annual Meetings held in Glasgow and Zürich respectively, all counting approx. 3,000 people in attendance.

AIMS

According to the present Constitution, the EAACI is aimed at:
- promoting basic and clinical research;
- collecting assessing and diffusing scientific information;
- being a scientific reference body for other scientific, health and political organizations;
- encouraging and providing training and continuous education;
- promoting good patient care

in allergology and clinical immunology within Europe.

MEMBERS

There are two types of EAACI membership:

1. Society membership, for official Societies of allergology and clinical immunology of different European countries. Twenty-two societies are at present part of the Academy with a total number of member of approx. 15,000.
2. Individual membership, for individual scientists and doctors operating in the area of allergology and clinical immunology. The number of EAACI individual members progressively increased in these last years up to the present number of 1,300 approx.

ACTIVITIES

Congress and meeting organization is still a major activity of EAACI. The next EAACI Congress will be held in Paris, in May 1992, while next Annual Meetings have already been planned for 1993 in Rotterdam and 1994 in Stockholm.
However, the increasing number of members and demands because of changes in Europe after 1992 and of the increasing relevance of allergy and clinical immunology in science and medical practice, prompted the EAACI to extend its activities beyond the area of congress organization.
The following 13 sub-committees were created:
- Adverse reactions to food
  chaired by C. Ortolani
- Aerobiology of inhalant allergens
  chaired by G. D'Amato
- Anti-allergic drugs
  chaired by J. Bousquet
- Editorial and audio-visual
  chaired by Cl. Molina
- Epidemiologic, social and economic aspects of allergic diseases
  chaired by P. Burney
- Immunotherapy
  chaired by B. Weeke
- Insect venom hypersensitivity
  chaired by U. Müller
- In vitro allergy tests
  chaired by S.G.O. Johansson
- Occupational allergy
  chaired by P. Maestrelli
- Provocation tests with allergens
  chaired by G. Melillo
- Skin tests and allergen standardization
  chaired by S. Dreborg

- Teaching and organization of allergology and clinical immunology
  chaired by J.M. Cortada Macias
- Allergic inflammation
  chaired by F. Levi-Schaffer

The sub-committees are aimed at stimulating individual members to take part in the Academy activities, increasing understanding of topics of current interest and relevance, reaching position statements on important or controversial fields.

With reference to this last aspect several relevant documents were recently published by some EAACI sub-committees, such as those on "Immunotherapy", "Skin tests and allergen standardization", "Aerobiology of allergenic pollens and mould spores" and "Occupational allergy". These are available, on request, from the EAACI Secretariat.

No specific sub-committee is at present devoted to the many allergy and clinical immunology problems of military medicine. EAACI should warmly welcome any proposal for new activities in this area which was successfully covered by other similar associations such as the American Academy of Allergology and Immunology.

The good financial situation due to the high incomes from Congress and meetings made also possible to develop a series of initiatives in favour of members while maintaining the subscription fee at extremely low levels; Travel grants and awards were established for young scientists, reduced subscription fees were obtained by many of the major allergy and clinical immunology journals, special programmes are in course aimed at favouring exchange of ideas,doctors and research workers. Moreover, since January 1992 the EAACI will have its own official journal: Allergy - The European Journal of Allergy and Clinical Immunology made available by Munksgaard with a 65% discount to EAACI members.

Finally, special attention was devoted to some problems of special actual relevance such as recognition of specialty, pregraduate, postgraduate and continuing medical education, contacts with EEC in view of the new guidelines and regulations after 1992.

We do look forward to your active participation in the European Academy of Allergology and Clinical Immunology.

THE SCREENING OF INHALANT ALLERGIC DISEASES IN THE SELECTION OF CANDIDATES FOR AIRCRAFT PILOTING

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INTRODUCTION

Respiratory hypersensitivity to airborne allergens affects over 10% of the population in industrialized nations (1) and is a source of substantial social and health-care costs (2). By virtue of the negative effects of allergy on professional performance and the current nature of attacks, sufferers are necessarily excluded from certain occupational activities involving considerable risk, physical/psychological stress, and high cost training, such as aircraft piloting (3). Allergic rhinitis and asthma prevent admission of a candidate to enrolment as a pilot in the Italian Air Force. As a consequence, candidates allergic to inhalants must be identified during selections. It is generally stated that anamnesis is the cornerstone for a correct diagnosis of an allergic disease in a clinical setting. On the other hand, anamnesis is almost always unreliable as a diagnostic tool for selection purposes of candidates to aircraft piloting, at least in Italy. In fact, candidates are interested in denying any kind of disease. Furthermore, the accessual characteristic of the allergic respiratory diseases makes these diseases difficult to be disclosed by physical examination during intercritic periods. A standardized diagnostic procedure, mainly based on objective data, should be adopted in order to avoid employment of candidates with (and even at risk for?) allergic asthma and/or rhinitis as military aircraft pilots.

Several thousand candidates wishing to become pilot are screened every year by the Italian Air Force Medical Corps. This very large number creates the need for a rapid, reliable and cost-effective screening test for the detection of inhalant-allergic subjects.

Measurement of total serum IgE (PRIST) has proven to be insufficiently sensitive (4,5), while RAST and (SPT) are very efficient (6), but they are time-consuming and relatively expensive procedures. Several multiple-antigen RAST assays have been proposed in the past few years as screening tests for inhalant allergies (7-10). An assay of this kind, «Phadiatop» (Pharmacia), has recently become commercially available as a paper-disc assay (11), and adapted to a new, automatized test system (CAP system, Pharmacia)(12). The Phadiatop CAP test is based on the reaction between a balanced mixture of inhalant allergens absorbed on a solid phase and specific IgE in the serum sample. Results are obtained by subsequent incubation with antibodies labelled with ^{125}I or ß-galactosidase-generating fluorescence by splitting the fluorogenic substrate 4methylumbrelliferyl-ß-D-galactoside (12). The former Phadiatop assay has already proven to be a valuable screening aid in clinical studies in which allergic subjects comprised 50% or more of the total test population (11, 13-14). However, the accuracy of any screening procedure is contingent on several variables related to the group being tested and in particular, false positives may increase as the actual number of affected subjects diminishes (15).

Furthermore, test performance in screening for inhalant allergy may vary according to the geographical area of application, due to possible differences in the most relevant airborne allergens responsible for respiratory atopic diseases in a given country. The aim of this study was to test the validity of Phadiatop CAP as a mass-screening procedure by administering it to 1815 young men who had previously qualified for enlistment in the Italian Air Force, but in whom no allergy testing had been performed.

SUBJECTS AND METHODS.

Subjects.

A group of 1815 male subjects (age range 17-24 yrs) all attending the Italian Air Force NCO Training Course in Caserta were studied after obtaining informed consent. On the basis of medical histories, physical examination, skin prick tests (SPT) for the seven airborne allergens most common in Italy (mixed grass pollens, Parietara judaica, Artemisia vulgaris, Olea europaea, Alternaria tenuis, Dermatophagoides pteronyssinus, and cat epithelium) and, if necessary, RAST for the same allergens, each subject was classified as «allergic to inhalants» (with asthma, oculorhinitis or both), «non allergic to inhalants», or «atopic asymptomatic»; all the subjects with SPT positivity to at least one allergen but who had never experienced clinical signs of disease were included in this category.

Bronchial provocation test (BPT).

In a subgroup of 98 subjects a bronchial provocation test with methacholine was also performed; for this test increasing amounts of predosed methacholine had been administered and FEV1 has been calculated with an automatized spyrometer (Pony,Cosmed). For statistical purposes a 20 per cent reduction in FEV1 with a dose lower or equal to 1200 ug of methacholine was considered positive.

Phadiatop.

Phadiatop CAP immunoassay was performed in the serum obtained from each subject by venipuncture.

CAP is a new type of solide-phase radio- or enzyme-immunoassay representing a further development of Phadiatop RAST (12). It consists of a solid-phase immunoassay, instrumentation and information-management software for the automated measurement of total and specific IgE in undiluted serum or plasma. The assay can be performed as an RIA or a fluorimetric assay. In this study, the fluorimetric assay was used. The system is built around a new type of solid-phase consisting of a flexible hydrophylic carrier polymer encased in a capsule (CAP). The carrier consists of a CNBr-activated cellulose derivative. The solid phase is in principle similar to that of the paper disk, but it has a three-dimensional structure increasing the surface and allowing a closer contact between allergen and serum IgE antibodies. In the case of Phadiatop, reaction occurs between a balanced mixture of airborne allergens (bound to the solid phase) and specific serum IgE. The system uses ß-galactosidase antibodies generating fluorescence by splitting the fluorogenic substrate 4-methylumbrelliferyl-ß-D-galactoside. Positivity or negativity is determined when sample fluorescence emission results above or below the reference serum value, respectively.

The level of fluorescence is given as FU/ml of serum sample and is a direct function of the specific serum IgE bound to the solid phase.

Statistical analysis.

Specificity, sensitivity, efficiency, positive and negative predictive values of Phadiatop with respect to subjects classification and to BPT were determined (15). For frequency distribution analysis, FU values were converted to ln.

RESULTS.

Two-hundred and seventy subjects (14.88%) were found to be allergic to inhalants on the basis of history confirmed by skin tests and/or RAST (Fig. 1). Twenty-one point three-eight per cent of subjects had at least one skin test with class 1 positivity but had never experienced any symptoms of allergy.

Six-hundred and twenty-three (34.27%) subjects resulted positive to Phadiatop. In fig. 2 the frequency distribution of ln FU in the whole population examined is shown. Interestingly, a bi-modal curve was obtained, indicating the presence of two distinct subgroups, the non atopic one (mode = 4.64 ln FU) and the highly atopic one (mode = 9.32 ln FU).

Phadiatop identified 265 of the 270 subjects with inhalant allergy, indicating a test sensitivity of 98.14 %. However, specificity and positive predictive values of the test were lower (76.89 % and 42.60 %, respectively), due to the finding of 357 subjects with no allergic disease in the positive group (Tab. I, left side). The vast majority of these subjects had at least one positivity to the seven allergen tested with SPT and/or RAST. In Fig. 3 comparisons between Phadiatop assay and SPT are reported. Subjects were grouped accordingly to the highest allergen reactivity resulted to SPT. As shown, the Phadiatop clearly discriminated between subjects negative to SPT and those with a 3+ or 4+ reaction to at least one allergen. Subjects with

a 1 + or 2 + reaction were broadly distributed from 5 ln FU to 9.75 ln FU.
In order to evaluate if Phadiatop CAP quantitative result correlated with the clinical expression of an atopic tendency we analyzed the frequency distribution of Phadiatop ln FU according to the category of each subject (allergic, atopic, non-atopic) (Fig. 4). This analysis shows that very few allergic subjects have a ln FU lower than 6.5-7.0 (660-1100 FU). Furthermore, the probability that one subject is affected by allergic symptoms linearly increases with its Phadiatop ln FU.
On the basis of this observation we evaluated the concordance of Phadiatop CAP with the diagnosis of allergic disease using a new reference cut-off placed at 900 FU (Tab. I, right side). The increase in specificity and positive predictive value were appreciable above all, while only a slight decrease in sensitivity and negative predictive value were observed with respect to the results obtained with the cut-off given by the reference standard serum (tab. I, left side).
Table II shows results obtained in the subgroup of 98 subjects also examined with the BPT. This group included 6 subjects with mild to moderate asthma. Sixteen subjects had a positive BPT to methacholine. Comparison between Phadiatop and BPT is also reported. Fourteen (85%) of the 16 subjects with bronchial hyperresponsiveness were also positive to the Phadiatop assay. Interestingly, all the six subjects affected by asthma resulted positive to both tests.

DISCUSSION.

This study confirms the high prevalence of allergic diseases to inhalants especially in young male adults (16). Phadiatop CAP was found to be highly sensitive although not highly specific. This low specificity is not due to the presence of false positives, but rather to a considerable percentage of subjects in the general population who have specific anti-inhalant IgE titers and yet are asymptomatic. In any case, many of the latter subjects presented multiple but low or moderately positive SPT reactions (mainly class 1 or class 2). These reactions have little, if any, clinical significance but often correspond to low levels, multiple RAST positivities, which are able to react with the solid phase of Phadiatop CAP in summation, producing a positive result.
In a clinical setting this sensitivity of the test may be helpful to the general practitionner in order to clarify the atopic background of symptoms whose etiology is not well defined. In large screening programmes of unselected populations a better resolution between atopic subjects with or without clinical signs of allergic disease may be obtained by increasing the cut-off of the test, as we have shown in tab. I. The proportion of subjects with no symptoms and Phadiatop positivity will be reduced to those with higher levels of circulating specific IgE antibodies. Prospective studies performed by other authors have demonstrated that in young healthy adults the higher the score of SPT for inhalants, higher is the probability of developing respiratory allergic diseases over the course of time (17). The higher Phadiatop quantitative results, which we have shown are correlated to the highest SPT score (Fig. 4), may therefore prove to be, in these cases, a useful indicator in allergy prediction.
In the subgroup of 98 subjects also examined with the methacholine BPT we observed that bronchial hyperreactivity was strongly associated to a Phadiatop positive result. Our data is in agreement with that reported by Cockroft (18), who found a strong association between bronchial hyperresponsiveness and atopy in young adults. BPT with methacholine is one of the most used screening tests during selection of candidates to aircraft piloting but it requires time and the collaboration of the subject. On the basis of our data it may be proposed that BPT be restricted to the Phadiatop-positive population only.

Conclusions.

In over 99% of cases in our population study, Phadiatop negativity corresponded to a lack of allergic disease to inhalants and in over 97% of cases to a lack of bronchial hyperresponsiveness. On the other hand, Phadiatop positive subjects need to be further examined to reach a complete clinical diagnosis. We conclude that Phadiatop CAP may be proposed for use in the selection of candidates to aircraft piloting, provided that a quantitative evaluation of the test results is performed.

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SUMMARY.

The validity of Phadiatop CAP as a tool in the mass screening for inhalant allergies was investigated. Two-hundred and seventy of 1815 Italian recruits were classified as allergics to inhalant allergens on the basis of history, physical examination, SPT for inhalants and/or RAST for the seven most common aeroallergens in Italy. Phadiatop was positive in six-hundred and twenty-three (34.3 %) subjects: in 265/270 allergics and in 357 subjects which had never experienced allergic symptoms; the vast majority of these subjects were also positive to SPT and/or RAST. The level of Phadiatop reactivity was lower in this group with respect to the allergic one. In a subgroup of 98 subjects bronchial hyperresponsiveness was also examined. A very high percentage (85%) of the subjects with bronchial hyperresponsiveness were also positive to Phadiatop, suggesting that atopy is one of

the major etiologic factors of bronchial hyperreactivity in our population sample. We conclude that Phadiatop CAP is extremely useful in the screening of inhalant allergies in candidates to aircraft piloting.

Fig. 1 - Sensitivity to inhalant allergens in 1815 italian recruits.

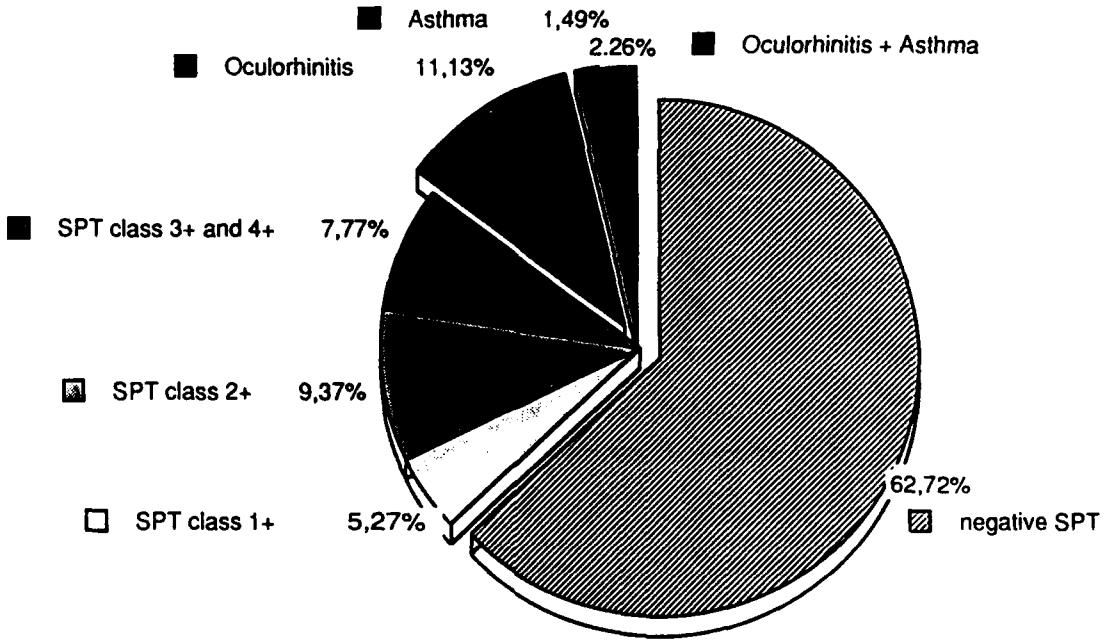


Fig. 2 - Distribution of Phadiatop CAP ln FU in 1815 italian recruits.

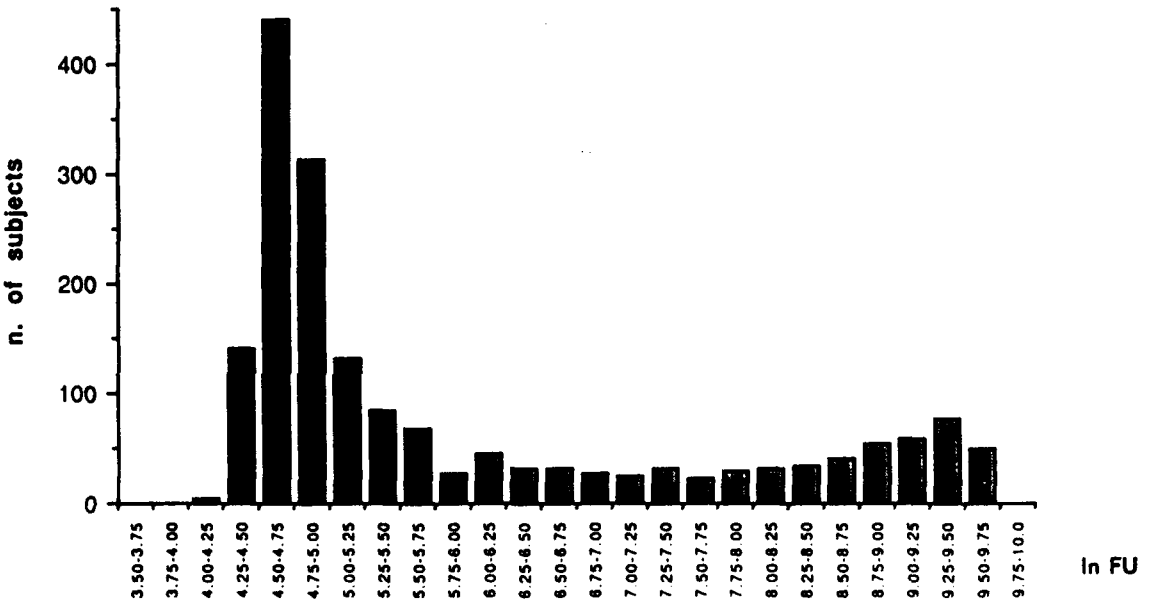


Fig. 3 - Relationship between Phadiatop and SPT.

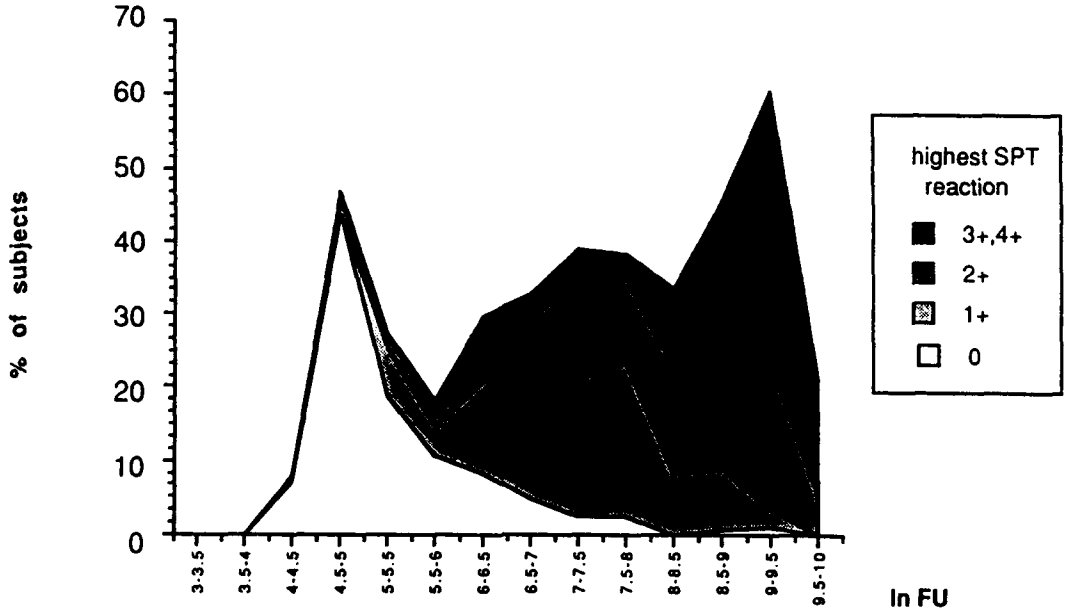
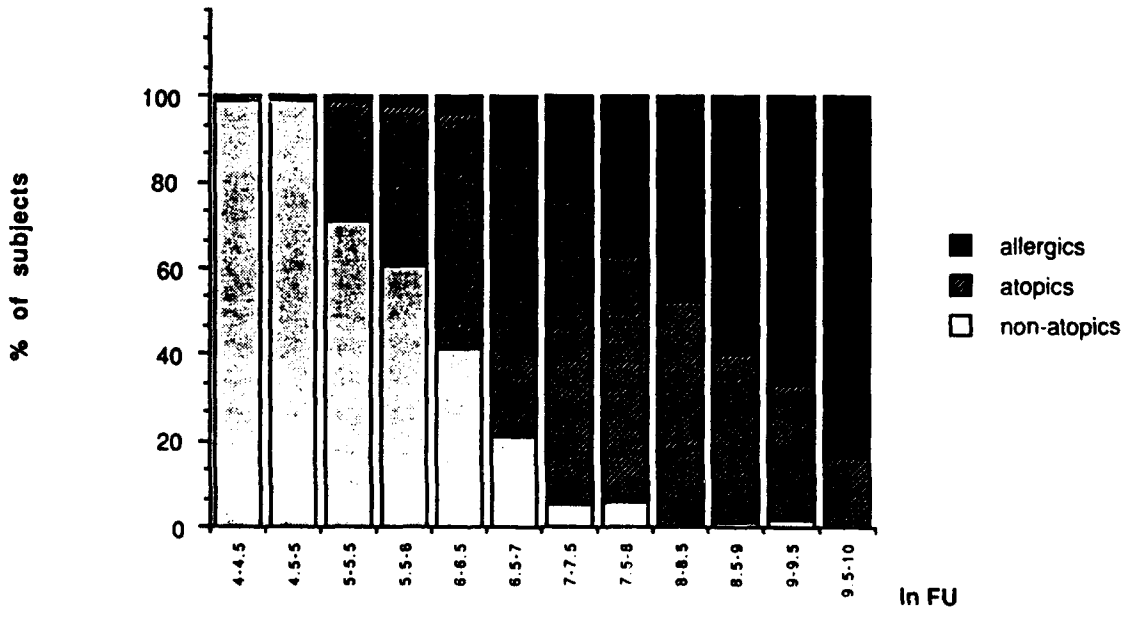


Fig. 4 - Relationship between Phadiatop CAP ln FU and allergy to inhalants.



Tab. I - SCREENING OF INHALANT ALLERGIES IN 1815 RECRUITS USING PHADIATOP CAP FOR DETECTION OF SPECIFIC SERUM IgE.

	PHADIATOP CAP (cut-off = reference serum)		PHADIATOP CAP (cut-off = 900 FU)	
	Positive	Negative	Positive	Negative
Allergics	265	5	260	10
Non-allergics	357	1188	219	1326
Sensitivity (%)	98.14		96.29	
Specificity (%)	76.89		85.82	
Efficiency (%)	80.05		87.38	
Positive predictive value (%)	42.60		56.16	
Negative predictive value (%)	99.58		99.25	

Tab. II - SCREENING OF INHALANT ALLERGIES IN 1815 RECRUITS. ASSOCIATION BETWEEN PHADIATOP CAP AND BPT

subjects examined	98
positive to Phadiatop	26
positive to BPT	16
affected by asthma	6

PHADIATOP CAP (cut-off = reference serum)

	Positive	Negative	
POSITIVE BPT	14*	2	
NEGATIVE BPT	12	70	
Sensitivity (%)	87.5		
Specificity (%)	85.4		
Concordance (%)	85.7		
Positive predictive value (%)	53.8		
Negative predictive value (%)	97.2		

* including all subjects affected by asthma

PHADIATOP, A SCREENING TEST FOR INHALANT ALLERGY

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SUMMARY

The Phadiatop® test, a new in vitro test for inhalant allergy, was evaluated in relation to a panel of seven RAST tests with the common inhalant allergens in The Netherlands, and in comparison with the PRIST for total IgE determinations, in two populations: one in which the prevalence of inhalant allergy was expected to be high and one in which it was expected to be low. The Phadiatop was classified positive or negative according to percentage binding, total IgE was considered elevated at values >=200, >=150, and >= 100 kU/l at ages 12-14, 15-16, and 17 years and over respectively. The RAST panel as reference was considered positive when at least one RAST result was class 2 or more. From the predictive values (which depend on the prevalence of the disease in the population) and the accuracies of the Phadiatop® and the PRIST for the RAST, it can be concluded that the Phadiatop® is a highly efficient test, much more so than the PRIST, in correctly classifying atopic and non-atopic subjects as judged by the reference RAST panel.

INTRODUCTION

Allergy is a common condition in the general population with a prevalence of about 30 per cent, of which about half develop allergic symptoms. However, a clear age-relation exists, with a peak between 20 and 30 years of age with a gradual decline thereafter.
Until recently screening for immunoglobulin E (IgE) mediated allergy could be carried out by limited skin prick testing, or by laboratory tests measuring total IgE concentration using the paper-radioimmunosorbent test (PRIST) or determining the presence of specific IgE-antibodies against a panel of specific allergens, using the radio-allergosorbent test (RAST). The newly introduced Phadiatop® test is a single yes/no test based on the allergosorbent principle. It covers a balanced mixture of relevant allergens causing common inhalant allergy. In this study the Phadiatop® and PRIST were evaluated in relation to a panel of seven RAST tests on inhalant allergens common in the Netherlands. Two separate populations were studied in which different prevalences of inhalant allergy could be expected.(1,2)

PATIENTS AND METHODS

Patients

Population A:
Serum was obtained from patients, aged 12-64 years, who had been prescribed anti-asthmatic drugs in the year previous to the start of a study on the prevalence of respiratory allergy in patients in General Practice in a community in the Western part of the Netherlands. The following drugs were concerned: β_2-agonists, anticholinergics, xanthine derivates, inhaled steroids, and sodium cromoglycate.

Population B:
Serum was obtained from adult patients (minimum age 30 years in the study period) treated in General Practice for chronic airways obstruction, based on American Thoracic Society criteria. The patients were recruited for a medication intervention study, taking place in the Eastern part of the Netherlands, and inclusion criteria were defined in terms of lung function values and histamine threshold values.

Laboratory tests.

The following tests were performed: Phadiatop® for determination of IgE antibodies against a balanced mixture of relevant inhalant allergens not further specified by the manufacturer, PRIST for determination of total serum IgE, and RAST for determination of specific IgE antibodies against the following inhalant allergens common in the Netherlands: house dust mite, grass pollen, cat dander, dog dander, weed pollen, tree pollen and moulds (Phadebas RAST D1, Gx1, E1, E5, Wx3, Tx9 and Mx1, Table 1). The tests were carried out with reagents of Pharmacia Diagnostics AB, Uppsala, Sweden, according to the instructions of the manufacturer. The Phadiatop® and RAST were performed as single tests, the PRIST as a double test. The percentage binding of the Phadiatop® was determined and classified as a positive or negative result. The percentage binding of each RAST was determined and classified semiquantitatively in Phadebas RAST classes 0-4. Total IgE concentration was expressed in kU/l, taking the mean of two determinations, and considered elevated at values >=200, >=150, and >=100 kU/l at ages 12-14, 15-16, and 17 years and over respectively.(3-5)

Data analysis

The RAST panel served as reference. It was considered positive when at least one RAST result was class >=2,(6,7) and negative when all seven RAST results were class 0 or 1. The predictive values and accuracy of the Phadiatop^R and the PRIST for the RAST were computed.(8)

RESULTS

Population A:
Blood was sampled in 248 patients (113 males and 135 females), mean age 34.9 years and standard deviation (SD) 15.6. The number of patients who had a positive RAST panel (at least one RAST result class 2 or more) was 148 (59.7%). The predictive value of the Phadiatop^R for one or more positive RAST results was 100% and its predictive value for all RAST results being negative was 97.1%, while these predictive values for the PRIST were 84.7% and 71.2% respectively. So the accuracy of the Phadiatop^R was 98.8%, and of the PRIST 78.6%. Table 2.

Population B:
Blood was sampled in 204 patients (115 males and 89 females), mean age 52.8 years, SD 12.5. In this population 52 (25.5%) of the patients had a positive RAST panel. The predictive value of the Phadiatop^R for one or more positive RAST results was 90.9% and its predictive value for all RAST results being negative was 98.7%, the accuracy of the test being 96.6%. For the PRIST these predictive values were 46.8% and 88.0% respectively with the accuracy of the PRIST being 72.1%. Table 3.

CONCLUSION

The Phadiatop^R can be used as a very reliable screening test in populations with varying prevalences of inhalant allergy, either to confirm or to exclude sensitization to common inhalant allergens. Additional RAST tests can be carried out with the same serum sample, if stored at a temperature of at least -20°C.

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Table 1. Allergens used in the RAST

D1 House dust mite:
- Dermatophagoides pteronyssinus

Gx1 Grass pollen mix:
- G3 Cocksfoot
- G4 Meadow fescue
- G5 Rye-grass
- G6 Timothy
- G8 Meadow grass, Kentucky blue

E1 Cat dander

E5 Dog dander

Wx3 Weed pollen mix:
- W6 Mugwort
- W9 English plantain, Ribwort
- W10 Goosefoot, Lamb's quarters
- W12 Golden rod
- W20 Nettle

Tx9 Tree pollen mix:
- T2 Grey alder
- T3 Common silver birch
- T4 Hazel
- T7 Oak
- T12 Willow

Mx1 Moulds mix:
- M1 Penicillium notatum
- M2 Cladosporium herbarum
- M3 Aspergillus fumigatus
- M6 Alternaria alternata (tenuis)

Tabel 2. Predictive values and accuracy of Phadiatop and of PRIST (total IgE) for a panel of seven RAST tests in population A, with panel positive when at least one RAST result class > =2

RAST panel	Phadiatop			PRIST (total IgE)		
	positive	negative	total	elevated	normal	total
positive	145	3	148	116	32	148
negative	0	100	100	21	79	100
total	145	103	248	137	111	248
Predictive values:						
positive:	145/145 = 100 %			116/137 = 84.7%		
negative:	100/103 = 97.1%			79/111 = 71.2%		
Accuracy:	245/248 = 98.8%			195/248 = 78.6%		

Tabel 3. Predictive values and accuracy of Phadiatop and of PRIST (total IgE) for a panel of seven RAST tests in population B, with panel positive when at least one RAST result class > =2

RAST panel	Phadiatop			PRIST (total IgE)		
	positive	negative	total	elevated	normal	total
positive	50	2	52	37	15	52
negative	5	147	152	42	110	152
total	55	149	204	79	125	204
Predictive values:						
positive:	50/ 55 = 90.9%			37/ 79 = 46.8%		
negative:	147/149 = 98.7%			110/125 = 88.0%		
Accuracy:	197/204 = 96.6%			147/204 = 72.1%		

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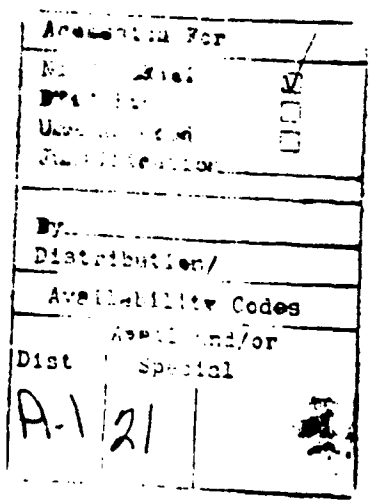

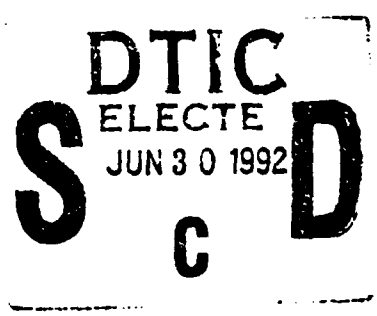

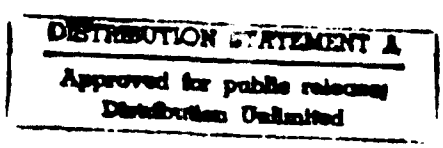

DTIC FORM 463

OPI: DTIC-TID

IN VIVO AND IN VITRO DIAGNOSIS OF ALLERGIC RESPIRATORY DISEASE DURING SCREENING PROCEDURES IN THE ITALIAN NAVY; COMPARATIVE EVALUATION OF A RECENT QUANTITATIVE AUTOMATIZED ENZYME IMMUNOASSAY METHOD TO DOSE SPECIFIC IgE.

by

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INTRODUCTION

Atopy is very common among the Italian population. Recent evaluations of the Italian Society of Allergology and Clinical Immunology indicate that about 17% of the inhabitants are affected by allergic diseases, and a considerable amount of subjects are suffering for bronchial asthma.
Being the prevalence of these pathologies particularly high in the first three decades of life and considering their increasing incidence, allergies became a remarkable problem in the selection of military personnel (1.2.3).
In the military frame allergic patients could be exposed to many trigger factors acting on their organism (4). The increased risk for airborne diseases in the community (moreover viral infections) and the prolonged exposure to the air conditioning systems (for example serving aboard the ships (5) are surely situations able to negatively influence asthmatic patients. In the meanwhile it is very difficult imagine patients affected by food allergy following a specific diet during military service. And we could propone many other examples.
But , if till now we examined the possible negative effects for the allergic patients deriving from military life, we also must consider that allergic military personnel can put themselves and their buddies at risk and/or determine damages to expensive military means and the abortion of particular operations (6).
For the above mentioned reasons in the Italian Navy we make a strict selection of allergic patients during the enrolment activities (7).
Considering the possible ways to join the Navy, we can be in three different situations:

a. Conscripts obliged by the law to serve for 12 months in the Armed Forces as soldiers or sailors;

b. Conscripts who asked to do their mandatory service as Officers,

c. Volunteers who wish to follow a career as Officer or P.Officer.

In case a. whe conscripts have interest in manifesting their pathologic condition to avoid being enroled, on the opposite in cases b. and c. the interest is to occult physical problems making them unable as Officers or P.Officers.
The required diagnostic pathway is quite different in the two cases. In the first, all our clinical knowledge must be implemented on selected subjects to reach a confirmation of the diagnosis for which they claim (8,9,10,11), in the second, we must search for possible allergies on a large number of subjects without any information. In this last case there is an indication to widely use an in vitro system to detect specific IgE against the main inhalant and food allergens.

MATERIALS AND METHODS.

a. During 1990 in the Department of Allergology and Pneumology of the Naval Hospital of La Spezia we observed 1180 male subjects, conscripts and already in service personnel, aging from 16 to 30 years, who came to us declaring that they suffered from respiratory allergies or with asthmatic symptoms. All patients were treated strictly implementing the protocol showed in fig.1.
Skin prick tests (SPT) were performed by using Dome-Hollister-Stier extracts and Morrow-Brown lancets. The results were interpreted comparing the wheal produced by the allergen with histamine-prick, according to the following rule:

wheal from 1/4 to1/2	+
wheal from 1/2 to 1	++
wheal from 1 to 2	+++
wheal >2	++++

Doubtful results induced in vitro research of specific IgE by the mean of different Enzyme-Linked Immuno Sorbent Assays (ELISAs).

A computerized spirometer Microloop BBC was used to obtain basal spirometry. In those cases who required a deeper study of lung functions (12), a computerized spirometer Autolink-P.K. Morgan and a Body-box Pletismography-P.K. Morgan were used to measure Residual Volume, Carbon Monoxide Diffusion and the airways Resistences.
The not-specific bronchial challenge was performed by a methacholine dosimetric method using a MEFAR dosimeter (13,14), according to the protocol by G.BALZANO and Co.(15).
The specific bronchial challenge was performed by inhaling allergen solutions at Tidal Volume from a Wright-like nebulizer, according to the protocol proposed by D. KILLIAN and Co. (16).

b. On 98 subjects taken at random from the above mentioned case-record, we made a comparative study between the results of the SPT and those obtained by using a semi-automated instrument system for immunoassay panel testing (ABBOTT MATRIX, it consists of an analyzer with an enclosed ten-position carousel, wash and waste system, reader, printer, keypad, display and computer). Assays are run in analyte-specific test cells using color enzyme immunoassay methodology. Test cells are embossed with a dot array, each dot containing a unique assay. The first reagent and sample are pipetted into a test cell which is loaded into the analyzer by the operator. The Abbott Matrix Analyzer will then automatically incubate, wash, request additional reagents, read and calculate results. The total required time is about 20 hours, with approximately 1 minute hands-on-time per patient sample.
The statistic data analysis was performed by using a specific software (Biomedical Data Package) and applying the "kappa" statistic test to make a measure of agreement between "in vivo" and "in vitro" results. The global results (allergic/not allergic) and the

absolute differences between the classes of positivity "in vivo" and "in vitro" for each allergen were particularly compared. Values of less than 0,40 reflect poor agreement. With between 0,40 and 0,75 agreement is fair to good. Values of above 0.75 indicate strong agreement.

RESULTS

a. In the Table 1 the results concerning the age of the patients, the duration of the disease, the familial anamnesis, the characteristics of symptomatology, the diagnosis of asthma, the sensitizations, and the degree of bronchial hyperreactivity in the not-symptomatic patients are shown.
One hundred fifty-three out of 1180 subjects didn't show any sensitization to airborne allergens, and in 53 of them the diagnosis of intrinsic asthma was adopted. 100 subjects out of 1180 were neither allergic nor asthmatic (fig.2).
Figure 3 shows how the condition of polisensitation in our case-record doesn't seem to induce symptomatology more than monosensitation although many Authors in the past concluded in the opposite way.
Figure 4 stresses that a significant amount of the pure pollinosic patients who came to our observation out of the pollen season presented symptoms confirming the sensitivity of bronchial mucosa of allergic patients to the not-specific noxae.
Data about therapies are shown in fig.5.

b. Reparding the comparison between SPT and the MATRIX System, 14 out of 98 subjects resulted negative to SPT (14,29%), while the same 14 plus other 12 (26,53%) were negative to MATRIX (fig.6). Statistic value on the full sample was 0.33, indicating a poor global agreement between the two methods.
The degrees of agreement for the single allergens are shown in Table 2.
The agreement is fair to good for all allergens except than for Aspergillus (k=0,379), while it is very strong for Perietaria and Dermatophagoides Pteronyssinus.
Sera from 13 subjects positive to olive in vivo and/or in vitro were controlled in double by MATRIX and RAST in front of a low, although significant, concordance between the first two methods (k=0.529). The agreement between MATRIX and RAST was quite total (k=0.99).

DISCUSSION

The protocol we applied to screen respiratory allergic diseases showed to be easily appliable to a considerable number of subjects (we observed a mean of 8-10 patients for working day) and it gave us a large amount of information on respiratory allergy and bronchial asthma in youth.
With the exception of 100 subjects who resulted neither allergic nor asthmatic, 805 on 1080 allergic patients were affected by bronchial asthma so that they were declared unable to do military service as conscripts, or they were treated and monitorized for a long time if already enroled Officers or P.Officers. Whenever their physical conditions allowed us they were left in active duty taking into care to avoid particular environmental exposures and working categories at risk.
We never observed undesired reactions to the methacholine or allergen bronchial challenges.
Allergy to house dust mites by alone or in association with pollens was obviously prevalent (774 patients). Among pollens the majority of sensitizations were against Graminae (477), followed by Parietaria (297) and Olive (196). Remarkable for Italy the high frequency of sensitizations against Birch (109), moreover in relationship to that against olive, a more common pollen in our country.
A not allergic asthma, the so-called Intrinsic Asthma, was diagnosed in 53 out of 805 asthmatic patients. This datum must be considered in relationship to the young mean age of our case-records, as the allergic actiology is prevalent in the first three decades.
A peculiar consideration is related the previous therapies. While only 29,7% of patients referred to having practiced pharmacological or similar therapies, more than 51% have been treated with Specific Immnunotherapy, confirming the widely diffusion of such a therapy in Italy.

b. The comparison between SPT and an "in vitro" test for specific IgE, ABBOTT MATRIX, is of course influenced by many factors like variability among the different operators in interpreting "in vivo" results (the variability in performing SPT is significantly reduced by using standardized lancets for prick testing), and the differences in specifity and sensitivity existing between the two methods. This is foundamentally the reason for which the global comparison of data (positivities to the panel of allergens in vivo VS MATRIX Aero-panel) is statistically unsatisfactory (k=0.33).
Nevertheless if we consider each single allergen, the degree of agreement between the two methods, is quite good. Only for Aspergillus there was a poor agreement between the 2 methods, but we know that in vivo answers to mould extracts are often unreliable. In fact also the agreement for Alternaria was positive, but it presented the lowest positive concordance of the all allergens panel (k=0.500).
The low, although good, agreement of Olive (k=0.529) struck us, so we decided to verify the MATRIX results repeating the test by Radio Allergo Sorbent Test (RAST) on 13 sera taken at random from patients positive "in vivo" and/or "in vitro" (MATRIX) to this allergen. The agreement between MATRIX and RAST was nearly total, confirming the tendency of SPT to give false-positive results (high sensitivity).

CONCLUSIONS.

The observation of a large number of young allergic patients gave us the possibility to acquire crucial information on the epidemiology, the aetiology, and the pathogenesis of respiratory allergies and particularly that of bronchial asthma.
The diagnostic protocol used accomplished in a satisfactory way the proposed tasks to screen allergic conscripts and to clinically study already in service patients.
Our evalutaion of the recently introduced immunoassay ABBOTT - MATRIX confirmed its characteristics of specificity and reproducibility. It also demonstrated to be an easy to use "in vitro" system with very poor requirements of intervention by the operator.
Concluding, we emphasize the need to routinarly introduce "in vitro" research of allergies for the preliminary screening of the candidates to the Military Academies, the schools for P.Officers, and to special categories with an expensive training and high risk, like pilots or divers.

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TABLE I:
CLINICAL CHARACTERISTICS IN 1180 MALE SUBJECTS OBSERVED AT THE NAVY MILITARY HOSPITAL OF LA SPEZIA.

age range		16-30
disease duration	less than 5 yrs	313 (26.6%)
	5-10 yrs	257 (21.8%)
	more than 10 yrs	610 (51.6%)
allergic familial history	positive	577 (48.9%)
	negative	603 (51.1%)
type of symptomatology	seasonal	575 (48.8%)
	perennial	605 (51.2%)
diagnosis of asthma	symptomatics	250 (21.2%)
	positive BPT	555 (47.0%)
	negative BPT	375 (31.8%)
PD 20 FEV, methacholine	≤ 200 µg	88 (15.8%)
	400 µg	85 (15.3%)
	800 µg	330 (59.4%)
	1200 µg	52 (9.5%)
sensitizations	mites + pollens	424 (42.2%)
(1007: 85.9%)	mites	350 (34.8%)
	pollens	189 (18.7%)
	pollens + danders	38 (3.8%)
	alternaria	6 (0.5%)
sensitization to perennial allergens	D. pteronyssinus	697
	D. farinae	633
	Cat	173
	Dog	45
sensitization to pollens	Grass	477
	Pellitory	297
	Olive	196
	Birch	109
	Hazel tree	71
	Alder	69
patients without sensitization	symptomatics	25 (16.3%)
(153: 14.1%)	positive BPT	28 (18.3%)
	negative BPT	100 (65.4%)

Table 2.

Allergen	Agreement SPT/MATRIX (kappa statistics)
DERM. PTERONYSSINUS	0.770
DERM. FARINAE	0.744
CAT	0.684
DOG	*
PARIETARIA	0.824
GRAMINAE (thimoty+bermuda grasses)	0.647
OLIVE	0.529
BIRCH	0.628
ALTERNARIA TENUIS	0.500
ASPERGILLUS	0.379

* Although for dog we observed a percentage of differences in the classes values contained from 0 to 1 in 92.9% of cases, being absent differences of levels 2 and 4, the software paradoxically did not calculate statistics.

FIG. 1

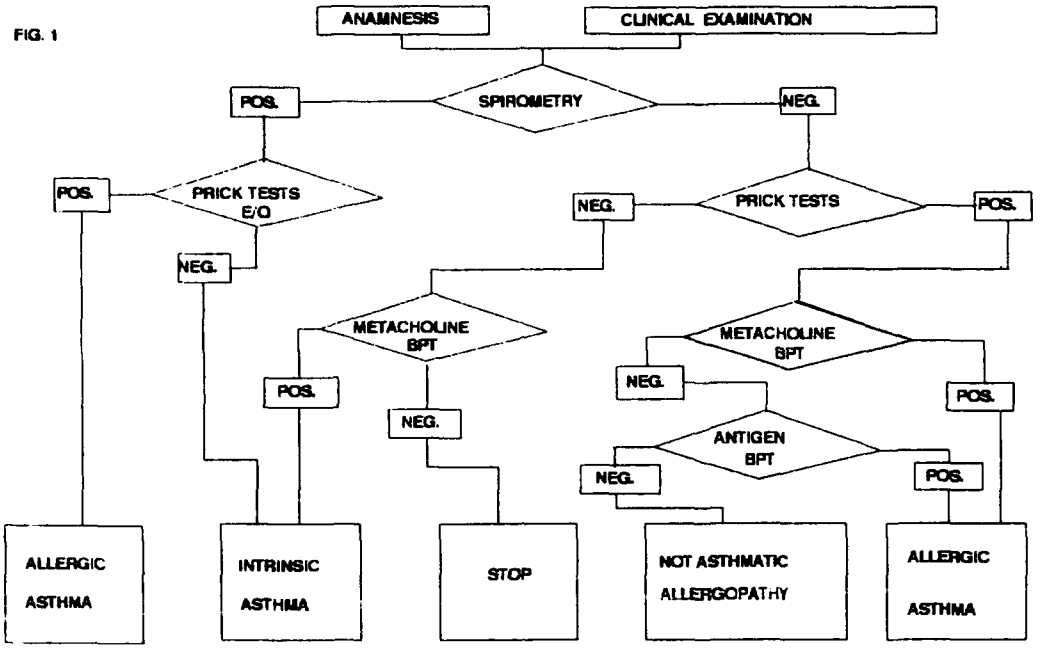
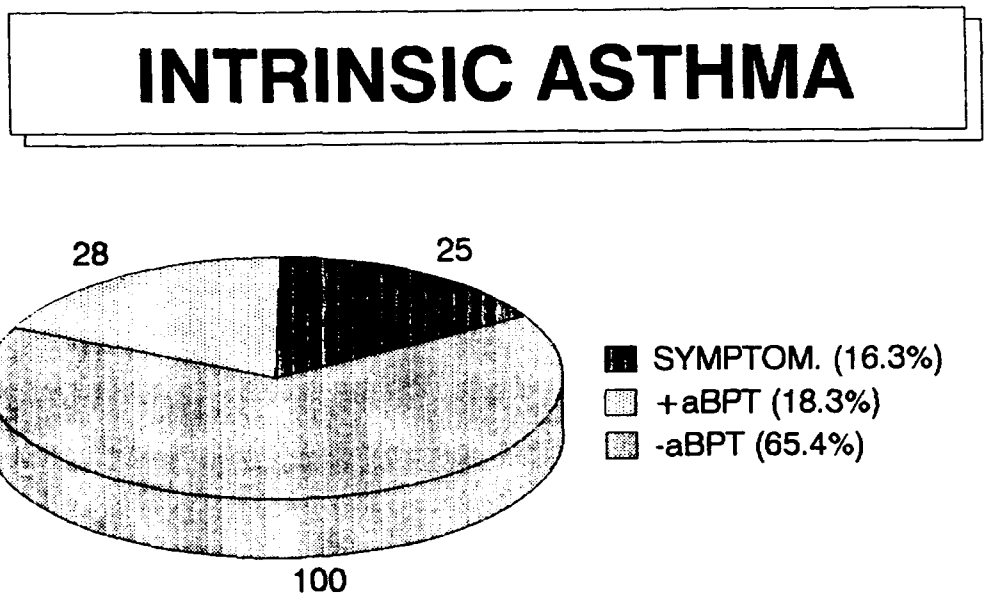


FIG. 2



NOT ALLERGIC PATIENS (153 ON 1180)

FIG. 3

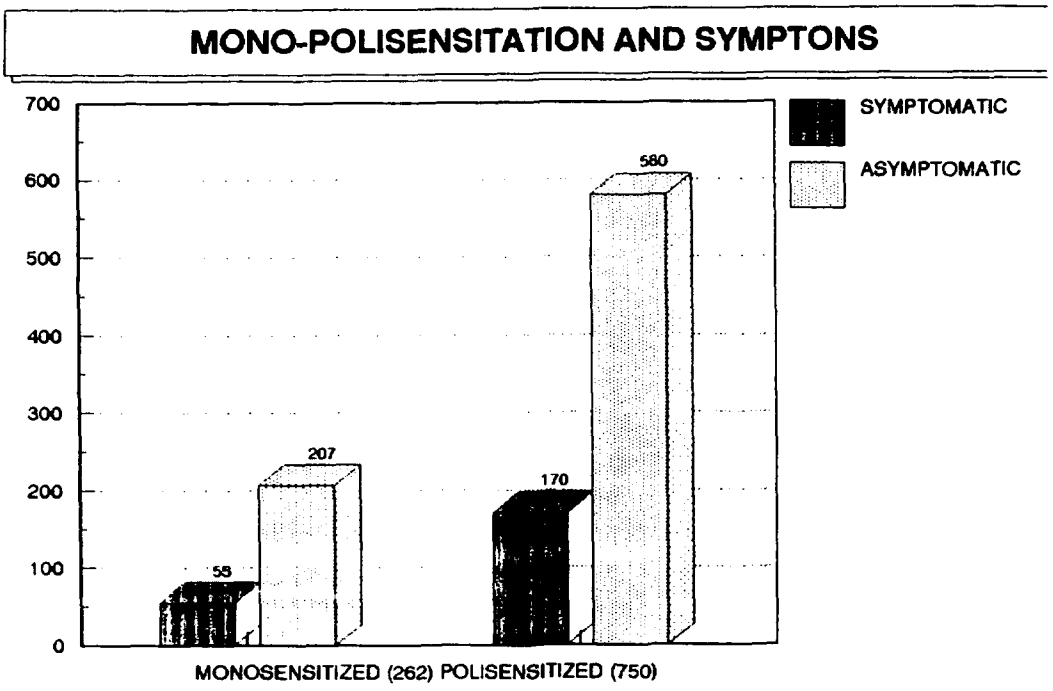

MONO-POLISENSITATION AND SYMPTONS
700
600
500
400
300
200
100
0
55
207
170
580
SYMPTOMATIC
ASYMPTOMATIC
MONOSENSITIZED (262) POLISENSITIZED (750)

FIG. 4

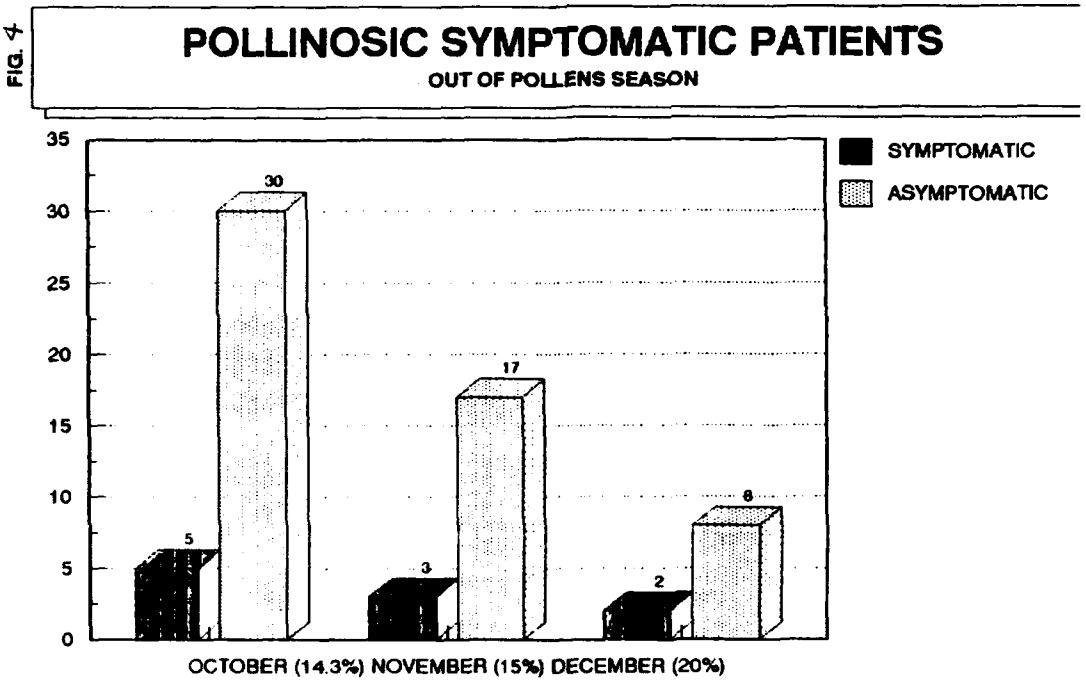

POLLINOSIC SYMPTOMATIC PATIENTS
OUT OF POLLENS SEASON
35
30
25
20
15
10
5
0
5
30
3
17
2
8
SYMPTOMATIC
ASYMPTOMATIC
OCTOBER (14.3%) NOVEMBER (15%) DECEMBER (20%)

FIG. 5

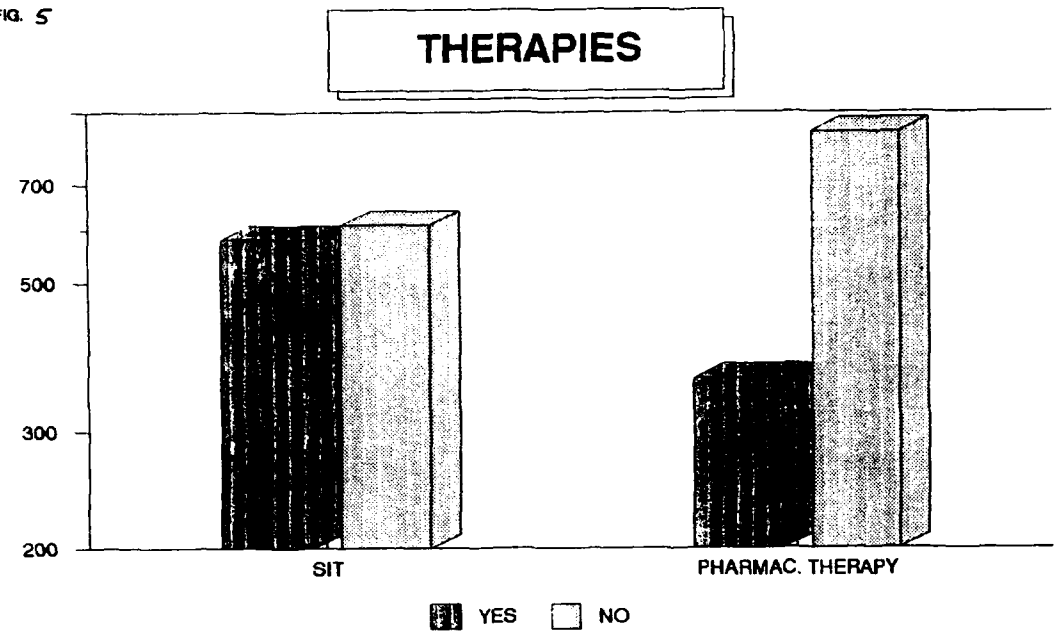
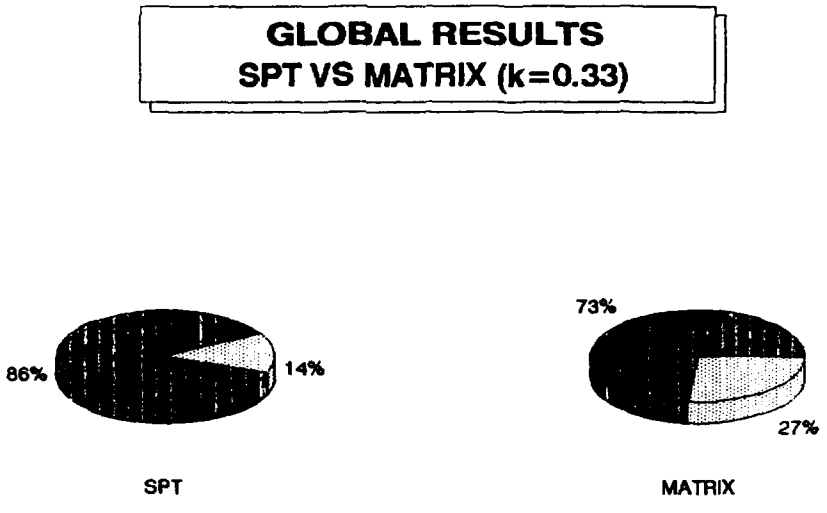

THERAPIES
700
500
300
200
SIT
PHARMAC. THERAPY
YES
NO

FIG. 6


GLOBAL RESULTS
SPT VS MATRIX (k=0.33)
86%
14%
73%
27%
SPT
MATRIX
POS.
NEG.

Asthma In Aircrew: Assessment, Treatment and Disposition

by

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SUMMARY

Asthma represents a spectrum of increased airway reactivity from mildly increased responsiveness through to severe life-threatening bronchospasm. Rational recommendations for treatment and aeromedical disposition require a careful assessment of bronchial reactivity through correlation of the clinical findings with results of pulmonary function testing including an objective measure of airway reactivity . Airway challenge testing with methacholine allows a safe, objective assessment of airway reactivity.

In the Canadian Forces, aircrew candidates with a history of wheezing, recurrent cough or bronchitis in childhood, or abnormal screening PFTs are further screened with an airway challenge test. Applicants with a PC20 less than 4 mg/ml are disqualified from pilot selection, and less than 2 mg/ml from other aircrew.

Trained aircrew who develop wheezing are assessed with full pulmonary function testing including a methacholine challenge test. Those with objectively confirmed mild bronchial hyper-reactivity requiring no treatment or controlled by inhaled anti-inflammatory agents (corticosteroids, sodium cromoglycate, nedocromial sodium) are returned to flying in other than fast jets where even minor small airway instability may worsen ventilation-perfusion mismatch caused by +Gz.

LIST OF ABBREVIATIONS

FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
FRC	Functional residual capacity
MMFR	Mid-maximal flow rate
MEF50	Maximal expiratory flow rate at 50% of the forced vital capacity
MEF75	Maximum expiratory flow rate at 75% of the forced vital capacity
MEF5	Change in MEF50 breathing heliox gas mixture
PC 20	The concentrationof methacholine in mg/ml which causesa 20% fall in the FEV1
TLC	Total lung capacity
URI	Upper respiratory infection

INTRODUCTION

The term asthma encompasses a spectrum of increased airway reactivity from mildly increased bronchial responsiveness induced only under unusual circumstances through to severe episodic bronchospasm requiring regular use of systemic medications. The latter situation is obviously incompatible with flying duties, while individuals with very mild bronchial hyper-reactivity may quite safely perform at least restricted flying duties.

The aeromedical concerns about asthma include acute incapacitation caused either by severe bronchospasm or at least theoretically, by gas trapping leading to pneumothorax or aeroembolism. Additional concerns include the various medications, many of which have aeromedically undesireable side-effects. Lastly, even mild bronchospasm may lead to ventilation-perfusion mismatching predisposing to hypoxia with a potential reduction in tolerance to +Gz, a concern in fast jet aircrew.

From an aeromedical standpoint, the problem lies in accurately identifying the degree of airway reactivity. The standard clinical tools of history and physical examination are often of little help since even severe asthmatics may be asymptomatic between episodes.

ASSESSING AIRWAY FUNCTION

Assessment of airway function and reactivity includes the following measures:

1. Careful clinical history
   - recurrent "bronchitis" or respiratory symptoms in childhood
   - history of other atopic disease; hay fever, eczema
   - family history of atopy or respiratory disease
   - wheezing with URIs, in unusual circumstances (eg around animals), or with exercise especially in cold air

2. Full pulmonary function assessment
   - maximum expiratory manoeuvers
     volume-time curves (FEV1, FVC, MMFR)
     flow-volume curves, air and heliox
     (V50, V75, V50)
   - lung volumes (TLC, FRC ? any gas trapping)
   - diffusing capacity
   - closing volume (single-breath nitrogen washout)

3. Repeat pulmonary function assessment after bronchodilator
   - improved flows
   - change in closing volume
   - change in lung volumes

4. Objective assessment of airway reactivity
   - standardized challenge tests with methacholine, histamine or cold air

5. Exercise testing +/- cold air

AIRWAY CHALLENGE TESTING

Assessment of airway reactivity through a standardized inhalation challenge test provides an objective measure of bronchial responsiveness. Such testing, when combined with other measures of pulmonary function and with clinical information may allow a rational recommendation about aeromedical disposition in a disease where the decision has historically been made more often on the inclination of the individual Flight Surgeon or Board than on any objective findings.

At DCIEM, we use the methacholine challenge test as developed and standardized by Hargreaves, Dolovich and Cockroft at McMaster University in Hamilton, Canada (1,2). After obtaining baseline expiratory flow parameters, subjects breathe nebulized solutions containing increasing concentrations of methacholine for a period of two minutes; repeat flow measurements are obtained after each concentration. The response is defined by the concentration of methacholine in mg/ml which produces a 20 percent fall in the FEV1, which is expressed as the PC20 (mg/ml). An example of a report is shown in Figure 1.

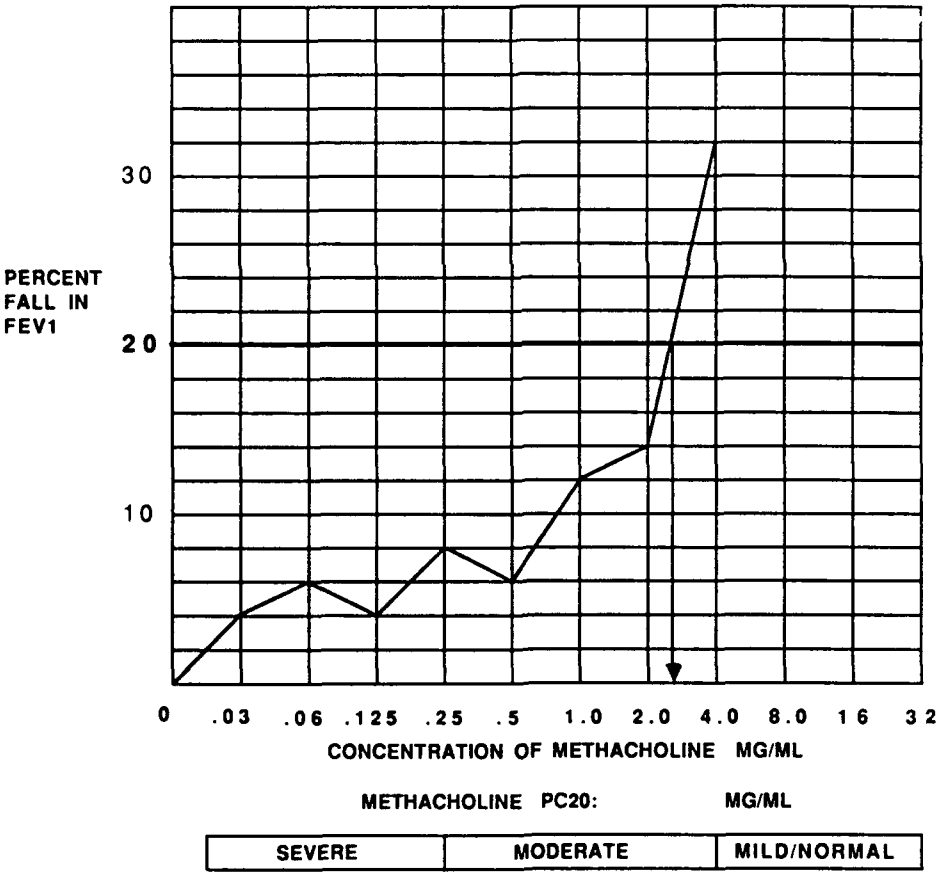
CENTRAL MEDICAL BOARD METHACHOLINE CHALLENGE TEST

SIN:_____ **RANK**_____ **SURNAME, Initials:** _____

PHYSICIAN: _____ **TECHNICIAN:** _____ **DATE:** _____

ASC/UNIT: _____ **DOB/AGE:** _____

DOSE RESPONSE CURVE



METHACHOLINE PC20: **MG/ML**

SEVERE	MODERATE	MILD/NORMAL

COMMENTS: **Mildly increased bronchial reactivity. PC20 3 mg/ml**

Figure 1

The test is relatively simple, inexpensive and easy to perform, with little risk to the subject. Particular attention must be paid to the calibration of the output of the nebulizer and the flow meter; errors in either can significantly affect the results. These should be recalibrated at regular intervals, especially in an aeromedical laboratory where decisions regarding an aviators career may hinge on the results.

AEROMEDICAL SCREENING/AIRCREW CANDIDATES

At the Central Medical Board at DCIEM, all candidates are screened with maximum expiratory flow-volume curves. Selected candidates are further screened with complete pulmonary function studies including methacholine challenge tests. The indications to proceed to complete pulmonary function testing are:

1. History of asthma or wheezing, or of recurrent cough or bronchitis in childhood.

2. Abnormal screening flow-volume curves (MEF50, MEF75, FEV1, or FEV1/FVC <80% predicted)

3. History of significant upper airway atopy.

The pass/fail criteria based on the methacholine PC20 are shown in Table 1.

TABLE 1

CMB METHACHOLINE STANDARDS

PC20 (mg/ml)	DISPOSITION
>4	Fit Pilot selection
2-4	Unfit pilot, fit other aircrew

Table 2 shows the CMB data for a two year period. Based on the above criteria, only a small percentage (3.5%) of the 1805 candidates required evaluation with methacholine challenge testing. Of the 63 candidates who underwent further evaluation, 57% were disqualified from pilot selection. The highest rejection rate was in the group with a past history of wheezing.

AIRCREW STATUS	DISPOSITION	No.
Fast Jet Pilots (8)	Unrestricted flying	6
	Removed from fast jets	2
Rotary Wing Pilots (6)	Unrestricted flying	5
	Restricted from CF5/CF18	1
Transport Pilots (1)	Restricted from CF5/CF18	1
Flight Surgeons (3)	Unfit fast jets	2
	Decompression chamber	
	Unrestricted	1
Navigator Students (2)	Ceased Training	2
Flight Steward (1)	Continue Duties	1

TABLE 3. DISPOSITION OF AIRCREW REFERRED FOR EVALUATION OF REACTIVE AIRWAYS DISEASE.

In many cases, demonstration of normal or very mildy increased airway reactivity on methacholine challenge testing allowed the aircrew to be returned to unrestricted flying duties. Two cases are given as examples:

Case 1. A 30 year old tactical helicopter pilot developed symptoms of wheezing and shortness of breath while renovating a house he had recently acquired. He had been cleaning and painting in the house, in which pets had previously lived. He went to the Base Medical Clinic and was found to be mildly dyspneic, with bilateral wheezes but good air entry on auscultation. He was given inhaled salbutamol via a nebulizer with rapid resolution of the symptoms. He was given a salbutamol inhaler but he did not require further treatment.

He gave a history of upper airway atopic symptoms as a child, and had been on a desensitization program. On occasion , he had developed mild wheezing on exertion in cold air and with lower respiratory tract infections. He had not had any such symptoms since the age of 8 until this particular episode.

Pulmonary function tests showed normal expiratory flow rates with a modest increase in flow rates at low lung volumes after bronchodilator. A methacholine challenge test showed normal airway reactivity, with a PC 20 of 4.0 mg/ml.

TABLE 2	No.	PERCENT OF TOTAL	NUMBER REJECTED	PERCENT OF CANDIDATES SCREENED
CANDIDATES SCREENED	1805			
AIRWAY CHALLENGE TESTS	63	3.5	36	57
Upper Airway Atopy	12	0.7	2	16
Wheezing or bronchitis	45	2.5	32	71
Abnormal PFTs	6	0.3	2	33

He was returned to flying duties as a tactical helicopter pilot.

Case 2. A 24 year old jet instructor pilot developed symptoms of mild intermittent dyspnea over a period of several weeks. He had awakened on several occasions in the night with this sensation. The problem was brought to the attention of the Flight Surgeon only after the instructor declared a physiological emergency during a local training flight after he became seriously dyspneic. Although the pilot was not wheezing after the incident, the question of mild asthma was raised.

Full pulmonary function tests were normal, with no change after bronchodilator. On methacholine challenge testing, the PC20 was found to be 16 mg/ml.

Having eliminated reactve airways disease as a likely cause for the problem, attention was directed to hyperventilation as a possible cause. His wife was expecting their first child within a few weeks, and the symptoms resolved following delivery.

TREATMENT OF REACTIVE AIRWAYS DISEASE IN AIRCREW

Current trends in the treatment of asthma reflect the recognition of the very significant contribution of inflammation in the pathogenesis of the disease . Chemical mediators of inflammation are released upon stimulation of mast cells triggering both a bronchoconstrictive and inflammatory response. (3). The drugs which act primarily as relaxants of tracheobronchial smooth muscle (bronchodilators) include the theophylline derivatives, beta-adrenergic agonists, and anticholinergics. Those that act primarily as inhibitors of inflammation include corticosteroids, sodium cromoglycate and nedocromil sodium.

In flight deck aircrew, because of the significant side-effects of the bronchodilators, only the inhaled anti-inflammatory agents are generally compatible with continuing flying duties. However, in individuals with mild to moderately increased airway reactivity, aggressive treatment with inhaled anti-inflammatory agents alone or in combination can result in a significant stabilization of airway hyper-reactivity. To achieve this the agents must be used meticulously on a regular daily basis. In Canadian Forces aircrew, these agents are used with no flying restriction required. Demonstration of improved or normalized airway reactivity on treatment may be carried out with challenge testing before and while on treatment.

Preventive measures are also important and worthwhile. Agents that precipitate symptoms should be removed from the environment whenever possible, including cigarette smoke, furry animals, feather pillows, down comforters, and rugs and other materials that collect dust or foster mould.

There is still considerable controversy about the role of immunotherapy and desensitization in the treatment of asthma. It remains of unproven benefit. Because of the complex logistics involved including a mandatory period of grounding required after desensitization it is not advised in Canadian Forces aircrew for the treatment of reactive airways disease.

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DISCUSSION SESSION IV

INHALANT ALLERGIC DISEASES (Papers 29 to 34)

W.D. CLARDY (USA): Dr. Gray: are you following any of the people who did not show positive on the methacholine test who were shown to be false negatives when they later demonstrated respiratory symptoms and if so, is it now working out?

G. GRAY (CA): We introduced the screening procedure that I outlined here about five years ago. Since then, none of the people we have screened have come back to see us because of asthmatic problems and that is the best answer I can give you, and it is a while before we will give you a negative or a positive predictive value on that test. We haven't had any problems with people screened so far.

R. D'AMELIO (IT): A question to Dr. Gray. In your diagnostic algorithm there is no serological approach. Do you think it is possible to identify all the allergic subjects during the screening procedures just by a functional approach? Is there a possibility in losing some of the subjects with allergic situations?

G. GRAY (CA): We have been relying on the history to detect people with upper airway allergies and I certainly think there is the potential error for subjects, to have taken antihistamines to have come in clear nosed with no symptoms of asthma and get through it.
I think there is a place for us to look at introducing one of the screening techniques test I've heard about here today, and looking at that, when go back to Canada, it will be helpful.

G. ANZALONE (IT): A question to Dr.Gray. If I have understood well, you allow subjects with a mild hyperreactivity to become part of an aircrew: but don't you think that there might be a variability in their degree of hyperreactivity according to the season, if allergic, or according to daily conditions?. Do you think it is better to follow them and to repeat challenges in time or not?

G. GRAY (CA): I think that the methacholine challenge test certainly looks at reactivity just at that particular time, it's not a necessary indication of what they are going to be like for ever. But usually we see them at a time when they have developed symptoms, so probably we have seen them at a time when they have been at their worse. If we can demonstrate mildly increased airway reactivity at that time when they have become recently symptomatic, we can say that they will not probably become any worse after this, but we can control this situation at the next check up. But your point is good, a challenge test is only a window of their reactivity, in that particular time so that, for example, if you put someone with moderately increased airway reactivity on an inhaled corticosteroid and leave them on until their airway inflammation cools down, and then check them again, you find an improvement in bronchial reactivity.

S. BONINI (IT): Prof. Bellanti, you have focused in your presentation on a very important point: that IgE-mediated reaction can progress to allergic inflammation through a late phase reaction. Now, there is a lot of interest in asthma for the presence of fibroblasts and collagen production, so that allergic inflammation is followed possibly by a thickening of basement membrane, or by a change of collagen structures. As a consequence, irreversible lesions appear. I would like you to comment on this aspect which is also very relevant for military medicine, because it also adds a point concerning medico-legal problems and litigations. In fact, while in the past allergy was considered a completely reversible disease, now there is evidence to claim that irreversible lesions can be caused by allergy.

J. A. BELLANTI (USA): Well, I think prof. Bonini has identified a very important point about the progression of allergic diseases and the question of reversibility or irreversibility. I can speak as a pediatrician interested in immunology and allergies and diseases which afflict the growing child. I think from the prospectives of this symposium which involves young adults which apply to military service that we must consider these diseases in a "continuum". I think that Dr. Gray alluded to that in his very important message about the history in childhood of having had some precursors of disease. As pediatricians we're very much interested in identifying the allergic insult at a time when intervention can be performed and before irreversible changes occur. I think it's a tragedy when we see end stage lung disease in the adult. I think we should always ask the question "was there something that could have been done previously to prevent that?". Medicine today is also prevention not just treatment of disease. So I think what you are referring to is an extremely important aspect and the message here is to identify allergic diseases early and to identify the offending substance in eliminating it and, if it's not possible to eliminate it, modulate the inflammatory response by the best treatment we have and that involves the combinations of agents which prevent the bronchospasm as well as the immediate and late response in terms of steroids and other antinflammatory agents.

A.M.J. WEVER (NE): One of the major factors in bronchial reactivity when you're not allergic is smoking. What is your opinion about aircrew who smoke a lot and are (slightly) hyperreactive?

G. GRAY (CA): Smoking is an important factor in airway reactivity and a number of studies quite clearly demonstrate that people who only give up smoking improve airway reactivity and change a mild hyperreactivity to a normal reactivity. Obviously we are against aircrew smoking, but we cannot oblige anyone to give up smoking. What we do though, is carry out a type of screening and obviously, if candidates have some type of serious abnormalities, we can exclude them from enrolement.

J. A. BELLANTI (USA): I did briefly allude to smoking as one of the causes of elevated IgE, but I think it opens up discussion on a very important point which we haven't covered, and that is, the neuroimmunology of allergic disease. We now know, and a lot as been written in the recent literature, that the neurologic system modulates the immune system, and when there is inflammation there is a mucosal denudation by viral infection or by non-specific trauma, as smoking or pollutants that opens up access to stimulating neuro-immune response, which can also play into the network of bronchial hyperreactivity.

G. ANZALONE (IT): There is also another answer to the question. Smoking is a classic dose-dependent toxic effect and in the first stage, mucociliar clearance is paradoxically increased by smoking. When smoke induces a defect in the bronchial mucosa, receptors become more superficial, so they are stimulated by nonspecific agents. The studies by Bienenstock you were referring to, demonstrate how during chronic inflammation in the bronchial phrame you can have proliferation of nerve terminations, so that you can have an increase in the response to non specific stimula. So mild hyperreactive persons which smoke could become

severe hyperreactive patients in time.

S. BONINI (IT): A brief comment on the paper presented by dr. Gray and a question. My comment refers to the need of distinguishing between tests for sensitization (which, as far as we understand by the very nice study of dr. Matricardi, are very closely related in young people to bronchial hyperreactivity), and tests to study target organs hyperresponsiveness. We have data showing that non-specific conjunctival hyperreactivity to histamine and non-specific hyperresponsivity of the bronchi are equally important as allergy in causing the final clinical picture. Therefore, we have to study both sensitization and the target organ response to non-specific agents. My question is: I enjoyed the fact that you are using mainly anti-inflammatory drugs and not beta-stimulants or bronchodilators as a first step for the treatment of asthma. This is also the position of NIH and of BTS. Now, asthma is inflammation, do you think that you can treat it completely, and, therefore, you don't have to put asthmatic people out of service; or do you think that even if you treat them with topical steroids for a long time they still remain hyperreactive, so that they must be excluded from service? We have a follow up study of 24 months of bronchial reactivity to histamine in a group of asthmatics treated with topical steroids or placebo. There is no doubt that topical steroids reduce bronchial reactivity. However, if the duration of asthma is more than 5 years or in some subjects with asthma lasting even less than 5 years, you may find persisting bronchial hyperresponsiveness to histamine also after prolonged topical steroid therapy. Therefore, from our study it seems that hyperreactivity is not only inflammation which can be reversed. The practical consequence of my question is that if you completely treat inflammation and hyperreactivity you have healthy subjects available again for work, but if it is true that "semel asmaticus, semper asmaticus" positive hyperreactivity tests may be very important for exclusion of asthmatics from training and service.

G. GRAY (CA): I think we have to distinguish between candidates for which you still have to spend money and trained aircrew, for which you have already spent money. If you have an experienced aircrew and a subject suffers from a type of wheeziness, and you get him to take antiinflammatory drugs and his wheeziness goes away, the subject returns to a normal state, so he may continue to fly, but if you have a candidate from author who is taking antiinflammatory drugs on a regular basis to obtain a normal airway responsiveness, it isn't worthwhile spending money in order to train him in becoming a pilot.

J. A. BELLANTI (USA): Just to underline this point. Isn't it true, Sergio, that the degree of hyperreactivity is correlated with the degree of inflammation, and if you can treat inflammation you can reverse the degree of hyperreactivity? So, this really comes down to the other point you made earlier, such as when inflammation becomes irreversible. It's a very difficult thing to quantitate in what period in life this occurs. I think it is a very variable thing. I looked at this thing statistically, that is, if you look at asthma in children you're dealing with a pure condition where there is more spasm and less inflammation. With repeated insults during the lifespan of that individual, you get subacute inflammation, you get chronic inflammation and the risk of irreversibility increases. At what point that occurs I think that no one has the crisper inpoints, but what I think is important is to try to intervene in moving it to reversibility. Whether steroids work or not, as your study shows, will be a question of how long that chronic inflammation has been going on and at what point they are irreversible. I wish we had crisper endpoints!

R.E. SPIER (UK): When you're talking about allergy you're talking about IgE, there are many companies now making monoclonal antibodies against IgE with the hope of controlling an acute system in the short term. Surely we have to start looking back, and saying where did the IgE come from in the first place, and may be in the changing of the cytokine profile is the thing one should be aiming at early in life, as in order to prevent that the eccessive production of those IgE against stimula which are not normal evokers of major response of this nature. One really has to understand the language of the cells, and in order to get that, we have to determine early in life in order to get into the preventive aspect of this, other than to doctor-out the symptoms, sort of patch it up and hope that it works for a few hours.

J. A. BELLANTI (USA): I think Prof. Spier has put the finger right on the point; there is now a cytokine cascade which appears to control the synthesis of IgE beginning with IL1 with macrophage T cell antigen presentation, IL4 seems to enhance the Pre-B cells before it is matured on its way, IL5 comes after in an intermediate level and IL6 comes in at the terminal differentiated plasma cells. There are other interleukins such as gamma-interferon which turn off the synthesis of IgE. Our Italian colleagues, Ricci and Romagnani, in Florence, have done some very nice work on this topic. It may be possible to intervene through the knowledge of the interleukins in the future to modulate this inflammatory response.

N. MORTIER (BE): I have a question for dr. Gray concerning the candidate pilots who were accepted although they had a history of wheezing during childhood. Is it known what happened later in their career? Did they have any problems?

G. GRAY (CA): We started the screening process about 5 years ago and so far we haven't had any people that we screened in the last five years who came back to us with wheezing. But I think it will take longer to follow-up before we can give you a true answer. So we haven't had any problems so far.

ALLERGY SCREENING AND FOLLOW-UP IN STUDENT PILOTS OF THE BELGIAN AIR FORCE (BAF)

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SUMMARY

The different methods used in the Medical Centre of Aerospace Medicine, Brussels (Med C Aerospace) to detect allergy in pilo applicants, are discussed. During the period 1987 until 1989 included, the Total Immunoglobuline E (IgE) levels were also determined at the start of pilot training. The aim was to assess if Total IgE could be used as an additional selection criterion. We conclude that it is not useful to determine the Total IgE in the selection of pilot candidates. The existing investigations and examination methods are sufficient to detect allergy.

1.INTRODUCTION.

An allergic reaction is defined as an unwanted and often noxious inflammation caused by an immunologic reaction against an external antigen (or allergen). One of the links in this reaction is IgE, produced by plasma cells. The reason why certain allergens cause IgE production is not known. Atopici have an increased production of IgE against inhalation and food allergens. Atopy is a hereditary trait and is more likely to cause allergic rhinitis, allergic asthma, atopic eczema and IgE mediated food allergy. Production of IgE is not an exclusive feature of atopici and IgE mediated allergic reactions against bee- and wasp-venom or against medication like penicilline are not correlated with atopy. Almost 20% of the population is estimated to be atopic. Only half of them have also clinical symptoms and by almost half of this last group the clinical symptoms are sufficient to seek for medical help (Ref 1).

2. ALLERGIC SYMPTOMS OF AEROMEDICAL IMPORTANCE.

2.1. Bronchial asthma can cause sudden incapacitation. According to studies by Hopkirk (Ref 2) of childhood asthmatics, about 50% will have prolonged remission, 40% will continue with mild symptoms and 10% will have troublesome symptoms. If follow-up is prolonged nearly 70% of the children who have had asthma in childhood will show symptoms later in adult life, though these symptoms may be mild. In subjects with adult onset asthma the prognosis is even less good.

Because of the uncertain prognosis of childhood asthma and the enormous investment in time and money made in training aircrew, candidates with a history of childhood or adult asthma are unfit for entry into pilot training.

2.2. Allergic rhinitis and conjunctivitis can cause temporary incapacitation by decreased vision due to itching, flood of tears and sneezing. There is also an increased risk of barotitis, barosinusitis and alternobaric vertigo. Medication against the allergic symptoms can cause decreased vigilance and decreased vision.

2.3. Eczema can incapacitate the pilot by the itching it can cause, the possible sudden aggravation or the surinfection.Prolonged missions and less good hygienic circumstances can increase the symptoms.

2.4. Urticaria are incapacitating by the itching and antihistamines can cause decreased vigilance.

2.5. Laryngeal edema causes sudden incapacitation and is life-threatening.

2.6. Allergy to medication can make foreign missions impossible for pilots who are allergic to the chemoprophylaxis needed.

3. SCREENING FOR ALLERGY.

Different clinical and technical investigations can screen for atopy. Different methods are used in our Med C Aerospace in the selection of pilots for the Belgian Forces.

3.1. A questionnaire with the personal and family history has to be completed by the applicants.The family history is of importance because when one parent is atopic, the child has a chance of 30% to have atopy and up to 50% when the 2 parents have atopy (Ref 1 and 3).
In the personal history, we ask for asthma, chronic obstruction of the nose, allergic rhinitis and conjunctivitis, skin diseases as eczema and urticaria. There is a specific question to former stays in a sanatorium.

3.2. The examining physician discusses the questionnaire with the applicant. Special interest goes to allergic symptoms, former medication or treatment such as desensitivity therapy, antihistamines or antiasthma medication.

3.3. During the general clinical examination increased attention goes to skin diseases: eczema, dermatographism, urticaria and scratch marks.

3.4. Allergic conjunctivitis and chronic blepharitis is checked by an ophtalmologist.

3.5. ENT-examination by an ENT-specialist.(*)
The anamnesis, the anterior and posterior rhinoscopia can trace an allergic rhinitis or nasal polyps. When there is suspicion of allergy, skin tests are performed by a prick test with BENCARD solution. The screening is limited to about 10 common allergens: house dust, house dust mite, cat fur, dog hair, a mixture of grass pollen and tree pollen, pollen of the stinging nettle and of the plantain. If for some reason skin testing can not be done, specific IgE (RAST test) is asked. Nasal provocation is not done as for the nasal allergy there is no good standardized procedure. The decision of aptitude is based on the anamnesis, the clinical examination and the skin testing. Skin testing is not done when anamnesis and clinical examination are both negative or both positive.Table 1 gives a summary.

Table 1: ENT-investigation and influence upon aptitude.

Anamnesis	Rhinoscopia	Skin test	Aptitude
negative	positive	negative	apt
negative	positive	positive	inapt
positive	negative	negative	apt
positive	negative	positive	inapt
positive	positive	--	inapt
negative	negative	--	apt

3.6. Laboratory testing.
3.6.1. Pulmonary function.
The forced vital capacity (FVC) and the forced expiratory volume in one second (FEV1) must reach at least 80% of the theoretical normal value (according to sex, age and length). The ratio FEV1/FVC (=Tiffeneau index) must be at least 70%. When there is suspicion of asthma in the history or when the Tiffeneau index is lower than 70%, we perform a provocation test with salbutamol:
- 2 or 3 puffs salbutamol (=200 to 300 mcg Ventolin 100) are administrated via spray.
- the lung function tests are repeated after 20 minutes and if FEV1 increases with at least 10%, the candidate is unfit for aircrew training.
When this salbutamol provocation is normal but there still is suspicion of hyperreactivity, a metacholine provocation test is performed on a second day: acetylcholine chloride 1% is inhaled during 2 minutes via aerosol at -15 cm H_2O.
If FEV1 decreases with 15 to 20% or if wheezing is observed, the candidate is unfit for entry.
3.6.2. Blood examination.
The total eosinophilia (eosinophils per mm^3) is influenced by multiple diseases and is not systematically measured in our -in principle- healthy candidates.
The relative eosinophilia (% of total leukocytes) is of little value in the diagnosis of allergy.
The method of High Pressure Liquid Chromatography (HPLC) to detect antihistamines or other medication in blood serum and urine is being adjusted but is at this time not jet operational.
We do not determine the Total IgE by all our applicants. To assess if our screening for allergy could be improved, we measured the Total IgE in student pilots at the start of their training. The Total IgE was not used as a criterion for exclusion. For further discussion see point 3.7.

(*) With special thanks to Lt Col VERTRIEST R., ENT-Consultant Med C Aerospace

3.7. Relation Total IgE and allergy in student pilots BAF.
From 1987 until 1989 included, the serum Total IgE was measured in student pilots at the start of their training. They had already passed all the medical tests as pilot applicants in our Med C Aerospace.
A total of 135 male trainees (age 20-29 years) were followed. Their medical file was checked for allergy symptoms. According to the laboratory (Military Hospital Brussels), a Total IgE of more than 200 IU/ml is highly suggestive for atopy. Group A is the group with low Total IgE (less than 200 IU/ml).Group B is the group with high Total IgE (more than 200 IU/ml).
From the total of 135, 84% (n=114) are in group A and 16% (n=21) in group B (situation end June 1991). The allergy symptoms are: allergic conjunctivitis and rhinitis, recurrent barosinusitis on allergic rhinitis, pansinusitis on allergic basis, angioneurotic edema on medication, severe local reaction to insect bite, dermatographism, positive skin tests and pulmonary hyperreactivity after provocation (see Table 2 and 3).

Table 2: Group A: Number of students and training level.

Total Group A: 114		
A.With allergy symptoms:	17 (15% of 114)	
a.Disqualified:	6	
(1)Ground course:		1
(2)Insufficient in-flight progression:		4
(3)Medical: allergic sinusitis and recurrent barosinusitis		1
b.In training: 1 student transient positive acetylcholine provocation	5	
c.Licensed:	6	
B.No allergy symptoms:	97 (85% of 114)	
a.Disqualified:	56	
(1)Ground course:		4
(2)Insufficient in-flight progression:		49
(3)Medical: 1 pathological EEG + 2 motion sickness		3
b.In training:	18	
c.Licensed:	23	

Table 3: Group B: Number of students and training level.

Total Group B: 21		
A.With allergy symptoms:	9 (43% of 21)	
a.Disqualified:	4	
(1)Ground course:		1
(2)Insufficient in-flight progression:		3
(3)Medical:		0
b.In training:	1	
c.Licensed:	4	
B.No allergy symptoms:	12 (57% of 21)	
a.Disqualified:	5	
(1)Ground course:		0
(2)Insufficient in-flight progression:		4
(3)Medical : motion sickness		1
b.In training:	4	
c.Licensed:	3	

In Group A,17 (=15% of Group A) had allergy symptoms. One student pilot had recurrent sinusitis and barosinusitis of allergic cause and became unfit. One trainee was temporary unfit because of bronchitis and temporary impaired lung function with positive acetylcholine provocation. A total of 97 (=85% of Group A) had no allergy symptoms. Three of them became unfit on medical grounds : 1 with pathological EEG and 2 with motion sickness.

In Group B, 9 (=43% of Group B) had allergy symptoms but none became unfit on medical grounds. From the 12 (=57% of Group B) without allergy symptoms, 1 had motion sickness and became unfit.

The 2 trainees who had difficulties because of allergy (sinusitis and temporary impaired lung function) both had low Total IgE.
One should expect the most failures on allergic grounds in group B but none of this group became unfit due to allergy, although 43% had minor allergy symptoms.
We can conclude that it is not useful to determine the Total IgE in the selection of candidate pilots.

4. CONCLUSION.

Special attention to allergy during the history (family and personal) and clinical examination (general, ENT and ophtalmological) is almost always sufficient to detect atopy. Pulmonary function with provocation and skin testing are a good completion of the clinical examination. The Total IgE gives little additional information on allergy, when used as screening of pilot applicants.

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Allergic and Nonallergic Rhinitis in Greek Pilots

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1. SUMMARY

In order to study the Allergic Rhinitis (AR) and Nonallergic Rhinitis (NAR) in Greek pilots we examined 144 Greek male pilots, aged from 23 to 50 years, with symptoms of chronic rhinitis. The diagnosis was based on: 1) detailed history 2) physical examination 3) Skin Prick Tests (SPT) on common aeroallergens (positive SPT>3 mm wheal) and 4) nasal secretions for assessment of eosinophilia (positive eosinophilia20% eosinophils in nasal smears). Also we measured serum total IgE (IV/ml) by PRIST method and expressed as geometric mean value. We excluded patients suffering from chronic rhinitis (infections, mechanical-anatomic, drug-induced, sinusitis, metabolic states) and bronchial asthma. Eighty-six patients (59,7%) had Seasonal Allergic Rhinitis (SAR), 30 patients (20,8%) had Perennial Allergic Rhinitis (PAR), 22 patients (15,3%) had Vasomotor Rhinitis (VR) and 6 patients (4,2%) had Nonallergic Rhinitis with Eosinophilia (NARE). One patient with PAR and 2 patients with NARE possessed nasal polyps. The frequency of major positive SPT in the SAR group was: 1) Grasses (80,2%), 2) Olive (69,7%), 3) Parietaria (50%) and in the PAR group was: 1) House dust (100%), 2) mite D. Farinae (93,3%), 3) mite D. Pteronyssinus (90%). The levels of IgE among the four groups were 174,6 IV/ml in the VR group and 39,2 IV/ml in the NARE group. It is concluded, that in Greek pilots: 1) AR (SAR and PAR) was more frequent than NAR (VR and NARE), 2) the major aeroallergens responsible were different in patients with AR, since pollens predominated in the SAR group and house dust-mites in the PAR group, 3) patients with NARE had a high prevalence of nasal polyps, 4) IgE levels were higher in patients with AR (SAR and PAR) than in patients with NAR (VR and NARE).

2. INTRODUCTION

Rhinitis is a general term for disorders of the nasal mucosa, it chronically affects about 40 to 50 million adults in the United States. Although Chronic Rhinitis (CR) is sometimes thought to be a trivial illness, the morbidity and economic burdens associated with it are immense. Allergic Rhinitis (AR) is one of the most common reasons for patients to seek medical attention. It affects roughly 10% to 20% of adults in the United States (Ref.1). With the appropriate diagnosis, most patients can have symptomatic relief with therapeutic agents.

The purpose of our study is to describe our experience regarding the diagnosis and differences of AR and Nonallergic Rhinitis (NAR) in Greek pilots.

3. MATERIALS AND METHODS

One hundred fourty-four male Greek pilots, ranging in age from 23 to 50 years, were examined from December of 1986 to December of 1990, in consultation for complaints of 3 months or more of nasal symptoms (watery thinorrhea, sneezing and/or nasal congestion and/or itching-swelling of the throat-eyes). The presence of symptoms for 3 months was felt to be sufficient to exclude viral respiratory tract infections.

Patients suffering from rhinitis secondary to medications, medication abuse (rhinitis medicamentosa), obstruction due to anatomic variation, acute sinusitis and metabolic states were excluded prospectively. Patients with bronchial asthma were also excluded from this study.

The patients gave historic information regarding exacerbation of nasal symptoms on exposure to inhalant allergens (seasonal or perennial). The physical examination was conducted with special attention to the colour of the mucous membranes of the turbinates. Nasal polyps were recorded.

All patients were routinely tested to histamine, saline, mixed grasses (six grasses), Bermuda grass, mixed weeds (four weeds), fat hen, nettle, plantain, mugwort, mixed flowers (four flowers), mixed trees (five trees), pine, cupress, parietaria pollen, olive pollen, House Dust (HD), mite Dermatophagoides Pteronyssinus (DPt), mite Dermatophagoides Farinae (DF), animal danders (cat, dog) and mixed molds (six molds). Allergy skin testing was performed by the prick technique as described by Pepys (Ref.2). A Skin Prick Test (SPT) reaction is considered positive when the mean wheal was ≥3 mm in diameter (Ref3). All SPT were interpreted in the presence of negative saline and positive histamine controls. Commercially available skin tests materials were used supplied by Allergopharma.

In all patients with a perennial history, for nasal symptoms and negative SPT, nasal secretions for assessment of eosinophilia were obtained by having the patient blow through each nostril into a polyethylene sheet. The secretions were transferred to glass slide and air dried. Staining was done according to Hansel's technique. Nasal eosinophilia was regarded as significant when ≥20% of the cells on nasal smear were eosinophils.

Serum total Immunoglobulin E (IgE) was measured in all patients by the Phadebas IgE paper disk radioimmunoassay (PRIST) technique (Pharmacia Diagnostics), in International Units per ml (IV/ml), using fresh or recently frozen sera. Serum IgE values were converted to $\log_{10}$ for calculation and

antilog or Geometric Mean Value (GMV) and Standard Deviation (SD) are used.

Seasonal Allergic Rhinitis (SAR) was defined as a syndrome presenting with only a seasonal (spring) history for allergen exacerbation and positive SPT correlated by history. Perennial Allergic Rhinitis (PAR) was defined as a syndrome presenting with a history for allergen exacerbation through the year and positive SPT correlated by history. Vasomotor Rhinitis (VR) is a descriptive term which is used to describe a "nonimmunologic, noninfectious chronic type of rhinitis". VR was defined as a syndrome with the following criteria: nasal symptoms with no history of allergen exacerbation, negative SPT and without eosinophils in nasal smears.

Nonallergic Rhinitis with Eosinophilia (NARE) was defined as a syndrome meeting all of the criteria of VR plus a nasal smear disclosing ≥20% eosinophils.

Student's t test and X^2 test - yate's correction was used to test for differences between groups.

4. RESULTS

Eighty-six patients (59,7%), mean age in years 29,8±6,8 were diagnosed as having SAR. Thirty patients (20,8%), mean age in years 28,6±6,3 were diagnosed as having PAR. Twenty-two patients (15,3%), mean age in years 34,3±9,4 were diagnosed as having VR. Six patients (4,2%), mean age in years 32,8±11,1 were diagnosed having NARE. Overall 116 patients (80,6%) were diagnosed as having AR (SAR and PAR) and 28 patients (19,4%) were diagnosed as having NAR (VR and NARE).

None of the patients with SAR and VR, possessed nasal polyps compared with the presence of polyps in 1 patient with PAR (3,3%) and in 2 patients with NARE (33,3%). This difference was not quite statistically significant when compared the occurrence of polyps in patients with AR and NAR (X^2 test-yate's correction = 1,82, P>0,05).

The frequency of major positive SPT in the SAR group was: 1) Grasses in 69 patients (80,2%), 2) Olive in 60 patients (69,8%) 3) Parietaria in 43 patients (50%). The frequency of major positive SPT in the PAR group was: 1) HD in 30 patients (100%), 2) DF in 28 patients (93,3%), 3) DPt in 27 patients (90%).

The highest IgE levels were found in patients with PAR (GMV±1SD: 267,9 IV/ml, 662,2 IV/ml, 108,4 IV/ml), followed by patients with SAR (GMV±1SD:174,6 IV/ml, 492 IV/ml, 61,9 IV/ml). Patients with VR (GMV±1SD: 30,3 IV/ml, 75,5 IV/ml, 12,1 IV/ml) and patients with NARE (GMV±1SD: 39,2 IV/ml, 168,6 IV/ml, 9 IV/ml) had a serum total IgE levels above the normal range. (SAR vs PAR, SAR vs VR, SAR vs NARE, PAR vs VR, PAR vs NARE, t-test, P<05).

5. DISCUSSION

AR is an immediate hypersensitivity reaction occurring in the nose. AR begins with the interaction of mast cell membrane-bound IgE and allergens (pollen, HD, mites, animal danders, mold spores) in the nasal mucosa and the release of histamine and other mediators of immediate hypersensitivity (prostagladin D2, Leukotriene D4 etc.). The results are rhinorrhea, nasal congestion, sneezing and itching of the nose. (Ref4)

The most useful diagnostic procedure in evaluation of patients with CR is the clinical history. The diagnosis of AR may be suspected from a history of typical symptoms arising from exposure to inhalant allergens and is substantiated by correlating the clinical history with demonstration of allergen-specific IgE on SPT. SPT with allergens usually provides clear delineation of positive from negative reactions, and results generally correlate better with clinical symptoms (Ref5). AR is customarily divided into seasonal and perennial forms. In seasonal form, symptoms and their annual pattern are striking. Thus the diagnosis is easily made. The patient can often date the onset of symptoms to a specific week or month of the year, usually in spring. SAR, commonly referred to as hay fever, is due to sensitivity to pollens. The perennial form of AR is something more of a diagnostic puzzle, as it is easier to confuse with NARE. In general, the symptoms of the perennial form are usually not as acute as they are in SAR. PAR is more often related to sensitivity to HD and mites. Our results are similar to those previously reported by others (Ref6, Ref7, Ref8).

We excluded patients with other nasal inflammatory and non-inflammatory conditions that must be considered in the differential diagnosis of AR (anatomic, drug-related, endocrine, infections, ciliary defect and cystic fibrosis). A form of NAR has received the name VR. This disorder is characterized by an apparent overactivity on the part of the parasympathetic innervation of the turbinates. Patients with VR have paroxysms of sneezing often for no known reason, or profuse rhinorrhea and/or nasal congestion. VR is a perennial condition affecting nasal membranes, which react to chilling of body surfaces and changes in environmental physical factors like temperature, humidity and barometric pressure (Ref9). In our study 15,3% of the patients met the criteria of VR. Another form of NAR has been called NARE, NARE is a condition that mimics AR, but SPT are nonreactive and eosinophils are abundant in nasal secretions (Ref10, Ref11). In our study 4,2% of the patients met the criteria of NARE. Mullarkey et al (Ref10) indicated that 15% of 142 patients with CR had NARE. Patients with NARE had a high prevalence of nasal polyps. Our data indicate that 2 patients (33,3%) with NARE had nasal polyps.

The levels of serum total IgE was higher in patients with SAR and PAR compared in patients with VR and NARE. High concentrations of serum total IgE are found mainly in patients with atopic diseases (AR, allergic bronchial asthma, atopic dermatitis). Although serum total IgE levels are elevated in the majority of individuals with IgE-mediated disease, this is not the case with all allergic patients. A substantial proportion of individuals with well-documented AR or IgE-mediated asthma have normal serum IgE Levels (Ref12).

Complications of AR are sinusitis, nasal polyps, otitis media with effusion (common in children) and perhaps asthma.

Sinusitis may be a cause of nasal polyps, which are common when sinusitis complicates AR and even more common in NAR (Ref 13). Pilots with AR or NAR are in high risk for such complications. The exact diagnosis of AR or NAR and with appropriate treatment, almost all patients obtain adequate relief of symptoms and complications.

It is concluded that in Greek pilots: 1) AR was more frequent than NAR, 2) the major aeroallergens responsible were different in AR patients, since pollens predominated in the SAR group and HD-mites in the PAR group, 3) IgE levels were higher in AR patients than in NAR patients.

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Correlation of serum Alpha$_1$ Antitrypsin with cigarette smoking and pulmonary function status in Greek pilots, for a ten year period

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1. SUMMARY

Quantitative and mainly qualitative aberration of the Alpha$_1$Antitrypsin (a_1AT) a major constituent of the human antielastase screen, is strictly associated with the development of lung emphysema. Certain factors like cigarette smoking and environmental polution may contribute to that. In this study we have correlated cigarette smoking with the serum a_1AT concentration as well with the trend of the predicted values of Forced Vital Capacity (FVC) and 1st Second Forced Expiratory Volume (FEV_1) over a ten years period.

The study population consisted of 113 randomly selected male pilots, of the Greek Airforce, being in flight duties grouped into non smokers (n=49, age x=36.3) and effective smokers (n=64, age x=38.4) smoking for more than ten years.

Serum a_1AT level was 176±36 mg/dl (mean±SD) and 199±32 mg/dl for non smokers and smokers respectively ($p < 0.001$). Serum a_1AT level for Greek male population was 190±35 mg/dl and the frequency of individuals with intermediate serum a_1AT level was 2.7%.

Recent and past (ten years ago recorded) FVC and FEV_1 predicted values were measured as follows (mean ± SD):

FVC(past):102±9, FVC(recent):95±9, FEV_1(past):82±6, FEV_1(recent):85±5 and FVC(past):102±9, FVC(recent):92±10, FEV_1(past):80±6, FEV_1(recent):83±5 for non smokers and effective smokers respectively. ($p < 0.05$ after comparison of differences between past and recent FVC values in non smokers and smokers).

There was generally a slight decrease of FVC volumes, significant in effective smokers, independently of intermediate a_1AT serum level, and a slight increase of FEV_1 volumes.

This data suggest that:

1) Cigarette smoking affects serum a_1AT level. 2) Intermediate serum a_1AT level cannot be employed as a predictive criterion for flight personnel's pulmonary status. 3) Ten years of cigarette smoking worsens the pulmonary function status significantly. 4) Extra physical exercise improves it.

2. INTRODUCTION

Human a_1-Antitrypsin (a_1AT) is an acute phase reactant and acts as an inhibitor of proteolytic enzymes inhibiting a variety of serine proteinases and hence is known as a_1-Proteinase Inhibitor (a_1-PI). It is produced by hepatocytes and phagocytes and along with a_2-Macroglobulin consist the basic human antielastase screen.

Alpha$_1$-Antitrypsin has a single polypeptide chain of 394 residues and three carbohydrate side chains with an overall molecule weight of 52000 D. The reactive site has been located on methionin at residue 358.
Regarding to the sequence homology, a_1AT belongs to a superfamily along with Antithrombin-III and ovalbumin. The protein has several isotypes due to differences in the structure of the side carbohydrate chains and several allotypic variants (more than 20) due to genetic polymorphisms.

Genetically the protein is encoded by two alleles inherited with an autosomal codominant way and a number of genetic variants caused mainly by a single point mutation, results in phenotypes with different electrophoretic mobility (Ref 1).

The piMM variant represents the homozygote normal M allele, while the common Z and S mutants in homozygosity are of particular interest because they give products in concentration equivalent to 15% and 60% of that of the normal M allele, respectively. Mixed heterozygotes like MZ, MS, and SZ give proportionately decreased concentrations (Ref 2).

Carriers of Z and S variants are frequent among Europeans with an average of 3% for the Z (pi MZ) and 7% for the S (piMS) mutants and a prevalence of Z carriers among Northern and S carriers among Southern Europeans.

There are two normal M haplotypes with a single difference, the substitution of Valine with alanine at residue 213, but similar in all other properties.

A structural difference between the Z,S variants and the normal M polypeptide is the replacement of glutamic acid by lysine at position 342 and by valine at position 246, for Z and S variants respectively. These substitutions, though far away from the reactive site of the molecule at methionine 358, affect the three dimentional configuration of the molecule (well established for the Z variant) (Ref 3). This ensues into (a) aggregation of the newly synthesized molecule within the cell and reduced production of the protein in the plasma and (b) vulnerability to its antielastase activity due to increased sensitivity to oxidation and instability of the formed complex a_1AT/Elastase (Ref 4).

The major function of a_1AT, that is locally produced or diffused from plasma, is to inhibit the alveolar neutrophil elastase conferring protection from elastolytic activity to the lungs and more specifically to the lower respiratory track (Ref 5).

Qualitative and/or quantitative a_1AT abberations have been associated with a number of diseases, the two most potent being at the lungs (emphysema) and liver (childhood cirrhosis) (Ref 6,7). Sixty per cent of those with severe deficiency will die of lung disease and 14% of those with the ZZ defeciency will die of liver disease.

Serum a_1AT levels of patients with a_1AT deficiency (ZZ homozygosity) are less than 50 mg/dl, while levels above 80 mg/dl protect the lung from an increased risk of emphysema.

A number of factors, besides the genetic pattern, may predispose to the raise of the disease (Ref 8). Such factors are cigarette smoking and environmental polution, which affect either directly by oxidation of a_1AT or indirectly by accumulation of macrophages in the lungs and consequent release of oxygen radicals (Ref 9,10).

In this assesment we have evaluated, serum a_1AT concentration, cigarette smoking and status of pulmonary function, for a ten year period in healthy male pilots, as a possible screening test for the selection of pilots and as a sign of predisposition to respiratory abnormalities.

3. MATERIALS AND METHODS

3.1 Subjects.

The population under study consisted of 113 Greek male healthy pilots, being in flight readiness with no evidence of chronic disease, who were going through their periodic medical examination at the Hellenic Airforce Center of Aviation Medicine in Athens. Among all pilots under examination within a certain period, were randomly selected for the study population those who had been serving in the airforce for more than 5 years and regarding the smokers, those who were smoking for more than 10 years.
They had an average age of 37.4 years and their mean serving time was 14.5 years. They were grouped into non smokers (n=49, age x=36.3, service time x=14.3) and effective smokers (n=64, age x=38.4, service time x=16.4) with the later smoking an average of x=21.0 cigarettes for 17.6 years.

3.2 Alpha$_1$-Antitrypsin measurements.

Serum a_1AT levels were assayed within a few days after blood collection in sera kept frozen at -20 C. Concentrations were measured by a liquid phase immunoprecipitation assay with nephelometric endpoint detection, employing a Turbox protein Analyzer (Orion Diagnostica Fin.).

3.3 Pulmonary function testing.

For pulmonary function testing the tests for Forced Vital Capacity (FVC) and 1st Second Forced Expiratory Volume (FEV1) were selected. These were performed according to the Epidemiology Standarization Project recommendations (Ref 11) by using an electronic spirometer (Autospiror HI-498, Chest Co.) and results were recorded as a percentage of predicted values, based on reference values for age, sex, and height.

The recent measurements were correlated to these recorded a decade ago. The average of three consecutive annual FVC and FEV1 measurements was employed in each case for higher accuracy. A less than 70% of the predicted FEV1/FVC ratio and a less than 80% of the predicted FVC value, were the criteria set for a pulmonary abnormality.

4. RESULTS

The study population, grouped into smokers (n=64) and non smokers (n=49) and their serum a_1AT level (mean ±1 SD) is shown in table 1. The groups age averages were similar x=38.4, and x=36.3 respectively. Taking into account that the pulmonary function measurement tests belong to two distant periods (past, recent) with a 12.2 year interval of sampling, one can conclude that the past pulmonary function test measurements are related to the beginning of the professional career as pilots of the population under study.

Table 1. The features and serum Alpha$_1$ Antitrypsin (a_1AT) of studied population.

	n	age ($\bar{x}$) (median)	service time ($\bar{x}$)	serum a_1AT $\bar{x}$±SD mg/dl
non-smokers	49	36.3 (36)	14.3	175±36 *
smokers	64	38.4 (39)	16.4	199±32
total	113	37.4	14.5	190±35

** p < 0.01 versus smokers by paired t-test.*

The a_1AT serum level in Greek male population is 190±35 with a significant difference (p<0.01) between non smokers (175±36) and smokers (199±32). Concerning the range distribution of the a_1AT levels no one had a low concentration, less than 50 mg/dl, three (2.7%) had an intermediate level and the rest of them normal concentration (figure 1).

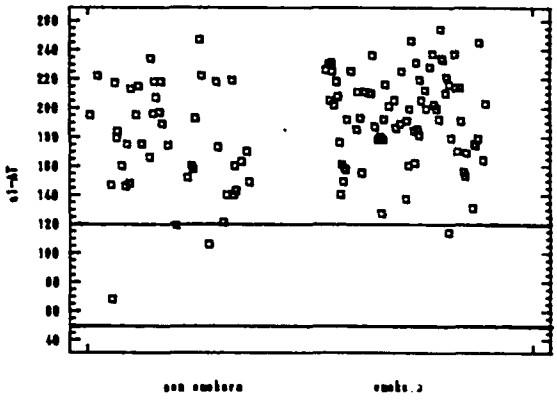

Figure 1. Distribution of the a_1AT levels.

Table 2 shows the pulmonary test values in the above mentioned periods. They had a normal Gaussian distribution and are expressed as past and recent percentages (x±SD) of normal predicted values. There is a decrease in FVC values for both groups, slightly higher for smokers and by comparing the differences between past and recent FVC values of non smokers and effective smokers they differ significantly (p<0.05).

Table 2. Lung function parameters measured in two distant, past and recent periods after a decade intervention.

	FVC		FEV_1	
	past	recent	past	recent
non-smokers	102±9	95±9*	82±6	85±5
smokers	102±9	92±10	80±6	83±5

FVC = Forced Vital Capacity as percentage of normal predicted values (x±SD)
FEV_1 = 1st second Forced Expiratory Volume as percentage of normal predicted values (x±SD)
** p < 0.05 between differences of past and recent FVC values in non smokers and smokers, by paired t-test*

Surprisingly, for the FEV1 values, there is a slight improvement of the recent values as compared to the past, for both groups. Given the limits of a pulmonary function abnormality FVC<80% and FEV1/FVC < 70%, table 3 shows their observed frequency. There is a relatively higher frequency of pulmonary function abnormalities among smokers as well a reasonable difference between past and recent frequences. Non of the above individuals had any clinical disorder and their a_1AT concentration ranged within the normal limits.

Table 3. Frequency of pulmonary function abnormalities.

	FVC < 80%		FEV_1/FVC < 70%	
	past	recent	past	recent
non-smokers	0	0	1	0
smokers	1	3	6	0

5. DISCUSSION

Cigarette smoking (Ref 12) and a_1AT deficiency are major causal factors that predispose to pulmonary obstructive disease. In this study we report the relatioship between serum a_1AT level and a decade's pulmonary status among Greek pilots.

Serum a_1AT level ranges from 155 mg/dl to 225 mg/dl (mean $\pm$1SD) in Greek male population. This is related to mixed phenotypes and subsequently slightly lower than that of around 220 mg/dl given for normal homozygous PiMM individuals. Non of the subjects had serum a_1AT level less than the 50 mg/dl limit that is invariably associated with homozygosity of the Z type. Three out of 113 (2.7 %) had an intermediate level,less than the 62% of the population mean, without having any pulmonary abberation.

Morse et al (Ref 13) have stressed that an intermediate serum level of a_1AT is not an important risk factor for the development of chronic obstructive lung disease, even though it is indicative of an heterozygous a_1AT deficiency.

This frequency of the intividuals with an intermediate a_1AT serum level seems to be close to the one found in a phenotyping study in Greek mixed population (Ref 14), that of MM 92.85%, MS 5.15%, MZ 0.2% and MP 0.4%, but smaller than the 7% corresponding to the S mutants common in the Southern European population.

Smokers had a higher a_1AT serum concentration ($p<0.01$) as compared to non smokers. Olsen et al (Ref 15) have found an increased level of a_1AT in total lavage fluid and in alveolar macrophages but have not reported any differences in serum level between smokers and non-smokers. Cigarette smoking increases the elastolytic activity locally and decreases the available functional a_1AT. An increased serum a_1AT level in smokers may be either the responce to the continous local inflammation or the positive serum feedback to the local decrease.

Concerning the lung function status, aging is generally followed by a shift to smaller FVC volumes, which become significantly smaller for effective smokers smoking an average of 21.0 cigarettes for more than 10 years. We found that the smoking period of 10 years for the given average of cigarettes in our population is the threshold beyond of which the FVC values dicrease significantly (data not shown). Possibly due to the influence of aging and smoking there is a tendency towards a widening in the distibution of the recent FVC values in the samples of smokers.

An improvement in FEV1 values, reflecting to the maturation of the skeletomuscular part of the respiratory system, occurs with physical aging. This is attributed to a special program of physical education that is followed by the given population at the beggining of their professional life.

We did not find any correlation between pulmonary function abnormalities and intermediate a_1AT serum concentration. It is worth outlining the prevalence of pulmonary function abnormalities among the smokers.

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DISCUSSION SESSION IV.

INHALANT ALLERGIC DISEASES (Papers 35 to 38)

R. D'AMELIO (IT): Only a consideration on the intervention of dr. Mortier. Our experience is like yours in the screening of pilots. Total IgE is not a good way of screening allergic subjects. This does not mean that serological screening is not good. But only as regards to total IgE as dr. Matricardi and dr. Wever showed. I think that all of us here are convinced that allergic pilots can't be enrolled. The problem is the right screening, because this question is open. There is no agreement on this problem. We have listened to the intervention of dr. Gray who proposed an algorithm based on functional and non-serological tests. This could be a right position. On the other hand, there is another position of just serological approach. Perhaps the right screen needs a good equilibrium between the two. We did not propose with the intervention of Dr. Matricardi a diagnostic algorithm, but in a survey among NATO countries that I did for this meeting, in the 14 NATO countries, only Italy is now starting the serological screening with the Phadiatop-CAP for student pilots. So, it would be possible to avoid in about 70% of cases the necessity in doing a more careful analysis. In the other 30% of cases, perhaps the best way is to utilise a traditional screening for allergy based on history, skin prick-test in addition to functional test. The problem in Italy is particular, because in our experience history is unreliable, it is not possible to base our diagnostic algorithm on clinical history because there are many cases of dissimulation. I don't know if the some problem is for Northern Europe and North America. But this is our experience, and based on our experience I think that the way we started recently, at first with the Phadiatop-CAP and then with a more careful analysis, has showed to be the best way in our country.

J. A. BELLANTI (USA): I would like to ask only one question. It seems to me that there are levels of impairment of allergic disease that can preclude or that will preclude, an individual from flying. There are some cases of allergic rhinitis that are clearly so mild, that there is no problem. And some cases may be so severe, so serious signs of sinusitis or middle ear disease might pose a problem, for the disease and the treatment. It just seems to me that what is needed is a multititered set of criteria that would encompass not only IgE testing but also the degree of clinical impairment. Is this not true, prof. D'Amelio? You would not use just the IgE screen alone in determining whether an individual is fit.

R. D'AMELIO (IT): I think this is a crucial point. I think that in our experience serological screening is able to make the history more reliable. So it is possible to obtain more information, more exact information after an objective screening. But my opinion is like yours. It is necessary to present a clinical symptomatology to avoid enrollment.

A.M.J. WEVER (NE): As a clinician I would say that if a patient is positive for IgE, RAST or Phadiatop you do methacholine challenge test or histamine challenge test and if the test is positive you may run into trouble with this patient. If these challenge tests are negative I think the only thing you can say is that the patient is sensitised to allergens and in the future he might get into trouble, and that at present he has no disease. Some investigators say that you can only develop disease, let's say asthma, if you have a positive provocation test for histamine or methacholine.
This is however a very extreme position. The problem lies with nasal allergy. There are provocation tests for nasal allergy. You can provoke this organ with house dust mites or with pollen and see what the reaction is, however there is no standardized histamine provocation test, so far as I know, for nasal challenge, and that is the problem.

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14. Abstract

These proceedings include the Technical Evaluation Report, the Opening Address and 36 papers of the Symposium sponsored by the AGARD Aerospace Medical Panel and held at the National Research Council, Rome, Italy, 21st to 25th October 1991. The theme of the Symposium was the fight against infectious diseases, mainly the vaccine-preventable ones, and the study of inhalent allergic diseases. Enormous development in the last few years has allowed us to understand and prevent many pathological conditions, whereas the setting up of laboratory tests has allowed us to monitor with great precision the immune function which may be depressed by the environmental stimuli to space or military flight.
Lastly, the development of military and civilian air travel, which favours the spreading of infectious and parasitic diseases, requires the setting up of correct prophylactic measures.

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