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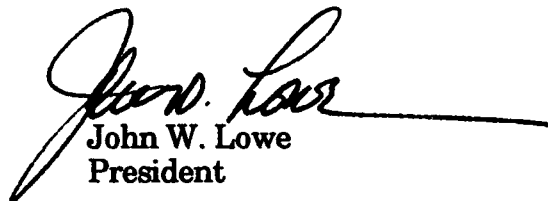
November 30, 1993

Commander
USAMRDC
ATTN: SGRD-PLA (COL Bancroft)
Fort Detrick, Maryland 21701-5014

Dear COL Bancroft,

Attached is the annual report for the HIV Research Program, Cooperative Agreement DAMD17-93-V-3004, covering the period April 1, 1993 through September 30, 1993. If you have any questions or issues regarding this report, please contact me at (301) 294-1202.

Sincerely,


John W. Lowe
President

cc: CDR USAMRAA ATTN: SGRD-RMA (Ms. Joyce Richardson)

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13. ABSTRACT (Maximum 200 words) A comprehensive HIV Research Program continued into its sixth year of scientific endeavors with new direction and focus guided by a Cooperative Agreement between the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional), and the Henry M. Jackson Foundation, signed on April 1, 1993. During this six month reporting period intensive planning represented a major effort; nevertheless, each program area continued its research mission. Significant results during this time period included (but were not limited to): Vaccines for Prevention, Behavioral Prevention, Drug and Gene Therapy, Vaccine Therapy, Intervention Assessment.				
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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the management of the Henry M. Jackson Foundation for the Advancement of Military Medicine and are not necessarily endorsed by the U.S. Army.

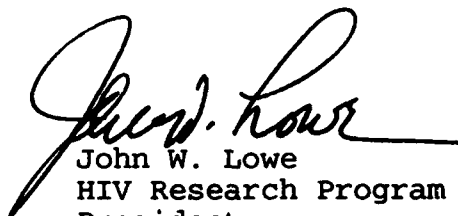
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In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.



John W. Lowe
HIV Research Program Director
President
Henry M. Jackson Foundation
for the Advancement of Military Medicine

November 30, 1993

Abstract

A comprehensive HIV Research Program continued into its sixth year of scientific endeavors with new direction and focus guided by a Cooperative Agreement between the United States Army Medical and Research Development Command and the Henry M. Jackson Foundation, signed on April 1, 1993. Five new research program areas constitute the science: Vaccines for Prevention, Behavioral Prevention, Drug and Gene Therapy, Vaccine Therapy, and Intervention Assessment. During this six month reporting period intensive planning represented a major effort; nevertheless, each program area continued its research mission. Significant results during this time period included (but were not limited to):

Vaccines for Prevention:

Foundation Scientific Director commended by the World Health Organization (WHO) for key role in organizing and characterizing an extensive network of HIV-1 isolates.

Developed a comprehensive research infrastructure in Bangkok, Thailand (AFRIMS) for the initiation of preventive vaccine trials.

Behavioral Prevention:

Foundation Scientific Director led working group at the WHO which addressed behavioral issues associated with international Vaccine trials.

Initiated focus groups to determine design of new Behavioral Interventions

Drug and Gene Therapy:

Furnished samples from a continued study of AZT Resistance which demonstrated that viral load is correlated with outcome (in collaboration with Intervention Assessment).

Initiated major collaborative effort with the National Institutes of Allergy and Infectious Disease (NIAID) for a large chemotherapy trial.

Vaccine Therapy:

Continued world's largest Phase II Vaccine Therapy trial, with a less than 1% missed visit rate for 601 volunteers.

Established the long term safety of rgp120 and rgp160 in volunteers with chronic infection.

Intervention Assessment:

Developed a quantitative PCR assay for RNA and DNA for which a patent was submitted.

Provided longitudinal historical clinical data to the FDA and NIH in assistance during a recent failed NIH Phase II study.

VACCINES FOR PREVENTION

USAMRDC Program Area Coordinator: MAJ John McNeil, M.D., MC,
U.S. Army

Foundation Scientific Director: Francine McCutchan, Ph.D.

Assistant Department Chief: To be determined

PROGRAM AREA OBJECTIVE: Develop and field test a preventive vaccine for HIV-1 that significantly reduces the risk of infection and/or decreases the severity of disease progression and potential for virus transmission. Research is conducted under this Program Area in accordance with integrated research plans as well as through human use and animal research protocols.

Protocols for Human Studies

Protocol No.	Protocol Title (Abbreviated)	Principal Investigator
RV68(P)	Phase I Seropositive - Thailand	Michael/Sorachai
RV69(P)	Phase I Seronegative-Thailand	Michael/Sorachai
RV69A(P)	Behavioral Aspects of RV 69	Jenkins
RV70	Prevalence/Incidence-Thailand	McNeil/Narongrid
RV87(P)	Protective Immunity	Artenstein
RV91(P)	Field Studies/Thailand	Carr*
RV91A(P)	Protective Immunity/Thai	McCutchan*
RVPro01(P)	HEPS Study: Collaboration	Mason/Chavalit
RVPro02(P)	Perinatal Transmission Events	Robb
RVPro03(P)	Phase III Cohort Exploration - Phayao	Markowitz*

Clinical Research Protocols (RVs)

Protocols for Animal Studies

Protocol No.	Protocol Title (Abbreviated)	Principal Investigator
RV-A1 (A)	Mouse Primate Chimeras SCID/HU and SCID/MAC	Rosenberg* Rosenberg
RV-A2	SIV-647 Peptide Vaccination	Lewis*
RV-A3 (A) (A) (A)	Vaccine for SIV (Peptides) Lymph Node Transfer from RVA3 HIV-2 Challenge of RVA3 Lymph Node Biopsy from RVA3	Lewis Lewis Lewis Lewis
RV-A4	Immunologic Potential (Galactosidase)	Lewis
RV-A5	HIV-2 of Rhesus Monkeys	Gartner*
RV-A6	Immunology with Individual Peptides	Lewis
RV-A7	Immunogenicity of HIV-1 Peptides	Lewis
RV-A8	Protective Effect of Neutralizing Antibody	Lewis
RV-A9	Evaluate Whole virus vaccine SIV	Lewis
RV-A10 (A)	Immunogenicity of SIV Transgenes Immune Response Against Expressed Antigen	St. Louis* St. Louis
RV-A11	Routine Antibodies Collection	Lewis
RV-A12	Vector Expression of SIV Env Gene	St. Louis
RV-A13	Heterologous Challenge/Developed Immunity	Lewis
RV-A15	HIV-1 Models in Primates	Gartner

Laboratory Work Units/Projects

Lab Protocol #	Protocol Title (Abbreviated)	Principal Investigator
LRP29	SIV Vaccines and Evaluation- Live Attenuated SIV Constructs	Lewis

LRP30	SIV Vaccines and Evaluation- Whole Killed Constructs	Lewis
LRP31	SIV Vaccines and Evaluation-Subunit Constructs	Lewis
LRP32	SIV Vaccines and Evaluation- Early Markers of Vaccine Efficacy	Rosenberg*
LRP33	HIV-1 Models-HIV-1, Pigtail Macaque Model	Gartner
LRP34	HIV-1 Models-SHIV for Cross Challenge	Gartner
LRP35	HIV-1 Models-Cross Species Adaptation	Gartner
LRP36	Protective Immunity-International Genetic Variation of HIV	Mascola
LRP37	Protective Immunity-Neutralization Characterization of International Human Immunodeficiency Viruses	Mascola
LRP37A	Protective Immunity-Pepscan Characterization of International Human Immunodeficiency Viruses	Lai*
LRP38	*Protective Immunity-Evaluation of Seronegative Vaccinee Sera	Mascola
LRP39	Protective Immunity-HIV-1 Enhancement	Mascola

*Foundation Principal Investigator (PI)

(A) = Addendum

(P) = Pending approval

Vaccines for Prevention

PREFACE:

It is a common occurrence in Science that concepts are discovered and then forgotten, only to reemerge later as "new" concepts. The preventive vaccine program takes inspiration from the work of Edmond Sergent, a French malaria researcher, who several decades ago put forth the concept of "premunition". This theory states that an organism's immunity to certain chronic infections can be assured only if it permanently carries the pathogen in an attenuated state. The scientists in this Program Area acknowledge their debt to this prescient colleague.

BACKGROUND:

Ten years of intensive research on HIV-1 and other primate lentiviruses has failed to yield a strong prophylactic vaccine candidate for efficacy testing or adequate long-term therapy options. Thus, it is appropriate to reexamine strategies and principles and to propose new approaches. Program Area Scientists and others recognize that certain commonly held concepts and assumptions may have lead research astray from the most productive paths. This Program Area proposes that vaccine prevention strategies for the HIV Research program should be based on information gained from successful vaccines against other viruses, on accurate and comprehensive understanding of the disease process itself, on a realistic appraisal of the course and nature of the global epidemic, and on an accurate assessment of the strengths and weaknesses of our research program. Over the past six months, Vaccines for Prevention, has restructured working hypotheses, assessed research and field testing capabilities, and linked program resources directly to program goals.

The construction and review of this plan occupied literally hundreds of hours of individual and collective effort, and every scientist in the program contributed to its development. Although much of the revised research plan awaits implementation, this Program Area report summarizes the development of the research plan and describes some of the proposed studies currently underway as well as research accomplishments from projects that were largely, if not entirely, conducted before the reorganization. Many of these projects are now reaching their conclusion, and others have been restructured and incorporated into the plan. These assembled materials in the report below, including manuscripts and abstracts of oral and poster presentations, reflect a period of significant productivity and testify to an extraordinary effort on the part of Vaccines for Prevention scientists and associates to complete ongoing research while formulating new plans.

Conceptual Plan:

Some commonly held concepts that have greatly influenced the course of HIV-1 vaccine research in the past and that are pertinent to this program are discussed below. The scientists in this program area contend that the positions they hold on these central issues are critical to defining a strategy for our program area.

1. A view is sometimes put forth that HIV-1 is somehow unique among human pathogens in ways that essentially preclude development of an effective preventive vaccine.

The scientists disagree with this view and accept as an article of faith that a preventive vaccine for HIV-1 will be developed. Protective immunity exists, and its correlates are definable and measurable. Further, they maintain that the development and field testing of an effective preventive vaccine is within the scientific capability of this laboratory unit and its collaborators within and outside the MMCARR.

2. Effective laboratory vaccines against primate lentiviruses should preclude the establishment of infection because infection inevitably leads to disease progression and a long period of transmissibility in infected individuals.

Program Area scientists disagree with this view and propose that the outcome of infection depends upon circumstances under which infection occurs. Establishment of an attenuated or abortive infection may provide significant protection against subsequent infection with virulent HIV-1. Both disease progression and transmissibility might be altered by pre-existing immunity. A completely protective vaccine must render subsequent infection with virulent HIV-1 harmless, both for the infected individual (no disease progression) and the public (no transmission). Incompletely protective vaccines would slow disease progression and reduce transmission. This is the Vaccines for Prevention's working definition of vaccine efficacy.

Recommendations:

2a. Emphasize laboratory vaccine studies that carry the highest likelihood of protection, including live attenuated and live vectored delivery systems. One must accept that the consequences of infection can be controlled to consider a live attenuated vaccine.

2b. Separate the laboratory goals of efficacy and safety - concentrating on efficacy first. The Program Area scientists find that laboratory vaccine safety concerns are premature until efficacy has been established and that much of the bias of research efforts

towards subunit vaccines has been driven by a (possibly premature but eventually essential) concern for safety.

2c. Develop objective laboratory criteria for reduced severity of disease and transmission potential using an integrated immunologic, virologic, and structural analysis of lymphoid tissues (including functional analyses). Access to lymphoid tissue is essential until peripheral blood markers which reflect the status of lymphoid tissue are validated.

3. Effective immunity against HIV-1 and related viruses may not be possible to attain with a vaccine because even natural infection does not provide effective immune control of the virus.

The Program Area scientists believe that vaccine-induced (pre-exposure) immunity may alter both virologic and immunologic aspects of subsequent infection. For example, initial viral burden may be decreased, certain viral variants may become preferentially established, the types and locations of infected cells may vary. The balance between cellular and humoral immune responses may be perturbed, and recall responses to specific antigens may differ quantitatively and qualitatively from primary responses. The researchers believe that protective immunity exists and that probing investigations of effectors of protective immunity is highly worthwhile.

Recommendations:

3a. Attain convincing, durable protection in SIV/HIV-1 animal model systems and determine, by comparison with nonprotective vaccines, correlates of protective immunity.

3b. Study immunologic and virologic characteristics of human subjects with prolonged and accelerated disease courses and high/low transmission potentials.

3c. Study primary immune regulation of HIV-1 during the acute infection and seroconversion period.

3d. Study intensively exposed but persistently uninfected human subjects.

4. A widely held paradigm holds that after the acute phase, viral replication is greatly diminished, and the disease enters a long latent phase only to reactivate later in the terminal phases of the infection.

The major site of virus-cell interaction is now thought to be lymphoid tissues, not circulating lymphoid and monocytoïd cells that

have been more intensively studied. The time course of CD4 decline, the CD4/CD8 ratio, and viral burden may not be accurately assessed by sampling of circulating cells. The scientists believe that virus replicates continuously and that pathogenic effects accumulate steadily during apparent clinical latency. Applied to clinical vaccine research, the scientists believe pre-clinical (virus-cell level) events can be defined which will prove to be valid markers of eventual disease course and survival. Efforts must be made to replace "time to death" as an endpoint for prevention trials with valid early endpoints. In so doing, the variety of preventive constructs tested could be correspondingly increased.

Recommendations:

4a. Emphasize research options which emphasize assessment of early, acute stages of infection and which permit sequential evaluation of lymphoid tissues.

4b. Develop validated, quantifiable markers of a slower or less severe disease course focusing on the period concurrent with and immediately following acute infection.

5. Many scientists are hesitant to employ analog models, such as SIV infection in Macaques, to develop HIV-1 vaccines because they use a different virus, do not show responses to the same immunologic determinants as humans exposed to HIV-1 (especially V3 loop) and do not mimic the disease course in humans. Various SIV strains have been described either as too virulent or avirulent to provide accurate parallels with HIV-1 infection in humans, and the range of international genetic variants of HIV-1 now identified is too difficult to model.

The Program Area scientists believe, based on findings from this laboratory, that the pathogenesis of SIV-induced disease in macaques is similar in virtually all respects to HIV-1 induced disease in humans and provides an excellent model for vaccine development. They believe that the accessibility of lymphoid tissues in animals is absolutely necessary to adequately determine the efficacy of vaccine candidates and strategies. With respect to strain virulence, we and other scientists have developed sufficient knowledge to make effective use of the range of pathogenic potential available in laboratory SIV strains. Concerns regarding differences in immune responses to SIV vs. HIV strains are premature until correlates of protective immunity have been determined.

Recommendations:

5a. Fully utilize the SIV model as a strategy for vaccine

development.

5b. Include SIV strains with different virulence, as appropriate, in vaccine studies.

5c. Explore a broad range of vaccine formulations including selected live attenuated, live vectored, whole killed and subunit vaccines. Structure studies such that more aggressive strategies are preferentially tested for the most dramatic effects. For example, live attenuated vaccines may protect from infection with highly virulent and/or heterologous strains while subunit vaccines may be expected only to delay disease development after challenge with homologous, less virulent strains.

5d. Develop strategies to promote increased lymphocyte function during vaccine administration. This may boost poor immunity of "safer" vaccines or may broaden immunity induced by any vaccine. For example, one could try to control and exploit T-cell activation properties of acutely lethal SIVPB1 variants to improve immunity developed by attenuated vaccines. Other immune modulators could be tested in this regard.

6. Any primate animal model that permits infection with HIV-1 is expected to provide more relevant information for HIV vaccine development than SIV models because the "right" virus is used and the exact vaccine formulation to be tested in humans can be evaluated.

HIV-1 infection in non-human primates has been generally difficult to attain. The most conclusive studies have used a single laboratory strain of HIV-1, and attempts to infect with clinical HIV-1 isolates have been disappointing. The persistence of infection and the pathogenic sequelae of infection are not well explored. The Program Area scientists believe that HIV-1 model development is in a preliminary stage and currently of very limited use.

Recommendations:

6a. The main requirements of the HIV-1 model in *M. nemestrina* are viral persistence and development of antiviral immunity. Consistent patterns of disease development are highly desirable. Expansion of the repertoire of HIV-1 strains that can be used as challenge stocks is also sought. At present, the primary use of this model would be evaluation of products shown to have had efficacy in their SIV analog system.

6b. For evaluation of HIV-1 vaccine cross protection, SHIVs provide a possible alternative strategy. Development of SHIVs with divergent HIV-1 envelopes that establish persistent infection is required. Disease development is highly desirable.

7. The relevance of HIV-1 genetic variation to vaccine cross protection is not determined and difficult to assess.

Several genotypes of HIV-1 have now been identified. Selection of genotypically diverse isolates for vaccine studies will increase the likelihood of developing broadly protective vaccines. Gaps in our knowledge include: the relationship between genotypes and immunotypes, the relationship of genotypes to vaccine cross protection, and the degree of genetic variation within regions important for protective immunity. No clear path to answering these questions exists but data from diverse sources may provide the necessary information.

Recommendations:

7a. Rely on heterologous challenge experiments as the most meaningful test of cross protection among viral strains. Some scientists favor a pentavalent challenge over pairwise heterologous challenges with the five major viral genotypes. These would be conducted in HIV-1 primate models.

7b. Attempt to document double infection of humans with disparate genotypes of HIV-1 in the field. If found, design experiments to determine if infection occurs serially or intercurrently.

7c. Use serum neutralization studies *in vitro* and passive protection studies *in vivo* (SCID) to help define the relationship between genotypes and immunotypes, with emphasis on reagents from human volunteers participating in vaccine trials.

8. Vaccine availability is currently limited to formulations based on virus genotype B which is prevalent in the US and Europe. This situation is expected to continue. Inexpensive, safe, and effective vaccines are a common goal of researchers in government, academic, and industrial laboratories. The Program Area scientists believe that cost, safety, and efficacy are separate goals and that they can be approached sequentially. They suggest that establishing efficacy against homologous challenge, without regard to cost, should be the primary goal. With established efficacy, elucidation of correlates of protective immunity should be facilitated. Safer products eliciting appropriate immunity would then be developed and tested for their durability and breadth of response.

Recommendations:

8a. For Laboratory studies with primates, establishing efficacy against homologous challenge should precede all other vaccine design considerations.

8b. Safety is the primary consideration. Only after this is accomplished should durability and breadth of immunity be investigated.

8c. Durability and breadth of protection should be considered by manipulating schedule/dose/route/adjuvant, etc.

8d. A global, long-term perspective should be included in cost considerations.

9. Preventive Vaccine field trials are not sensible/useful now because an effective vaccine is not at hand.

The scientists in this Program Area favor a parallel track approach to vaccine development in which field testing capability and vaccine candidate development proceed concurrently. In anticipation of preventive vaccine field trials the scientists should develop infrastructure, identify cohorts, develop epidemiologic data, prepare for behavioral/social/ethical aspects of trials, and gather data about safety and immunogenicity of potential candidates for efficacy testing in target populations. Site preparation and cohort definition and characterization requires 3-5 years effort. Given the exponential increase in global HIV-1 infections, early, partially successful vaccines could have more impact than delayed, but more effective vaccines. Thus, site development should not wait for a proven vaccine candidate. Trial site development is a current and critical program objective and is a parallel objective to the development of effective vaccines.

Recommendations:

9a. Invest in trial sites where partially protective vaccines can be most effectively evaluated.

9b. Develop vaccines closely matched to local strains to provide "homologous" challenge situation.

9c. Prepare for vaccine efficacy studies by conducting phase I trials with available vaccine candidates.

RESEARCH GOALS AND OBJECTIVES

Vaccines for Prevention is organized into four main components:

- | | |
|--------------------------|------------------------------------|
| I. SIV Vaccines | (Laboratory Research) |
| II. HIV-1 Animal Models | (Laboratory Research) |
| III. Protective Immunity | (Laboratory and Clinical Research) |
| IV. Field Studies | (Clinical Research) |

This section of this report is divided into discussions of each component. Objectives for each component are delineated. They are then followed by descriptions of Laboratory Research Protocols, and, Human Use Clinical Protocols and Animal Use Protocols which are conducted to meet these objectives. As previously mentioned, during the past six months, the five-year planning for this Program Area has represented a significant effort on the part of the scientific as well as technical staff. This strategic planning is represented in both current and proposed projects; therefore both current and pending projects and protocols are described below as well as significant results/anticipated results for each project/protocol. A summary compilation of significant achievements concludes each component.

I. SIV VACCINES & EVALUATION

OBJECTIVES

- a. Establish protection from homologous challenge in the SIV system.
- b. Build the capability to objectively quantitate vaccine effects using an integrated immunologic, virologic, and structural analysis of blood and lymphoid tissue.
- c. Establish and validate early quantifiable biological markers that predict subsequent clinical/survival endpoints.

Four laboratory research protocols comprise the major effort within this component:

1. (LRP 29) - Live Attenuated SIV Vaccines:

This protocol is conducted at the Foundation Retroviral Laboratories and the Frederick Research Center. Live attenuated

lentivirus vaccines have been attempted with SIV in two laboratories; both have used molecular clones of SIV_{mac251}.

Background:

- Study #1 The California Primate Center used the 1A11 molecular clone (J. Virol. 64:3644-3700, 1990) followed by challenge with SIV_{mac239}. All of the animals became infected with 239. The preexisting immune response altered the acute disease course and the animals lived significantly longer than the non-immune controls.
- Study #2 The New England Primate Center constructed a nef deleted SIV from the 239 molecular clone. This isolate infects rhesus, but causes no disease either acute or chronic. Animals first infected with nef deleted 239 and then challenged with virulent 239, were protected from infection with the virulent 239 (Science 255:1938-1941, 1992). Additional deletion mutants have been produced including a triple delete (nef, NRE and vpr) which has been used in vaccine studies and has had some degree of success in protecting against SIV_{mac251} challenge.
- Two additional related studies have also been performed.
- Study #3 Biberfeld et. al. (AIDS Res. Hum. Retroviruses 8:1511-1513, 1992) challenged cynomolgus macaques with HIV-2_{SBL-K135}. These monkeys became seropositive but virus could not be isolated from them following the acute phase of the disease. These monkeys were then rechallenged with SIV_{sm}, and followed for disease development. All of the animals became infected with SIV, but virus could be isolated for only a limited time. All of the control monkeys died (between 2-26 months) following challenge with SIV, while the HIV-2 infected monkeys survived >30 months. These two viruses have a nucleotide homology of approximately 70%.
- Study #4 In our laboratories, 8 rhesus macaques were challenged and infected with SIV_{ell1s}. These monkeys all became seropositive and were observed for over 6 months. All eight monkeys became routinely virus isolation negative during the observation period. These monkeys were then rechallenged with SIV₂₃₉ to determine if they were infectible with a slightly heterologous (93% homologous) SIV isolate. All eight monkeys yielded virus and had an SIV specific antibody increase following 239 challenge. Preliminary results indicated that at least some of the isolated virus was 239. None of these animals show any signs of acute infection (i.e. antigenemia, lymphadenopathy, loss of appetite), while both control animals showed classic acute syndromes of SIV infection.

Four of the eight animals are currently virus isolation negative which is not usually observed following SIV₂₃₉ challenge. This study suggests that preexisting anti-SIV immunity may protect from or alter disease development.

A number of strains of SIV with a wide range of pathogenic potential are available for proposed studies. These are summarized below:

Strain	Availability		Properties					
	<u>titrated</u>	<u>cloned</u>	acute		acute	chronic	<u>neuro</u>	<u>OIs</u>
			<u>Δnef</u>	<u>death</u>	<u>disease</u>	<u>duration</u>		
ells	yes	yes	no	no	no	>3 years	no	?
69R	no	no	no	?	?	?	?	?
251	yes	yes	yes	no	yes	<2 years	yes	yes
sm	yes	yes	no	no	yes	<2 years	yes	
	yes							
PBj14	no	yes	no	yes	yes	>2 years	?	?

Objectives:

1. To confirm and extend the research initiated by the New England Primate Center using the Δnef SIV₂₃₉ isolate. This will include:
 - a) Determination of immune system integrity of Δnef infected monkeys including assessment of cellular functions and immune responses to new antigens.
 - b) Determination the SIV specific immune responses of Δnef SIV infected animals as compared to SIV₂₃₉ infected animals.
 - c) Determination of virus load and virus presence in Δnef infected monkeys, including lymphoid tissues and brain.
 - d) Challenging Δnef infected animals with high and low infectious doses of homologous (SIV_{mac}) and heterologous (SIV_{sm}) strains of SIV as free virus.
 - e) Determination if Δnef infected animals have protective immune responses to cellular challenge with both homologous and heterologous (SIV_{sm}/B670) strains of SIV as virus infected cells.
2. To determine if Δnef constructs of another SIV molecular clone may yield superior protective responses. The researchers propose to construct a Δnef SIV_{PBj} isolate, believing that a Δnef SIV_{PBj} isolate will yield a virus that grows rapidly in vivo but will

not cause the acute disease observed in SIV_{PBj-14} infected macaques. The researchers propose to:

- a) Determine if a Δ nef SIV_{PBj} isolate will yield a virus that maintains the lymphoproliferative ability and rapid growth kinetics of the SIV_{PBj-14} isolate while not causing acute death.
- b) Determine the natural history of a Δ nef SIV_{PBj} isolate in macaques including virus load measurements, immune function and disease outcome.
- c) Determine if a Δ nef SIV_{PBj} isolate, if found to be avirulent, can generate a protective immunity to a more virulent SIV isolate such as 239, 251 or sm.
- d) Investigate the molecular determinants of SIV_{PBj} immune system activation for the purpose of providing a mechanism of rapid dissemination of attenuated viruses in the host.
- e) Determine immune system integrity of Δ nef infected monkeys including assessment of cellular functions and immune responses to new antigens.
- f) Determine if Δ nef infected animals have protective immune responses to cellular challenge by challenge with both homologous (SIV_{sm}) and heterologous (SIV_{mac}) strains of SIV as virus infected cells.

An integrated and intensive virologic, immunologic and pathologic analysis of the Δ nef infected and challenged monkeys is proposed. This will include an intensive study of both the acute phase (first 4 weeks) of the infection and the chronic phase. Assays to be performed will include studies of both the blood and lymphoid tissues compartments of the monkeys including:

PROPOSED ASSAYS

TISSUE PATHOLOGIC	VIRAL	IMMUNOLOGIC		
		CELLS	FUNCTIONS	
Blood Cells	DNA PCR RT PCR Virus isol	T cells B cells CD4 CD8 CD4/CD8 CD45RA	T cell prolif CTL	In Situ Immunohisto. Histochemistry
Plasma	RT-PCR Virus isolation p24 antigen		Antibody-SIV -neutralizing -ADCC	
Lymphoid Tissues	RT-PCR DNA-PCR Virus isol	T cells B cells CD4 CD8	cytokine RT-PCR FDC NK	SIV In Situ SIV Immuno- phenotype
Histochemistry		CD45RA		Immuno-EM

The timetable of these assays will include an intensive investigation of the developed immunity following nef virus infection and prior to challenge with virulent SIV. Following challenge with the virulent SIV, an intensive investigation of the acute and early phase of the infection will be performed in order to determine if long term outcome can be predicted. Long term follow up will generally be minimized if possible.

Projection of resources:

Objective 1.

<u>vaccine</u>	<u>challenge</u>	<u>expt</u>	<u># monkeys</u>
239Δnef	none	natural history	5
239Δnef	none	immune function	5
239Δnef	none	early sacrifice	3
239Δnef	mac251	protection	5
239Δnef	smB670	protection	5
239Δnef	mac251 cells	protection	5
239Δnef	smB670 cells	protection	5

Objective 2.

<u>vaccine</u>	<u>challenge</u>	<u>expt</u>	<u># monkeys</u>
PBjΔnef	none	natural history	5
PBjΔnef	none	early sacrifice	3
PBjΔnef	PBj6.6	protection	5
PBjΔnef	mac239	protection	5
PBjΔnef	smB670	protection	5

Timeline:

Winter 1993:

1. Establish infections with both 239Δnef and PBjΔnef viruses for natural history, immune function, early sacrifice and homologous challenge studies (31 monkeys).
2. Begin preliminary characterization of acute and early infections with the Δnef viruses.

Spring 1994:

1. Establish whether Δnef causes immune dysfunction in infected animals.
2. Establish infections with both 239Δnef and PBjΔnef viruses for heterologous and cell challenge studies (25 monkeys). Characterize the acute disease generated.
3. Determine the immunity derived from Δnef virus infection, both humoral and cellular.
4. Challenge monkeys with homologous viruses and follow for infection and acute disease development.
5. Determine virus load and areas of virus production in Δnef infected animals.

Winter 1994:

1. Determine the immunity derived from Δnef virus infection, both humoral and cellular for the animals involved with the heterologous and cell challenge studies.
2. Challenge monkeys with heterologous and cell associated challenge stocks. Characterize any acute disease generated.
3. Determine the degree of protection in the homologous challenged animals.

Summer 1995:

1. Determine the degree of protection in the heterologous and cell associated challenged animals.
 2. Establish long term safety of attenuated SIV vaccines.
-

2. (LRP 30) Whole Killed Virus Vaccines:

Background:

Whole virus vaccines have been attempted with some success in lentivirus infections. Unfortunately all of the reported trials using SIV as the immunogen have been thrown into question due to the presence of human cell membrane molecules in the SIV virions preparations. The presence of these proteins has been correlated with the observed protective responses of the immunized macaques when the monkeys were challenged with virus derived from human cells. Studies using these virions as immunogen followed by challenge with virus derived from macaques cells have shown that the vaccine does not protect from infection, but the disease course is significantly altered. Whole inactivated virus derived from the host species has been shown to be effective as an immunogen in the SIV model and with the type D retroviruses in macaques. This suggests that a whole virus immunogen may prove efficacious against SIV disease especially if it decreases or eliminates the pathogenicity of the infection or alters the transmission of the virus. This area has, under contract with ABL, determined the conditions necessary for UV-light psoralen or formalin inactivation of SIV and has developed methods to isolate large quantities of purified virions. Macaque derived virions are currently needed to be generated for this study. Two possible methods for this to occur would be either growth of the virus in macaque cells, either primary or transformed, or the production of pseudovirions from macaque cells using a vaccinia construct.

Objectives:

1. To determine if inactivated whole SIV_{mne/cells} generated in macaque cells when used as an immunogen in macaques induces a protective immunity following challenge with a macaque derived challenge virus. In order to perform these studies, titrated stocks of macaque derived challenge virus and virus infected cells will be required.
2. If a protective effect is observed this will determine:
 - a) what immune response(s) and to which virus protein(s) the protective immunity is correlated.
 - b) whether the immunity is specific for the homologous virus

c) if the vaccine can prevent the transmission of the virus from an infected macaque to a naive macaque.

PROPOSED ASSAYS

18

Projection of Resources

Objective 1.

<u>Expt</u>	<u>Vaccine</u>	<u>Challenge</u>	<u># Monkeys</u>
Titration	none	69R virus	9
Titration	none	69R cells	9
Vaccine	SIV _{mne/ells}	69R virus	5
Vaccine	control	69R virus	5

Objective 2.

<u>Expt</u>	<u>Vaccine</u>	<u>Challenge</u>	<u># Monkeys</u>
Vaccine	SIV _{mne/ells}	69R cells	5
Vaccine	control	69R cells	5
Vaccine	SIV _{mne/ells}	SIVsm	5
Vaccine	control	SIVsm	5
Transfer	none	tissues	10

Proposed Timeline:

Winter 1993:

1. Begin titration of 69R challenge stocks (18 monkeys). This will include deriving natural history data for these challenge stocks for the purpose of generating control data for future vaccine trials.
2. Develop methodology and produce macaque derived virus for vaccine.

Spring 1994:

1. Begin immunization of macaques for first vaccine trial (10 monkeys)
2. Continue to characterize disease development in the 69R challenged monkeys (6 monkeys).

Fall 1994:

1. Challenge immunized monkeys with titrated free virus stock.
2. Begin immunization of macaques for cell associated challenge (10 monkeys).

Winter 1994:

1. Immunize animals for heterologous challenge studies (10 monkeys) if protection is observed in free virus homologous vaccine study.

Spring 1995:

1. Challenge immunized macaques with cell associated virus.
 2. Perform tissue transfer studies if protection is observed in free virus challenged animals (10 monkeys).
 3. Challenge monkeys with heterologous free virus challenge.
-

3. (LRP 31) Subunit SIV Vaccines:

Background:

Subunit vaccines using SIV have been attempted by numerous laboratories and in most cases these vaccines have used envelope products derived from SIV_{mac239} and subsequent challenge with an SIV_{mac} virus. These studies have been uniformly unsuccessful when judged by whether or not the immunized animals were protected from infection. Little or no data has been generated to judge whether the immunization had any beneficial effects following infection. There are no reports of vaccines using non-SIV_{mac} derived proteins, due to the lack of availability of commercial products. This has been mostly due to the lack of availability of molecular clones of SIV isolates other than mac and because the vaccine testing groups have focused primarily on SIV_{mac} as the model system. Molecular clones of SIV_{sm} and SIV_{mne} have been produced, are biologically active and could be made available for use. Currently these clones are being used for the production of purified proteins in limited quantities. As far as the literature reports are concerned the only non-SIV_{mac} purified protein products in use are a baculovirus derived gp160 product derived from SIV_{mne/CL8} and a CHO cell derived gp160 product from SIV_{sm}. These products are available for use but their availability may be limited for the proposed studies.

Lentivirus peptide vaccines are currently being developed by laboratories associated with both HIV and SIV. The researchers have used conserved regions of the envelope of both viruses as immunogens, while other laboratories are using neutralization epitope (V3 loop peptides) or specific T or B cell epitopes. These studies currently have had some success following immunization including protection from disease development, generation of neutralizing antibody and generation of specific cellular immunity. Identified peptides can be produced, either alone or linked to a carrier and used as immunogens more easily than whole virus proteins, and commercial products are available.

Objectives:

1. To determine if a SIV derived envelope gene product(s) can protect from or alter disease development in immunized macaques. Studies are proposed to use SIV products derived from SIV. All commercially available SIV protein products are of SIVmac origin. Two other sources are from SIV molecular clones, mne/CL8 and sm/SmH4. Specific studies proposed will include:

a) Immunization of macaques with gp160 in adjuvant. Immunized animals and controls will be challenged with homologous virus and monitored for infection and disease development in an intensive manner to determine alterations in acute and chronic disease manifestations.

b) Immunization of macaques with three gp160 products: mac, sm and mne. Immunized animals will be challenged with SIV_{mac251} or SIV_{mne/69R}. Immunized animals and controls will be monitored for infection and disease development in an intensive manner to determine alterations in acute and chronic disease manifestations.

2. To determine if SIV derived peptide regions can alter disease development in immunized macaques. Studies are proposed to use peptides associated with regions shown or predicted to be associated with protective responses of immunized animals. Specific studies will include:

a) Immunization and characterization of macaques with SIV envelope peptides associated with the conserved regions of the primate lentiviruses (Shafferman peptides). These studies are proposed to be a repeat of the previous studies which involved 3 rhesus macaques that were immunized with b-galactosidase linked peptides followed by challenge with SIV_{e11s}. The researchers propose to increase the number of macaques immunized and to also determine if the developed immunity is protective against either e11s or a heterologous virus strain.

b) Immunization and characterization of macaques with peptide identified as either neutralizing, B cell specific or T cell specific epitopes, to determine their affect upon SIV disease pathogenesis.

For all of the proposed studies an integrated and intensive analysis of virologic, immunologic and pathologic parameters will be performed to determine if any correlates of protective immunity can be determined. This will include natural history data generation from all of the control macaques and an intensive study of both the acute and chronic phases of the infection. Assays will be performed when possible on both the blood and lymphoid tissue compartments of all immunized and control monkeys and are to include:

PROPOSED ASSAYS

TISSUE PATHOLOGIC	VIRAL	IMMUNOLOGIC		
		CELLS	FUNCTIONS	
Blood Cells	DNA PCR RT PCR Virus isol	T cells B cells CD4 CD8 CD4/CD8 CD45RA CBC	T cell prolif CTL	<i>In Situ</i> Immunohisto. Histochem
Plasma	RT-PCR Virus isolation p24 antigen		Antibody-SIV -neutralizing -ADCC	
Lymphoid Tissues	RT-PCR DNA-PCR Virus isol	T cells B cells CD4 CD8	cytokine RT-PCR FDC NK	SIV <i>In situ</i> SIV Immuno- phenotype
Histochemistry		CD45RA		Immuno-EM

Projection of resources:

Objective 1.

<u>vaccine</u>	<u>challenge</u>	<u>expt</u>	<u># monkey</u>
none	SIV _{mac}	control	5
mac-gp160	SIV _{mac}	protection	5
3 - gp160	SIV _{mac}	protection	5
3 - gp160	SIV _{69R}	protection	5

Objective 2.

<u>vaccine</u>	<u>challenge</u>	<u>expt</u>	<u># monkey</u>
none	SIV _{mne/ells}	control	5
4 peptides	SIV _{mne/ells}	protection	5

Proposed Timeline:

Fall 1993:

1. Begin immunization of peptide monkeys (10 monkeys).
2. Obtain and characterize monkeys for gp160 studies (20 monkeys)
3. Determine sources of gp160 products.

Spring 1994:

1. Challenge peptide immunized monkeys with SIV_{mne/e11s}.
2. Begin immunization of gp160 monkeys.

Fall 1994:

1. Challenge gp160 monkeys with either SIV_{mac} or SIV_{69R}.
2. Continue follow up of peptide monkeys to determine degree of protection including transmission studies.

Spring 1995:

1. Continue follow up of gp160 immunized monkeys to determine virus load and degree of protection including transmission studies.

4. (LRP 32) Early markers of Vaccine Efficacy:

Background and Significance:

Understanding of the immunological and virological events occurring during SIV and HIV infections has focussed on mid and late stage disease. More recently, acutely infected HIV positive patients have been studied; but the actual date of infection is frequently not known, and the early kinetics are lacking. SIV infection of macaques will provide necessary information in terms of early viral replication and the very effective immune response which appears to eliminate the majority of cells containing replicating virus. The nature of this early immune response is crucial to the development of vaccines designed at preventing infection or reducing viral burden sufficiently to maintain long lasting immunity. These studies will be used as a method of assessing vaccine efficacy in a shorter time frame than end stage disease. SIV infections also offer an advantage in that different isolates and macaques species can be chosen which induce variable and consistent disease courses further enabling dissection of SIV and HIV acute infections.

Objectives:

(1) To understand the virological and immunological markers of early SIV infections to ascertain the efficacy of various vaccine strategies.

(2) To understand the immune mechanisms (cellular or humoral) underlying the rapid decrease in viral replication during the acute phase of infection.

(3) To develop methodologies and read outs ie. CTL, PCR, *in situ* hybridization assays to evaluate the protective immune responses induced during early disease.

(4) To characterize the acute infections induced by different SIV isolates in order to have models appropriate for the different disease courses observed following HIV infections.

Protocols:

Infections with PBj-14 of pigtailed macaques (acutely lethal), PBj-14 in rhesus macaques (usually non lethal) as well as 251 and 69R infections of rhesus (delayed acute phase, non lethal) are proposed. The following regimens will be performed.

(i) Pig-tailed macaque- PBj-14 (D3,6,9)

	MON	TUES	WED	TH	FRI	//MON	TUES	WED	TH	FRI	//MON	-FRI//MON
#1	Inf						Sac					
#2	Inf						Sac					
#3	Inf										Nec	

(ii) Rhesus macaque- PBj-14 (5,10,17)

	MON	TUES	WED	TH	FRI	//MON	TUES	WED	TH	FRI	//MON	-FRI//MON
#4					Inf				Biop		Biop	Nec
#5					Inf				Biop		Biop	Nec
#6					Inf				Biop		Biop	Nec

(iii) Rhesus macaque - Mac-251, Mne-69R (D6,12*,19,30)

	MON	TUES	WED	TH	FRI	//MON	TUES	WED	TH	FRI	//MON	-FRI//MON
#7 (251)		Inf					Biop				Biop	Biop
#8		Inf					Biop				Biop	Biop
#9		Inf					Biop				Biop	Biop
#10		Inf					Biop				Sac	
#11 (69R)		Inf					Biop				Biop	Biop
#12		Inf					Biop				Biop	Biop
#13		Inf					Biop				Biop	Biop
#14		Inf					Biop				Sac	

PROPOSED ASSAYS

TISSUE PATHOLOGIC	VIRAL	IMMUNOLOGIC		
		CELLS	FUNCTIONS	
Blood Cells	DNA PCR RT PCR Virus isol	T cells	T cell prolif	In Situ
		B cells	CTL	Immunohist.
		CD4		Histochem
		CD8		
		CD4/CD8		
		CD45RA		
Plasma	RT-PCR Virus isolation p24 antigen		Antibody-SIV	
			-neutralizing -ADCC	
Lymphoid Tissues	RT-PCR DNA-PCR Virus isol	T cells	cytokine	RT-PCR SIV In Situ
		B cells	FDC	SIV Immuno-
		CD4	NK	phenotype
		CD8		
Histochemistry		CD45RA		Immuno-EM

SIV VACCINES - Research Accomplishments and Achievements

As a summary, ongoing studies have produced the following results/accomplishments in the SIV Vaccine component of this Program Area:

- Production of experimental vaccine under GMP:

The purification procedure for the b-galactosidase fusion proteins containing sequences from conserved regions of HIV-1 gp160 has been improved over that developed by Shafferman. Using the procedure, pilot scale productions of the four fusion proteins were carried out at the Forest Glen Annex of WRAIR under Good Manufacturing Practices (GMP) conditions. From about 0.5 kg of respective bacterial paste, 3 - 5 g of HIV88-bGal, HIV500-bGal and HIV647-bGal, and 350 mg of HIV582-bGal were obtained, over 2-fold yields hitherto achieved. The purities, as judged by the SDS-PAGE and Western-blot analysis, were over 98%, and the endotoxin levels were less than half that of Shafferman's preparation served as a reference. The Univax Co., the original contractor, provided the material support as well as the quality assurance and the environmental check before and after each preparation. The

purified proteins have been retained by Univax for formulation study.

- A new method for the covalent attachment of peptides for ELISA:

Carbopol, a polymer of acrylic acid, has been used as an "arm" for the covalent attachment of peptides onto well bottoms for ELISA. Carbopol is first attached to the NH₂-surface of Costar plates. Excess carboxyl functions on the Carbopol molecules are then activated and reacted with peptides with free NH₂-groups. The plates made this way were found to yield much better reaction characteristics with antibodies than that with peptide directly attached. The plates can be reused after stripping off antibodies with a "stripping solution". Reproducible results have been obtained for up to 5 times. (Manuscript in preparation).

- Shedding of surface glycoprotein (Gp120) by cell-free Simian Immunodeficiency Virus (SIV):

Most of the surface glycoprotein of SIV has been found detached from the virus particles in the cell-free culture supernatant; when virus particles were pelleted by ultracentrifugation, practically all infectivity was found associated with the pelleted fraction, and yet, over 80% of the envelope protein was found in the supernatant. Partially purified envelope protein appeared to enhance the infectivity of the pelleted virus.

- Conducted the most extensive survey of HIV DNA burden in human lymphoid and nonlymphoid tissues.
- Study of cytolytic activity of CD8+ cells in SIV-infected lymphoid organs and their role in disease progression.
- studied RNA expression in SIV-infected lymphoid organs and association with DNA.
- studied presence and role of viral DNA and RNA in thymuses of SIV-infected macaques.
- studied differences in mechanisms leading to death in SIV-infected macaques which either do and do not make anti-SIV antibody.
- Demonstrated that dual infection with two pathogenic SIV isolates can occur in rhesus macaques. The degree of infection by the second isolate is correlated with the levels of immunity developed during the infection with the primary isolate.
- Developed a colormetric assay for performing *in situ* hybridization in tissue sections for SIV RNA and continued these studies to develop assay systems for performing dual analysis in tissues for the determination of SIV RNA and antigen or cell surface markers.

- Performed preliminary studies to determine the levels of TH1 and TH2 related cytokine mRNA in tissues collected from SIV infected macaques.
-

II. HIV-1 ANIMAL MODELS

The second component of this Program Area is described in detail below. Primary objectives and laboratory research protocols, as well as proposed protocols/projects are also detailed. Summaries of results/accomplishments follow as a conclusion.

OBJECTIVES

- a. Establish persistent infection and development of antiviral immunity in *M. nemestrina* with HIV-1 virus(es). Disease development is desirable.
- b. Develop SHIVs for homologous and heterologous challenge of HIV-1 vaccines in monkeys.

The following laboratory research protocols comprise the major effort within this component:

1. (LRP33) *HIV-1 Pig-Tailed Macaque Model:*

Background and Significance:

The SIV macaque model is an excellent animal model for human immunodeficiency disease in that the nature and course of the clinical disease are comparable and the immune responses generated to the virus appear to be similar. Also, the fact that SIV is a naturally occurring pathogen for macaques is important in that it circumvents the need to consider additional variables that present themselves in heterologous systems. The one major concern that has been raised relative to this model has been that it does not utilize the human pathogen (HIV-1). Thus far, efforts to develop animal models that are based on HIV-1 have not yielded definitive, reproducible systems. The chimpanzee model is highly virus isolate restricted, and the pig-tailed macaque model is yet to be reproducibly defined. Given that it is anticipated that many putative HIV-1 vaccine products will be available for evaluation within the next five years and that it is highly unlikely that each of these will be developed in parallel for evaluation in the SIV macaque model, there remains a need to develop animal test systems for the direct and meaningful evaluation of putative HIV-1 vaccine products.

Principal Investigator: Suzanne Gartner
Co-Investigators: Yiling Liu, Mark Lewis, Yvonne Rosenberg, C.Y. Lai & Gerald Eddy

Objective 1:

Attempt to generate strong, broad immune responses against HIV-1 viral proteins in macaques. This proposed work should complement and extend findings from investigations of immune responses in HIV-1 infected individuals and subunit-vaccinated uninfected human volunteers. Better characterization of the immunogen and, consequently, more precise dissection of the resulting immune responses can be achieved in this proposed system as compared to studies of infected individuals.

Study Design:

Immunogens

1. Purified viral proteins (initially, gp120 and g41; possibly later, extended to include p24, p17 and RT). These could be column-purified protein preparations or proteins expressed in the various systems currently being used.
2. Whole virus lysates- The researchers propose to prepare lysates of pelleted virus particles. These could be particles derived from infected normal human T cells or monocyte/macrophages from the HLA-DR-negative neoplastic line Molt-3 and if possible, from a CD4+ macaque transformed cell line(4GNTS)*. Other human cell lines could also be screened to identify ones that do not express MHC class II molecules and are permissive for HIV-1. Other potential host cells could be considered based on the findings of Stott and others currently trying to determine the nature of the anti-human cell "protective response".
3. immunization schedule - to be determined (initial approaches include transfection of this cell line with cloned HIV-1 DNA and selection of clones stably maintaining provirus and hopefully capable of at least some viral protein expression.

Immunologic evaluations:

1. Humoral response

initial assessment - detection of neutralizing antibodies

Sera from immunized macaques would be evaluated in neutralization assays using 3 kinds of host cells: normal human T cells, normal human monocyte/macrophages and neoplastic cells(both H9 and Molt-3 would be used since one is HLA-DR positive and the other negative.)

2. Cell-mediated response

initial assessment - detection of specific cytotoxic T cells

Peripheral blood T cells from the immunized macaques would be evaluated for cytotoxic activity *in vitro*. Initially, transformed autologous peptide**-pulsed cells would be used as targets. We might also want to use SIV infected transformed autologous targets to look for evidence of SIV/HIV cross-reactive responses. Other appropriate targets would be included should they become available.

**selected on the basis of the particular immunogen

Animal considerations:

Given that rhesus and pig-tailed macaques differ in several important respects that might impact significantly on the proposed study (susceptibility to HIV-2, course of disease with some SIVs), the researchers propose to use both species for this study, 2 animals of each species per immunogen.

Timeline:

1-2 years depending on choice and availability of the immunogens

Other potential applications of this proposed work:

1. Should an appropriate HIV-1 challenge virus become available, the researchers could consider challenging the animals after they have some idea of the nature of any anti-HIV-1 response generated. The researchers could also consider an SIV challenge of animals immunized with HIV-1 viral lysates should the data generated indicate the potential for SIV/HIV cross-protection.

2. To better characterize the immunogens, small samples of the whole virus lysates could be analyzed also by HPLC using the methodology already worked out by Henderson and Arthur. Such analyses could shed more light on the nature of the anti-human cell "protective response".

Objective 2:

Better adapt HIV-1 to pig-tailed macaques. Minimal criteria for meaningful adaptation includes persistence of infectious, recoverable virus for at least 6 months accompanied by a sustained, significant humoral immune response against at least HIV-1 p24, gp41 and gp120. The experimental approach is based on the premise that a certain level of HIV-1 replication and expression *in vivo* can provide for the natural selection of a variant(s) having the requisite features. The initial goal, then, is to develop cell systems *in vitro* whose members can serve as appropriate cell-associated viral challenges.

Study Design:

in vivo selection of monkey-tropic HIV-1 variants**

1. Inoculate pig-tailed macaques with HIV-1 recovered from animal 255L (week 34 or week 36 isolate or another); both cell-free and cell-associated challenges will be considered.
2. Inoculate pig-tailed macaques multiple times with pelleted virions of multiple isolates (both highly cytopathic, highly replicative as well as noncytopathic; low replicative isolates will be included). Inoculation schedule to be determined. Plans include injection of virus directly into lymph nodes and immunomodulation (both activation and suppression) of animals prior to and during the acute infection interval.
3. Inoculate pig-tailed macaques with HIV-1 genome-bearing autologous and allogeneic transformed CD4+ macaque cells; STLV-I, HTLV-I and H. saimiri will be used to generate the transformants; the transformants will then be transfected with HIV-1 genomes and clones will be selected on the basis of provirus stability and HIV-1 particle expression.
4. Inoculate pig-tailed macaques with monkey/chimp heterokaryons permissive for HIV-1; the experimental approach will be to select 6-thioguanine-resistant mutants of CD4+ macaque transformants, fuse them with normal chimp CD4+ cells and culture in selective medium. (A scientist in this program area has considerable experience with this methodology.) Stable hybrids will be assessed for susceptibility and permissibility to HIV-1.

**Most successful adaptations of retroviruses to heterologous hosts have depended upon the presence of a helper (retro)virus. Given the complexity and nature of HIV-1 infection and our current lack of understanding, the introduction of additional retroviruses into the system could confuse data interpretation. For this reason, the researchers choose not to explore experimental approaches based on coinfection with xenotropic or other retroviruses in spite of reports in the literature pointing to the viability of such an approach. In spite of this, the researchers propose to use STLV-I and HTLV-I transformed cells as viral challenges. Such viruses would not actually serve as helper viruses in the classic sense, but rather, could serve to maintain cell proliferation in vivo, thereby allowing for potential continued expression of HIV-1 and the possibility of selection of a more adapted variant. It should be appreciated that HTLV-I appears to confer some unknown advantage relative to HIV-1 permissivity in that many of the cell lines used to propagate HIV-1, especially those used for plaque assays, are HTLV-I transformed.

methods of evaluation:

virus isolation
DNA PCR
immunophenotyping
antibody response (ELISA and Western blot)

Timeline:

year one - develop the cell lines and inoculate monkeys with 255L-passaged virus; also, begin the multiple isolate/multiple injection arm of the study.
year two - evaluate the cell lines *in vivo* and continue the multiple isolate/multiple injection study.

2. (LRP 34) SHIV for Cross Challenge:

Background and Significance:

While SIV infection and disease in macaques represents an excellent animal model for HIV-1-associated human immunodeficiency disease, it is limited in that currently, it cannot serve for the direct testing of human (HIV-1) vaccine products. One promising approach that could circumvent this problem for at least subunit-based vaccines is to use SIV/HIV-1 chimeric viruses for challenge. Several groups are presently constructing such chimeras, and a few have progressed to the point of determining infectivity *in vivo*. Three groups have already expressed interest in collaborating with Vaccines for Prevention researchers, those headed by Drs. Phil Johnson, Joe Sodroski and Paul Luciw. With respect to the HIV-1 structural gene products, the Sodroski and Luciw groups have focused primarily on *env*, and the Johnson group on *env* and *pol*. SIV239 is serving as the backbone for the Sodroski and Luciw group constructs and SIVsm/PBJ6.6 is being used by Johnson's group. Clearly, the availability of an *in vivo* test system for assessing protective and/or other immunologic responses to HIV-1 *env*-based vaccines would be of great value to our program goals.

Objectives:

1. To assess the ability of SIV/HIV-1 chimeric viruses to establish persistent infection and induce disease *in vivo* and to assess the consequent immune response.
2. To determine the ability of HIV-1 *env*- based vaccines to protect against challenge with the SIV/HIV-1 chimeric viruses. If protection is achieved, the challenge scheme would be expanded to include different isolates of SIV.

Study Design:

Objective 1: Chimeric viruses for testing (to be determined)

Animals:

The species will be dependent partly upon the chimeras to be evaluated. Most probably, in initial experiments, rhesus macaques for the Sodroski and Luciw viruses, and pig-tailed macaques will be used for the Johnson chimeras. A minimum of three monkeys per chimeric virus is proposed.

Clinical monitoring:

The animals will be followed routinely for evidence of infection and disease.

Virologic assessments:

Virus isolation and DNA PCR will be performed routinely; genetic characterization of monkey-passaged isolates may be included if deemed important and appropriate.

Immunologic assessments:

Immunophenotyping, ELISA, western blot and neutralization assays are planned for the initial phase of the proposed study. Assays to measure cell-mediated immunity will be included when appropriate.

Objective 2: to be determined based on results from the Objective 1 studies.

Timeline:

Objective 1: 12-24 months

Objective 2: To be determined

3. (LRP35) Cross-Species Adaptation:

Background and Significance:

(Animals currently covered under RVA 5; study includes monkey Ig-2 from RVA 3.2)

This Program Area currently has seven rhesus macaques who were inoculated close to four years ago with two different isolates of HIV-2. Three of these (37D, 62D and 70D) are definitively persistently infected as evidenced by continued virus isolation and a strong, broad humoral immune response. Given the importance of workable animals models of persistent infection with human immunodeficiency viruses, the length of time frequently required to develop overt symptoms of immunodeficiency, the surprising genetic similarities between nonpathogenic (in monkeys) HIV-2 and disease-inducing SIV, and the acknowledged potential for successful

retrovirus adaptation *in vivo* to a heterologous host, it is important to continue this study in spite of the fact that this program is focused on the development and testing of an HIV-1 vaccine. Prior infection with HIV-2 has been shown to retard disease development in monkeys subsequently challenged with pathogenic SIV. This suggests that HIV-2 can behave in a fashion somewhat analogous to the SIV nef-delete variants. Given plans to pursue live-attenuated vaccines, information gathered from this HIV-2 study can assist both our overall interpretation of data from the nef-delete studies as well as help guide the future course by providing a greater understanding of the long-term interaction of virus and host organism.

Objectives:

In general, these objectives focus on detection and characterization of cross-species adaptation. This kind of information is vital to Program Area plans and the need to develop meaningful animal models for evaluation of human HIV-1 based vaccine products.

1. To determine if viruses recovered from these long-term infected animals manifest a greater ability to acutely infect macaques. (Persistent infection in the initial host monkeys was accomplished via two inoculations with infected autologous cell challenges six months apart.)
2. To determine if cross-species passage can provide for the selection of more pathogenic variants. This goal is very important relative to attempts at HIV-1 adaptation to monkeys, because success at increasing infectivity and pathogenicity in this study can provide insight and encouragement for our HIV-1 adaptation work. (Enhanced pathogenicity has been observed in cross-species infection in some retrovirus systems.)
3. To determine the nature and extent of genetic changes associated with persistent HIV-2 infection in macaques.
4. To continue to follow those animals currently persistently infected for evidence of disease development. This work is both important and meaningful, especially given that clinical immunodeficiency disease can take years to develop in both virus infected humans and macaques. Also, the overwhelming value of such animals to our program, as well as to the AIDS effort in general, is obvious.

Study Design:

Objective 1

To determine whether the monkey-passaged isolates can more readily infect macaques, two of the four "uninfected" rhesus monkeys from RVA

5 will be inoculated with virus recovered from animal 37D (initially inoculated with HIV-26669SBL) and the other two "uninfected" monkeys with virus recovered from animal 70D (initially inoculated with HIV-21711H). (Previous work for this Program Area indicated that prior exposure to the virus should not negatively impact on susceptibility.) In addition, the researchers propose to include one, or preferably two, pig-tailed macaques in each group, since evidence from other groups has indicated that this species is significantly more susceptible to HIV-2. Also, these pig-tails could then serve as the initial cross-species recipients for Objective 2. Unless otherwise determined based on forthcoming information, animals will be followed for 6-12 months only. Particulars of the plan include cell-free virus challenge and monitoring of infection using the usual parameters (virus isolation, DNA PCR, immunophenotyping, serologic evaluation).

Objective 2

As noted in Objective 1, pig-tailed macaques will be inoculated with 2 isolates of rhesus-passaged HIV-2. Infectious virus recovered from the pig-tailed macaques will then be used to inoculate additional rhesus monkeys. This zig-zag inoculation scheme will be pursued for a maximum of four passages (rhesus---- pig-tail---- rhesus---- pig-tail). The interval between passages is yet to be determined and obviously will be somewhat determined by the findings as the study progresses. It is anticipated that four to six months between inoculations will be satisfactory. Particulars of the plan include cell-free virus challenge and monitoring of infection using the usual parameters (virus isolation, DNA PCR, immunophenotyping, serologic evaluation). The animals will also be closely followed for clinical symptoms and signs of infection and disease.

Objective 3

The researchers plan to begin this aspect of the study by genetically examining viruses recovered from animal 37D, since the parental isolate HIV-26669SBL has been molecularly cloned and sequenced and is therefore available for appropriate comparison. Initially, the most recent isolate available will be examined (four years post first inoculation). Attempts will be made to obtain a full-length clone using the strategy previously successful for this isolate. In addition, cloning and sequencing of PCR amplimers of selected regions of the genome previously shown or thought to be important will be performed. Genome regions initially targeted will be the LTR, and the env and nef genes. Viruses recovered from animals 70D and 62D could also be included and compared to their parental strain, should the findings from the 37D study indicate that this would be important to pursue.

Objective 4

The animals will continue to be monitored clinically, virologically and immunologically. Virus isolation, DNA PCR, immunophenotyping and serologic evaluations will be performed. Additional investigations could include *in vitro* determinations of enhanced susceptibility, permissivity and cross-species transmissibility of the persisting virus variants.

Timeline:

- Objective 1 - maximum of one year
- Objective 2 - 2 years
- Objective 3 - 12-24 months depending on whether we restrict our study to an analysis of only one parental isolate and its monkey-passaged variants
- Objective 4 - maximum of three years continuation

II. HIV-1 ANIMAL MODELS - Significant achievements

The following is a summary of the recent significant results from the ongoing science in this component of Vaccines for Prevention:

- Developed and characterized immortalized lines of macaque T and B lymphoid cells.

These cells are critical reagents for assessing the efficacy of vaccines being tested in macaque SIV and HIV models. They have potential application to assessment of both cellular (cytotoxic T cells) and humoral (neutralizing antibodies) immune responses. This represents a significant accomplishment in that several technical difficulties in establishing the lines (i.e. rampant spumaretrovirus expression, etc.) had to be overcome. The methodology for developing these lines is now well established in our laboratory and can be readily applied to cell populations recovered from additional monkeys.

- Continued virologic and immunologic characterization of pig-tailed macaques infected August, 1992 with HIV-1 and continued *in vitro* efforts towards cross-species adaptation of HIV-1.

While one group previously reported infection of pig-tailed macaques with HIV-1, several other investigators have had difficulty reproducing this work. Because of the potential importance of the workable model of persistent HIV-1 infection in monkeys, the researchers, too, have pursued this line of investigation. Following extensive *in vitro* experiments aimed at determining the susceptibility and permissivity of T cells and monocyte/macrophages from various macaque species to HIV-1, five pig-tailed macaques were reinoculated and one cynomolgus monkey with HIV-1. During this reporting period, some of the animals

reached the post inoculation mark. Based on the longevity of HIV-1 specific antibody responses, the continued presence of detectable HIV-1 viral DNA in uncultured peripheral blood and lymph node cells, and the isolation of HIV-1 from one monkey at late time, it was concluded that pig-tailed macaques can become persistently infected with HIV-1. This work represents a significant advancement of this animal model, particularly because of success with the choice of isolate and the nature of the viral inoculum (autologous, virus-expressing cells) we used. The researchers are continuing to attempt to better adapt the late-recovered, monkey-passaged HIV-1 using approaches that have been successful with other retroviruses, including HIV-2.

- Molecular cloning and sequencing of HIV-1 gp120 from isolates independently recovered from brain macrophages, thymic T cells, peripheral blood T cells and peripheral blood monocytes from the same animal at the same point in time.

This work is being conducted to determine the *in vivo* relevance of the *in vitro* phenomenon of HIV-1 macrophage tropism. This knowledge may be crucial to our consideration of which kinds of isolates need to be included in an efficacious vaccine preparation, especially given the propensity of macrophage-infecting microbes for escaping immune surveillance. The sequencing the five clones from each of a total of 10 isolates has almost been completed, and the data will be analyzed within the next two months.

III. PROTECTIVE IMMUNITY

The following objectives constitute the primary focus in this third component of the Vaccines for Prevention Program Area.

Objectives

- a. Perform comprehensive, detailed evaluation of cellular and humoral immunity induced by efficacious vaccines in the SIV model system.
- b. Determine whether sequential infection with more than one HIV-1 variant is occurring in humans.
- c. Employ reagents from seronegative volunteers participating in current vaccine trials to assess quality, durability, and breadth of protective immunity *in vitro* (neutralization/ enhancement, etc) and *in vivo* (PBL-SCID).
- d. Employ an integrated approach to the evaluation of virus-host interaction in the context of: disease progression, acute primary immunoregulation, transmission events,

highly-exposed persistently-uninfected humans.

The following laboratory research projects as well as proposed projects represent the science in this major component:

1. (LRP36) Genetic and Antigenic Characterization of Internationally Collected HIV-1 strains:

Description:

This protocol, organized and funded mainly by the Navy Medical Research and Development Command, is designed to collect HIV strains from a wide range geographically diverse regions. Genetic and antigenic characterization will be performed in collaboration with the Foundation and WRAIR. This protocol will primarily be conducted at Naval Medical Research Institute (NMRI), and will include multiple overseas Navy Medical Research Units and will be a collaborative effort between NMRI, the Foundation and WRAIR.

Research Hypothesis:

There are multiple genetic subtypes of HIV-1. If these subtypes represent antigenically distinct groups, a broadly effective vaccine may have to generate an immune response to numerous subtypes. Definition of the scope and prevalence of HIV variation will be an important component of HIV-1 vaccine development. To arrive at a more complete understanding of worldwide viral variation, a large number of complete specimens (virus and serum) from various parts of the world will need to be analyzed.

Objectives:

- 1) Establish a comprehensive international collection of HIV isolates and associated serum from infected individuals including regions with new or emerging HIV epidemics.
- 2) Characterize genetically and antigenically, viruses in collaboration with The Foundation and WRAIR.

Timeline:

This protocol has just been approved by the Naval Medical Research Development Command Human Use Committee (July 1993). Protocol initiation (collection of specimens) should begin September 1993.

2. (LRP 37) Antigenic characterization of Internationally collected HIV-1 strains:

Description:

A neutralizing antibody assay is used to attempt to antigenically distinguish internationally collected HIV-1 isolates that have been genetically characterized.

Research Hypothesis:

1. Genotypes of HIV may indicate antigenically distinct serotypes.
2. A broadly effective vaccine may need to include multiple HIV-1 serotypes.

There are multiple genotypes of HIV-1. If these subtypes represent antigenically distinct groups, a broadly effective vaccine may have to generate an immune response to numerous subtypes. Antigenically serotyping viruses based on reactivity to a functional antibody (e.g. neutralizing Ab) have, in many viral systems, proven useful for vaccine development. To study the potential antigenic (immunologic) diversity of distinct genetic subtypes of HIV-1, this study uses a neutralizing antibody assay to attempt to antigenically characterize divergent HIV-1 isolates. About 70 internationally collected viral isolates have been genotyped, expanded in PBMCs and are available for antigenic studies.

Objectives:

- 1) To determine if genetically distinct subtypes of HIV-1 represent antigenically distinct serotypes.
- 2) To antigenically categorize a large international collection of HIV-1 isolates in order to define the scope and prevalence of HIV-1 antigenic variation world wide.
- 3) To evaluate for potential Neutralizing Ab epitopes which are conserved between divergent HIV-1 subtypes.
- 4) To establish, in future animal and human vaccine studies, if inclusion of multiple genotypes or serotypes of HIV-1 is a necessary component of a broadly effective vaccine.
- 5) To establish if "dual infection" of HIV exists; i.e can an individual infected with one genotype of HIV-1 be infected by a genetically divergent strain.

Significant Findings:

- 1) A group of ten field isolates from Thailand and the U.S. were evaluated in a neutralizing antibody assay. These viruses each represent 1 of 2 distinct genotypes. Using a panel of U.S. and

Thai plasma, Program Area researchers were able to antigenically distinguish the two genotypes. This represents the first clear evidence that genotypes of HIV-1 correlate with antigenic subtypes.

2) An additional finding was that lab strains are much more susceptible to Neutralizing Ab than primary isolates. Neutralizing antibody assays run with laboratory strains of HIV instead of field isolates gives a potentially misleading impression that there is strong cross reactivity between divergent HIV-1 strains.

3) Preliminary results of PCR typing of infected Thais suggests the possibility that some individuals are infected with both the "A" and B" Thai strain. These results need to be confirmed by more rigorous molecular analysis.

Relevant Publications:

1. Mascola J, Louwagie J, McCutchan F, et al. Distinct genetic subtypes from Thailand and the U.S. can be Distinguished in a Neutralizing Antibody Assay (Submitted- June 1993).

2. McCutchan F, Louwagie J, Mascola J, et.al. Genetic Subtypes of HIV-1. AIDS Research Hum Retroviruses 1993;(in press)

3. Mascola J, Gartner S, Groenendaal C, et al. Cross Neutralization of HIV-1 Isolates from Northern Thailand and the United States in a Human Peripheral Blood Mononuclear Cell Assay. Annual Meeting of the Laboratory of Tumor Cell Biology. 1992;[Abstract]

Proposed projects:

1. Serotyping of HIV-1 isolates from each of the described genotypes using a neutralizing antibody assay.

To date, viruses from only two genotypes have been evaluated. The researchers propose to perform cross neutralization studies with viruses from all 5 (or more) genotypes of HIV-1. About 70 internationally collected HIV-1 isolates have been evaluated and are available for this study. Additional samples can be obtained from Navy overseas laboratories and/or the WHO collection network.

Timeline: This project was initiated in 1992. Completion is anticipated by the end of 1994.

2. Evaluation for conserved neutralization epitopes among different HIV-1 genotypes:

a) Analysis of sera with neutralizing antibody that cross reacts to divergent HIV-1 subtypes using peptide competition and adsorption studies.

b) Construction of HIV chimeras with gp120/160 of various genotypes cassetted into an HXB2 backbone. Compare neutralization characteristics of chimeras to parental strains.

c) Mapping neutralization epitopes using epitope specific human monoclonal antibodies (in collaboration with Susan Zolla-Pazner) or by type specific animal sera.

Timeline: Begin early 1994; Two years

3. Evaluation of Thais for "dual infection". Using nested PCR from primary PBMCs as a screen, identify individuals who appear to be infected with both Thai genotypes.

a) Analyze multiple env or gag gene clones from individuals with possible dual infection.

b) Viral Isolation - attempt to isolate both genotypes.

c) If dual infection is proven to exist, evaluate serial blood specimens from individuals to prove serial infection.

Timeline:

Sample collection in Thailand is ongoing; Molecular analysis to confirm dual infection 6-12 months. Cohort study to prove serial infection - 1 additional year.

3. (LRP 37A) *Use of pin-linked peptides for sero-typing of international HIV samples and of SIV challenge strains used in vaccine studies:*

Background:

Antibody patterns in the sera of HIV-infected individuals will be analyzed with a method of PEPSCAN, and correlation between the genetic variation and sero-types is sought. A similar study will be carried out for monkey sera from the SIV vaccine studies, using peptides sequences from the envelop protein of the challenge strain.

With the development of the technique commonly called PEPSCAN, hundreds of peptides can be synthesized simultaneously on polystyrene pins in sufficient purity for reaction in ELISA (1). Drastic reduction in the cost and time of multiple peptide synthesis has made epitope mapping of a variety of proteins possible. Loomis et al have recently studied the humoral response to gp160 of HIV, and found

changes in antibody patterns to epitopes during the rgp160 vaccine trial(2).

In the proposed study, peptides with sequences covering the regions of interest in the gp120, of the predominant variant of each HIV subtype, will be synthesized on the pins and used for sero-typing. In contrast to hexa-peptides with 5 residue overlaps used in the original study(1), 15 amino acid peptides with 8-residue overlaps will be synthesized for the study. Use of the larger peptides would increase the chance of recognition by the natural antibodies (2,3), and overlaps of the minimum epitope length would ensure coverage of the entire region of interest. The number of peptides to be synthesized would be 1/7 of that done in the original method, permitting more samples to be analyzed in shorter time.

Extensive studies on the genetic variations of HIV isolates (McCutchan et al) have revealed surprisingly large proportion of conserved sequences in the envelop protein gp120; 206 residues in the stretches of more than 7 amino acids in the total of 480 residues. Within a subtype, the proportion of conserved sequence is probably higher. With proper choice of peptide sequences from the dominant variant of a subtype, antibody patterns correlating with genetic variations within the geographical location may be obtained. Scanning of only the variable regions, with part of flanking conserved regions, will reduce the number of peptides to be synthesized, and more serum samples can be analyzed with a set number of pins.

The technique will also be applied for sero-typing of SIV strains used in the vaccine study. Changes in antibody patterns after virus challenge will provide useful information pertaining to the protective immunity.

Research site: Henry M. Jackson Foundation Research Laboratory.
Principal Investigator: C. Y. Lai
Co-investigators: John Mascola, F. McCutchan, M. Lewis, S. Gartner, G. Eddy.

Research Hypothesis:

Within an epitope, sequence deviation from the reference (sequence of the dominant variant in a subtype) will cause reduction of its affinity to the natural antibody and thus a decreased ELISA signal.

Objectives:

1. To set up the laboratory facility and protocol for the proposed assays, including experimental design and training of personnel.
2. To survey international sera of HIV infected individuals with the aim to obtain correlation between sero-typing and genetic variations.
3. In conjunction with clinical data of the individuals tested, detect markers that correlate with the presence of protective

immunity.

4. Provide insight into protective immunity, examining quantitative and qualitative change in antibody patterns after a live virus challenge in the SIV-model.

5. Design and develop an effective vaccine based on the information provided in this and other studies. (A long range objective.)

Research plan:

Selection of sequences and overlaps will be carried out based on:

- a. structural information obtained in the genetic typing of international HIV isolates, e.g. prevalent structure in a given geographic locations.
- b. hydrophilicity and secondary structure prediction,
- c. sequences that had been reported to be epitopes(2,3) and to elicit neutralizing antibodies(4).

Advantages of using the limited span of sequences rather than that of the whole gp120 are: a) much smaller number of peptides needs to be synthesized, b) more specimens may be analyzed with a fix number of pins, and c) saving of time and cost. Further literature search and review of relevant publications will be made in order to make a successful selection of sequences for the experiment.

This type of study will also be performed for SIV challenge strains used in the SIV vaccine trial. Initially peptides with overlapping sequences covering the entire envelop protein will be synthesized for the peptide mapping. If necessary, the information obtained will be used to select sets of peptide sequences for analysis as described before for HIV sero-typing. The changes in antibody patterns after a live virus challenge may then be correlated with the incidence and severity of infection.

While results of these studies may not reveal the nature of immune response that protects individuals (or animals) from the virus infection or transmission, presence of antibodies directed to a particular epitope in the protected individual would indicate their importance in combating viral infection. Studies on their structure, including state of glycosylation and conformation will further provide important information for the design of an effective third generation vaccine.

Timeline:

It is estimated that setting up the facility, protocol and personnel training will take approximately 3 months. Synthesis of a set of 96 14-amino acid peptides will take 2 weeks, though more than

2 sets may be synthesized simultaneously (experience of Dr. Larry Loomis (PA 4), who has agreed to collaborate, suggests that as many as 10 sets can be synthesized in two weeks). The time for analysis depends on the number of sets of pins available. Past experience with ELISA indicates that up to 12 microtiter plates can be manually processed per week per technician. With PEPSCAN, processing of 6 samples using 2 sets of pins will be possible per week.

Once the protocol and procedures are set-up, the assay can be performed for the proposed vaccine trials.

Reference:

- 1) Geysen, HM, et al. (1986), Proc. Natl. Acad. Sci., 81: 3998-4002
- 2) Loomis, L, et al. (1993), manuscript submitted.
- 3) Neurath, AR, et al (1990), AIDS Res. Hu. Retrovirus, 6: 1183-1192
- 4) Ho, DD, et al. (1988), Science, 239: 1021-1023

4. (LRP38) *Evaluation of sera from Phase I vaccinees for the presence of neutralizing antibodies to genetically diverse HIV-1 field isolates:*

Description:

Sera are evaluated for neutralizing antibody in a PBMC based assay. Human vaccinee sera are obtained either from the AVEG of NIAID, directly from biotechnology companies (Chiron, UBI) or from our own phase I trials. Sera from gp120 immunized baboons (various adjuvants, multiple immunization schedules) and rabbits immunized with various strains of gp120 are also available.

Research Hypothesis:

Based on animal vaccine studies, the Neutralizing Ab response generated in human vaccinees by current subunit vaccine products is likely to be strain specific.

There are multiple HIV-1 vaccine candidates in Phase I studies. All current vaccines are based on strains of genotype "B" (i.e. IIIB, MN, SF2). A broadly effective vaccine will have to generate an immune response to multiple genotypes (5 or more). Currently, analysis of neutralizing Ab in vaccinees has focused almost exclusively on homologous "lab strain" neutralization. In this study, sera from human phase I vaccine volunteers and some immunized animals will be evaluated for breadth of neutralizing antibodies; i.e. they will be assayed against a panel of primary HIV-1 isolates representing several different genotypes.

Objectives:

- 1) To determine if Phase I human vaccinees have neutralizing Abs to primary (clinical) isolates of the same genotype as the vaccine strain (i.e. non-homologous PBMC grown virus of the same genotype)
- 2) To determine if Phase I vaccinees have neutralizing Abs to primary (clinical) isolates representing a different genotype as the vaccine strain.
- 3) To test sera from animals vaccinated with various vaccine products/adjuvants/regimens not yet evaluated in human studies.

Proposed Projects

1. Evaluation of AVEG panel of sera (34 sera from human vaccines) against a panel of laboratory and primary HIV-1 isolates including genetically divergent isolates.

Do vaccinee sera neutralize primary HIV isolates?
If yes, does this extend to viruses of divergent genotypes?

2. Similar evaluation of human and baboon sera provided by Chiron and human sera provided by UBI.
3. Similar evaluation of human vaccinees from our own trials in Thailand as well as future phase I/II vaccine trials run by NIAID/AVEG and Biotech companies.

Significant Findings:

Studies of sera from rabbits immunized with recombinantly expressed gp120 or gp160 revealed that the serum contained high titers of neutralizing Ab against the homologous lab strain, but lacked neutralizing antibody activity against several field isolates from the U.S. which were of the same genotype as the vaccine strain. Preliminary results from similar studies with sera from human vaccinees reveals the same result; i.e. the neutralizing Ab response is strain specific.

Timeline:

- 1) This AVEG study was approved in February 1993. The first serum samples were received in May 1993. Study is in progress. Estimated completion of this initial project - October 1993. Other serum samples from AVEG are anticipated.
- 2/3) An Material Transfer with Chiron has been signed and serum samples from immunized humans and baboons will be available shortly. The researchers have a verbal collaboration with UBI from which serum will obtain serum from human phase I trials which UBI is conducting. Estimated start: September 1993. An ongoing evaluation of sera from human trials is anticipated.

5. (LRP39) Does Antibody-Dependent Enhancement (ADE) pose a risk

in HIV Vaccine Trials?:

Description:

The ADE activity of serum from Phase I vaccines and naturally infected individuals is assessed for ADE of infection of human lymphocytes or macrophages.

Research Hypothesis:

1. HIV vaccines may pose a risk to vaccinees by causing immune-mediated disease enhancement.
2. The antigenic variation (serotypes) of HIV-1 may contribute to the risk of ADE *in vitro*. ADE of HIV infection of human cells is a well described phenomenon. There are examples of immune-mediated enhancement in other viral diseases. In Dengue, the presence of distinct viral serotypes prevents cross protection by neutralizing antibodies and puts individuals secondarily exposed to a different serotype at risk for enhanced disease. Enhancement is mediated, *in vivo*, by ADE of infection of M/M cells and can be predicted by an *in vitro* ADE assay. In EIAV, animals immunized with a recombinantly expressed glycoprotein vaccine show enhanced (more severe) clinical disease than controls after EIAV challenge. Therefore the evaluation of HIV vaccinees for ADE *in vitro*, may impact significantly on our assessment of the potential risk of ADE in human vaccine trials and subsequently on the types and priority of animal studies needed to evaluate this risk.

Objectives:

- 1) To determine if Phase I vaccinees have enhancing antibodies to primary (clinical) isolates of homologous and heterologous genotype as the vaccine strain.
- 2) If antigenically distinct serotypes of HIV are identified, studies will be conducted to determine if serum lacking cross neutralizing activity between serotypes can mediate ADE of divergent serotypes.
- 3) To establish, through animal model studies, if *in vitro* ADE of vaccinees signifies a risk for *in vivo* enhancement.

Significant Findings:

- 1) Preliminary studies with sera from one cohort of Phase I vaccinees (recombinantly expressed gp120) revealed that the serum had neutralizing activity against the homologous viral strain but enhanced *in vitro* infection of PBMCs by closely related

heterologous strains.

2) A 2 day workshop titled: "Does Antibody Dependent Enhancement pose a Genuine Risk in Human HIV Vaccine Trials?" sponsored by WRAIR and the Division of AIDS, NIAID was held at the Rockefeller Institute in NY in December 1992. A group of investigators with expertise in ADE and related issues was invited and participated in drafting proposals for experiments to address the potential risk of ADE.

Relevant Publications:

Mascola J, Mathieson B, Zack P, et al,. Summary Report: Does Antibody Dependent Enhancement pose a Genuine Risk in Human HIV Vaccine Trials?, AIDS Research Hum Retroviruses (In Press).

Proposed Projects:

1. Naturally infected individuals: using primary PBMCs and Monocytes, determine if serum lacking cross neutralizing activity between serotypes can mediate ADE of the divergent viral isolates.

2. Phase I/II seronegative vaccinees: Using primary PBMCs and monocytes, determine if there are enhancing antibodies present to heterologous and/or divergent strains of HIV. Specifically, evaluate for the following potential scenario: Sera from a type B vaccine product vaccinee does not neutralize a divergent (Thai) virus but instead enhances its growth. This may discourage the field from pursuing large scale trials with this product.

Timeline:

This study is in preliminary phases. Serum from Phase I vaccinees will be evaluated for ADE over the next year - 1993. Partial completion of currently planned *in vitro* studies is anticipated by the end of 1994.

6. (RV91A - proposed): *Evaluation of Possible Dual HIV-1 Infection:*

Background:

In the absence of other human models for protective immunity to HIV-1, vaccine researchers have hypothesized that infection with HIV-1 protects from subsequent infection and have used the immunologic markers of infection as a model for protective immunity to HIV-1. The theory that infection with HIV protects against subsequent

infection, either with a homologous or a heterologous strain, has not been rigorously demonstrated. To address this question one should study a population with a high incidence rate, having therefore a high chance of reinfection, and with divergent strains of the virus in circulation so that one could look for re-infection with heterologous strains. Such a population exists in Thailand where infection with HIV is increasing rapidly and where two divergent strains of HIV-1 are prevalent. Investigating the occurrence of infection with two different strains of the virus in such a population would refute (or help confirm) the hypothesis that infection with HIV-1 protects from subsequent re-infection with a heterologous strain. If dual infection is confirmed to exist, prospective studies in high risk groups would be able to address the question of whether double infection occurs concomitantly or sequentially and, if sequentially, whether the sequence is definable and explainable.

If double infection can be demonstrated, it presents the opportunity to study another important aspect of HIV-1 vaccine immunology: antibody-dependent enhancement. In a few other viral diseases, most clearly Dengue virus, the presence of low-level antibodies to the virus of one serotype enhances disease when infected with a different serotype. The possibility that this could happen with HIV-1 is a serious concern in preparing for vaccine trials. The presence of people infected with both genotypes would be valuable for the study of *in vivo* enhancement with HIV-1.

OBJECTIVES:

1. To determine whether people can be dually infected with HIV-1 by studying the occurrence of infection with two distinct genotypes of HIV-1 in people living in Thailand.
2. If dual infection occurs, design prospective research studies to address:
 - a) correlates of protective immunity.
 - b) HIV-1 enhancement (*in vivo*).

PROPOSED TIMELINE:

Fall 1993:

1. Collect sera and PBMCs from seropositives in Thailand.
2. Characterize HIV-1 using nested PCR typing.
3. Characterize the serotype of HIV-1 using V3 peptide ELISA.
4. Clone and sequence selected isolates to confirm double infection.

5. Use heteroduplex gel shift to confirm double infection.

Winter 1993-1994:

1. Design research studies to explore protective immunity.
2. Design research studies to explore antibody-dependent enhancement of disease.

7. (RV_Pro01) Evaluation of a HEPS (Highly-exposed, Persistently-seronegative) Cohort: A Multicenter Collaboration:

Background:

Recently, several research units prospectively following cohorts of individuals at high risk of HIV-1 infection have discovered that some individuals remain seronegative (and purportedly uninfected) in the presence of significant exposure. It is possible that these individuals have some form of protection to the virus, either virus-specific or non-specific. Since there is currently no adequate animal model for infection with field isolates of HIV-1, there are no good *in vivo* immunologic markers of protective immunity to the virus. It is possible that the people who have continued high exposure to the virus and who remain uninfected may have developed protective immunity specifically directed against HIV-1 and if so, immunologic markers for that protection would be of critical value in the development of effective preventive vaccines.

The prevalence of HIV-1 is accelerating in Thailand and is especially high in northern provinces, particularly in and around Chiang Mai and Chiang Rai. In 21-year-old males recruited into the Royal Thai Army, prevalences are around 15%, and in commercial sex workers (CSWs) the prevalences are between 40% and 80%. Chiang Mai University, in collaboration with the local Ministry of Public Health, has started a prospective study of seronegative CSWs working in northern Thailand. They are collecting detailed exposure information including detailed sexually transmitted disease (STD) histories. Using both the exposure histories and STD incidence, they will define a group of highly exposed, persistently seronegative (HEPS) CSWs. Blood from these women will be analyzed to detect HIV-1 infection. If women are confirmed as both highly exposed and uninfected, research will be planned to aggressively explore their immunologic status. A similar cohort and strategy is being explored with the US CDC - Thai Ministry of Public Health HIV Collaboration in Thailand.

Timeline (Phase 1):

December 1993: Complete the documentation of the existence (and extent) of HEPS CSWs.

8.RV Proposed 02 Perinatal Transmission Events:

Outline of Research Proposal:

I. Baseline Data and Samples:

A. Maternal evaluations in each trimester, delivery and up to three months post-partum.

1. A standard obstetrical history and physical exam to document overall health and HIV clinical stage.
2. 20 ml of heparinized whole blood, separated by ficoll and cryopreserved.
3. 7 ml of blood in a clot tube for serum stored at -20°C

B. Infant evaluations at birth at one month, and at every three months until 24 months of age.

1. A standard history and physical is obtained at each visit to identify HIV or other disease manifestations.
2. 5 ml of heparinized whole blood, separated by ficoll and cryopreserved at birth and 7 ml at all subsequent visits.
3. 3 ml of blood in a clot tube for serum stored at -20°C at birth and 5 ml at all subsequent visits.

II. Sample processing (* reflects optional item):

MATERNAL	cells/sera	purpose
PBMC co-culture	2 x 10 ⁶ cells	viral stocks
Plasma co-cult.	0.5 ml	viral stocks
Quant. PCR	2-3 x 10 ⁶ 1 ml plasma	viral burden and sequencing
T cell line*	2 x 10 ⁶ cells	identify T cell clones to env
lymphoproliferation	10 x 10 ⁶ cells	env responsive T cell clones
neutralization of maternal stocks	500 ul of sera for each stock	autologous neut.

neutralization of infant stocks	500 ul of sera for each stock	autologous neut.
epitope mapping of antibody response	2 ml of sera	characterize V3 antibody profile
<u>INFANT</u>	<u>cells/sera</u>	<u>purpose</u>
PBMC co-culture	2 x 10 ⁶ cells	viral stocks
Plasma co-cult.	0.5 ml	viral stocks
Quant. PCR	2-3 x 10 ⁶ 1 ml plasma	viral burden and sequencing
T cell line*	2 x 10 ⁶ cells	identify T cell clones to env
neutralization of maternal stocks	500 ul of sera for each stock	autologous neut.
epitope mapping of antibody response	2 ml of sera	characterize V3 antibody profile

Viral stocks of both infant and mother will have the following:

SI/NSI characterization
resistance to nucleosides where applicable
sequence analysis pre and post neutralization

III. PROTECTIVE IMMUNITY - Significant Achievements

The following is a summary of significant achievements that have resulted from the science detailed in this component.

- Completed a comprehensive study of HIV-1 envelope genes from international isolates. Demonstrated the existence and broad geographic dispersal of at least six genetic subtypes of HIV-1.
- Provided first comprehensive description of HIV-1 genetic subtype F and demonstrated presence of two distinct subtypes, B and F, in Brazil.
- Developed a rapid genotyping assay for HIV-1 genetic subtypes using heteroduplex gel shift, in collaboration with Drs. Mullins and Delwart at Stanford

- Characterized by PCR typing (39 isolates) and envelope sequencing (two isolates) samples from the WHO network for HIV-1 isolation and characterization. Initiated participation in the World Health Organization effort on virus neutralization which will implement and supplement ongoing efforts in this laboratory.
- Antigenic characterization of Internationally collected HIV-1 strains:

A group of ten field isolates from Thailand and the U.S. were evaluated in a neutralizing antibody assay. These viruses each represent 1 of 2 distinct genotypes. Using a panel of U.S. and Thai plasma, the Program Area researchers were able to antigenically distinguish the two genotypes. This represents the first clear evidence that genotypes of HIV-1 correlate with the antigenic subtypes. An additional finding was that lab strains are much more susceptible to Neutralizing Ab than primary isolates. Neutralizing antibody assays run with laboratory strains of HIV instead of field isolates gives a potentially misleading impression that there is strong cross reactivity between divergent HIV-1 strains. (manuscript in press- Journal of Infectious Diseases).

- Genetic and Antigenic Characterization of Internationally Collected HIV Strains: Protocol has been approved by NMRDC human use committee (July 1993). Protocol initiation (collection of specimens) begins October 1993.
- Evaluation of sera from Phase I vaccinees for the presence of neutralizing antibodies to genetically diverse HIV-1 field isolates:

Studies of sera from rabbits immunized with recombinant gp120 or gp160 revealed that the serum contained high titers of neutralizing Ab against the homologous lab strain, but lacked neutralizing antibody activity against several field isolates from the U.S. which were of the same genotype as the vaccine strain. Preliminary results from similar studies with sera from human vaccinees reveals the same result; i.e. The Neutralizing Ab response is strain specific. (abstract accepted as a talk to NCVDG, October 1993).

- Does Antibody Dependent Enhancement (ADE) pose a risk in HIV Vaccine Trials?

A 2 day workshop sponsored by WRAIR and the Division of AIDS, NIAID was held at the Rockefeller Institute in NY in December 1992. A group of investigators with expertise in ADE and related issues was invited and participated in drafting proposals for experiments to address the potential risk of ADE. AIDS Research Human Retroviruses (in press).

IV. TRIAL SITE DEVELOPMENT & VACCINE TRIALS

This component has a primary focus in the development of international field sites for the conduct of vaccine trials. The WRAIR/Foundation research team has been working closely with researchers at AFRIMS to plan and implement a setting which will ultimately support large scale Vaccine Trials. There are three objectives for the current research in Thailand:

- a. To find an adequate cohort for a phase III vaccine trial. This research previously consisted of an investigation of the Royal Thai Army recruits, and that work continues. In August and September, for the first time, a field unit was dispatched to the site in order to improve follow-up. Based on those results, the feasibility of using recruits will be assessed. In addition to the recruits, however, plans are now ongoing to explore a community based cohort in a military/civilian community in an epidemic area in Northern Thailand.
- b. To perform phase I testing of HIV vaccine constructs already tested in the U.S. This research will be driven by 2 protocols, RV 68 and 69 (see below), which have completed approval processes in both the U.S. Army and the Royal Thai Army and have been approved by the Thai Ministry of Public Health. They are awaiting approval by the Thai National AIDS Committee. Preparations for these protocols are on-going.
- c. To discover markers for early HIV disease or infection which could be used for vaccine trial endpoints. This research is driven by the Natural History Protocol, RV91, which has passed institutional approval by the US Army and Royal Thai Army and the Thai Ministry of Public Health.

In addition to these protocols, there are two initiatives which are in early, exploratory phases:

- a. There have been reports of individuals who have had high exposure to HIV yet failed to become infected. It is possible that these individuals have developed viral-specific immunity to the virus and if so, this might be one of the only current models for protective immunity to HIV. In collaboration with Chiang Mai University, the researchers have identified CSWs with high exposure based on history and STD incidence and are in the process of testing the blood to confirm lack of infection. If the evidence confirms lack of infection, plans will then be made to investigate them intensively. Further description of this proposed project can be found below under the annotation - RVPro 01.
- b. In a small survey of 35 HIV positive people in Thailand, a cluster of people have been identified who appear to have been

infected by two different substrains of HIV. This has never been observed before, and the hypothesis has been that people infected with one strain are protected from subsequent infection. Further investigation of these possibly dually infected people is on-going and, if confirmed, would form the basis for a new research initiative. This proposed project is described below - RV91A.

Current and proposed protocols/projects:

Currently, one epidemiological protocol is active (RV70) and two vaccine protocols continue a lengthy "international" protocol process (17 steps to date). The following are descriptions of these protocols as well as pending protocols and projects.

RV-70 "Prevalence and Incidence of HIV-1 Infection among Young Men in the Royal Thai Army (Tahan Gan)":

Number of volunteers to be screened: Prevalence: 137,000
Incidence: 14,000- 16,000

Protocol summary:

1. To assess temporal, geographic and demographic correlates of HIV-1 infection among young men entering the Royal Thai Army (RTA).
2. To directly measure the rate of incident HIV-1 infection among young Thai men in the RTA during their two year service obligation
3. To explore the feasibility of geographically circumscribed cohorts as possible future participants in field efficacy trials of HIV-1 prophylactic vaccines in Thailand.
4. To identify a population of recently HIV-1 infected Thais for recruitment into RV-91.

Significant Findings:

This research, consisting of prevalence and incidence components, has described the HIV serostatus of RTA recruits when they entered the service and followed their status over time while serving in the army. A total of 28,087 recruits were screened in May 1993, with a seroprevalence rate of 4.0%. In August another incident follow-up of the 17,611 recruits in the incidence study was

performed. On this visit for the first time, a team was sent into the field to improve follow-up. The results from this visit are not yet available. From both studies it is clear that the upper north of Thailand is experiencing a very high level of HIV transmission.

RV 68 - "Phase I Study of the safety and immunogenicity of a recombinant envelop based product in Thai volunteers with early HIV infection":

Protocol Description:

This is a proposed double-blinded, randomized cross over Phase I/II evaluation of rgp160 in 60 Thai volunteers with early stage infection. The objectives are to assess the immunogenicity and safety of this product and to determine if Thai specific population factors exist in terms of safety or immunogenicity of HIV-specific vaccine products. Additionally, due to the reported predominant circulation of two highly distinct viral clads in this population, the role of infecting genotypes on the specificity of human adaptive immune responses to a particular immunogen can be assessed. Finally, a parallel Phase I trial concurrently conducted in seronegative volunteers would also offer further assessment of the impact that natural infection on the specificity of anti-HIV vaccine induced immune responses.

Significant Findings:

This protocol is still in the review process. It has passed institutional review by both the US Army and the Royal Thai Army and by the Ethics Committee of the Ministry of Public Health but is awaiting review by the National AIDS Committee.

RV 69 - "Phase I Study of the safety and immunogenicity of a recombinant envelop based product in HIV seronegative volunteers"-

Protocol Summary:

This proposed study will be an open Phase I/II evaluation dose finding trial of rgp 160 vaccine in 120 Thai seronegative low risk volunteers. This product was selected because it has previously been demonstrated to be safe and immunogenic in low risk seronegative U.S. volunteers. The primary purpose of this trial is to facilitate infrastructure development for the future execution of large scale prophylactic vaccine trials when candidate products meet appropriate criteria for large scale trial evaluation. Together with RV 68, this trial has an additional scientific objective to determine the impact of natural infection on the immunogenicity of a particular vaccine

product as compared to product immunogenicity profile in a non-infected population.

Significant Results:

This protocol is still in the review process: it has passed institutional review by both the US Army and the Royal Thai Army and by the Ethics Committee of the Ministry of Public Health but is awaiting review by the National AIDS Committee.

RV 69 Behavioral Addendum - *"A Phase I Study of Safety and Immunogenicity of a Recombinant Envelop-Based Product in Human Immunodeficiency Virus (HIV) Seronegative Thai Volunteers: Behavioral Side-Effects and Behavioral Factors in Study Recruitment, Participation and Adherence"*

Protocol Description:

This protocol represents a collaborative effort between this Program Area and the Behavioral Prevention Program Area. The importance of Behavioral Factors in International Vaccine Trials has been recently underscored and emphasized by such organizations as WHO, and this protocol will explore such issues. The primary purpose will be to develop and evaluate methods for the recruitment, accrual and retention of protocol participants; the development and evaluation of behavioral intervention programs to support protocol participation and adherence, as well as prevent any behavioral side-effects are also objectives. Such efforts will assist this Program Area in meeting its objective of establishing an infrastructure for future execution of large scale prophylactic vaccine trials in Thailand when candidate products meet appropriate criteria for a large scale trial evaluation. Sixty (60) volunteers will participate over a two year time frame to coincide with participation in RV 69.

Progress:

This protocol was approved by the Retrovirus Clinical Research Review Committee (RC)² on October 13, 1993 and will continue through the administrative and scientific approval process.

RV-87 *"Immunoregulation and pathogenesis of symptomatic, primary HIV-1 infection"* -

Protocol Description:

This is a proposed descriptive protocol designed to establish a collection of properly stored PBMC, sera and other body fluids from patients with acute symptomatic primary HIV infection and to use these samples to study the early immunologic and virologic events. Five patients will undergo phlebotomy twice weekly for the first 4

weeks, then twice monthly for the next 4 months and then every three months thereafter.

Progress:

This protocol passed Tri-Service Scientific approval and was forwarded to the WRAMC Department of Clinical Investigations in September. Two more approvals are pending.

RV-91 "The Natural History Of HIV-1 Infection in Thailand"-

Protocol objectives:

1. To characterize viral, immune regulatory, and clinical sequelae in recently HIV-1 infected Thai men, especially during the first 1-2 years post-infection. These data may form the basis for efficacy endpoints in future prophylactic vaccine trials in Thailand.
2. To characterize (genetically and serologically) circulating HIV-1 from recently infected Thais. These data may form the basis for selection of vaccine strain prototypes for use in development of Thai-specific vaccine constructs.
3. To assess virus specific and immune regulatory correlates of HIV-1 progression in Thais. These data will be considered largely hypothesis-generating.

Number of Patients to be enrolled:

Incident HIV-1: 200-300
Prevalent HIV-1: 150-200
Seronegative Control: 40

Additional visits to completions:

Incident HIV-1: 800-1200
Prevalent HIV-1: 150-200
Seronegative Control: 4

Progress:

As of the end of September, 1993, this protocol has received approval by the Royal Thai Army, the US Army and the Ministry of Public Health. It will commence during October, 1993.

Laboratory research projects are also conducted within this

component. Planning has been initiated for RV91A, RV Proposed Protocol 01 and Proposed Protocol 02, described on pages 46-50 which are conducted in coordination with Protective Immunity component.

FIELD STUDIES - Significant Achievements

An extensive investment of expertise and time has been contributed from this Program Area in preparation of the Thai Armed Forces Research Institute of Medical Science site for the vaccine clinical research which will start in the next year. This effort has resulted in a fully functional Joint Clinical Research Center which is capable of executing and supporting Phase I and Phase II vaccine trials, as well as Epidemiological and Behavioral studies. Planning for this represents a major achievement in international research infrastructure development. To insure coordination of such a complex effort, site visits by many teams and individuals were completed during this reporting period; brief trip summaries are provided below:

- April 24 - May 8:

A team from the Foundation Management Information Systems group visited to review the operations of the data system. This resulted in major improvements to the database and generated a concrete blueprint for how to proceed in the plans for the future.

- June - November:

A Foundation Senior Programmer visited on extended duty to design the database which will be used in the vaccine and natural history studies. This work is ongoing and is targeted to be complete by the beginning of the new year.

- April 29 - May 8: The Program Area Scientific Director and a Senior Scientist looked over the functioning of the AFRIMS PCR laboratory and made suggestions for improving performance and decreasing the chances of contamination.

- July 20 - Aug 15:

Foundation Senior Research Technologists arrived to give assistance in the PCR and cell proliferation laboratories respectively; improvements recommended during the April/May visit were implemented; cell proliferation assays were initiated and the serum and cell processing were improved.

- In total, these visits have consolidated the functioning of the laboratories at AFRIMS, improved the existing database and are in the process of creating the new database which will be needed for the research planned in the immediate future. In terms of staffing, the senior epidemiologist has returned to the U.S. and a replacement is

pending. Also in November, a computer site manager will join the Thai site.

CONCLUDING REMARKS - VACCINES FOR PREVENTION

Although this six months has represented a transition period for this Program Area and a time of intense reorganization and planning, ongoing scientific efforts have continued as evidenced by the 19 manuscripts, 22 abstracts and one report submitted during the reporting period. This Program Area has made significant contributions to the military as well as to the world-at-large effort in the search for the development of an effective vaccine for the prevention of HIV. As a key example, the Foundation Scientific Director was recently commended by the World Health Organization for her key role in organizing an extensive network and characterization of HIV-1 isolates. As detailed in text above, 60 of the 120 isolates have been characterized fully and 60 are extremely well characterized. This represents the best characterized set of viruses currently available to any research team. With the new five-year plan in place, this Program Area will continue its notable scientific progress towards its goal of developing a vaccine which will ultimately prevent infection with HIV.

Program Area 1 Publications: April 1993 - September 1993

Alving C, Detrick B, Richards R, Lewis M, Shafferman A, Eddy G. Novel adjuvant strategies for experimental malaria and AIDS vaccines. *Proceedings of the New York Academy of Sciences*. In press. 1993.

Birx D, Lewis M, Vahey M, Tencer K, Zack P, Jahrling P, Tosato G, Burke DS, Redfield R. Association of IL-6 in the pathogenesis of acutely fatal SIV_{smm}/PBj-14 in pigtailed macaques. Pending publication.

Black P, Downs MB, Lewis M, Ussery M, Dreyer G, Petteway S, Lambert D. Antiretroviral activity of protease inhibitors against leukemia virus and simian immunodeficiency virus in tissue culture. Pending publication.

Delwart E, Shaper E, Louwagie J, McCutchan F, Grez M, Rubsamen-Waigmann H, Mullins J. Evolutionary relationships between HIV env genes determined by a quantitative heteroduplex mobility assay. *Science*. In press.

Lewis M, Bellah S, McKinnon K, Yalley-Ogunro J, Greenhouse J, Elkins W, Brown C, Rosenberg Y, Gartner S, Eddy G. Dual infection of rhesus macaques with SIV_{E11₈} and SIV₂₃₉. Pending publication.

Lewis M, Bellah S, McKinnon K, Yalley-Ogunro J, Zack P, Elkins W, Desrosiers R, Eddy G. Titration characterization of two rhesus derived SIC_{MAC} challenge stocks. Pending publication.

Lewis M, Elkins W, McCutchan F, Benveniste R, Lai CY, Montefiori D, Burke DS, Eddy G, Shafferman A. Passively transferred antibodies directed against conserved regions of SIV envelope protect macaques from SIV infection. Pending publication.

Louwagie J, McCutchan F, Peeters M, Brennan T, Sanders-Buell E, Eddy G, van der Groen G, Fransen K, Gershby-Damet G-M, Deleys R, Burke DS. Phylogenic analysis of gag genes from 70 international HIV-1 isolates provides evidence for multiple genotypes. *AIDS*. 7:769-780, 1993.

Novembre F, Johnson P, Lewis M, Anderson D, Klumpp S, McClure H, Hirsch V. Multiple viral determinants contribute to the pathogenicity of acutely lethal simian immunodeficiency virus SIV_{smm}PBj variant. *Journal of Virology*. 2466-2474, May 1993.

Mascola J, Louwagie J, McCutchan F, Fischer C, Hegerich P, Wagner K, Fowler A, McNeil J, Burke DS. Two antigenically distinct subtypes of human immunodeficiency virus type 1: Viral genotype predicts neutralization serotype. *JJD*. In press.

Mascola J, Mathieson B, Zack P, Walker M, Halstead S, Burke DS. Summary report: Workshop on the potential risks of antibody-dependent enhancement in human HIV vaccine trials. Pending publication.

McCutchan F. Interim report to the WHO technical working group on HIV-1 isolation and characterization.

Rosenberg Y, Kosco M, Lewis M, Leon E, Greenhouse J, Bieg K, Eddy G, Zack P. Changes in follicular dendritic cell and CD8+ cell function in macaque lymph nodes following infection with SIV₂₅₁. *Dendritic Cells in Fundamental and CLinical Immunology*. New York. 1993.

Rosenberg Y, Zack P, White B, Papermaster S, Elkins W, Eddy G, Lewis M. Decline in the

CD4+ lymphocyte population in the blood of SIV-infected macaques is not reflected in lymph nodes. *AIDS Research and Human Retroviruses*. Vol 9:7, 1993.

Shafferman A, Lewis M, McCutchan F, Benveniste R, Jahrling P, Burke DS, Eddy G. Prevention of transmission of simian immunodeficiency virus from vaccinated macaques that developed transient virus infection following challenge. *Vaccine*. Vol 11:8, 1993.

BEHAVIORAL PREVENTION

USAMRDC Program Area Coordinator: Capt. Richard Daniell, MD, US Navy

Foundation Scientific Director: Dr. Lydia Temoshok

Assistant Department Chief: To be determined

PROGRAM AREA SUMMARY: The primary goal of the Behavioral Prevention Program Area is to conduct scientific behavioral research for the prevention of HIV exposure and transmission, and the reduction of neuropsychiatric deleterious consequences associated with HIV.

Protocols for Human Studies

Protocol No.	Protocol Title (Abbreviated)	Principal Investigator
**	Army Wide HIV/AIDS Survey	Temoshok*
RV26	Tri-service HIV Biopsychosocial Study	Temoshok*
RV56	STD patterns/Ft. Bragg	McKee
RV56A	Laboratory addendum - RV56	
RV62	Foundation for Aviator Performance	Mapou*
RV69A(P)	Behavioral Addendum - RV69 (assigned to Vaccines for Prev)	R. Jenkins*
RV72(P)	Using Interactive Media- HIV Seropositive Cohort	R. Jenkins
RV76(P)	Using Interactive Video - USAF	Zachary*
RV81(P)	Prevention of exposure of High Risk Soldiers to HIV/STDs	P. Jenkins*
RV82(P)	Phase I/II Study of Safety, Behavior Responsiveness, Efficacy of Behavioral Interventions for HIV Infected Military Medical Beneficiaries (MMBs)	Temoshok
RV89(P)	OB/GYN affective reactions to uncertainty and discussing HIV/AIDS	Goldschmidt*

*Foundation Principal Investigator (PI)

**Separately funded by the Office of the Surgeon General,
Department of the Army

(P) = Pending approval

OVERVIEW:

The Behavioral Prevention Program Area has been actively involved in HIV Research Program research activities since July, 1990. The mission of this Program Area is the prevention of HIV exposure and transmission, and the reduction of neuropsychiatric deleterious consequences associated with HIV. Beginning with the new cooperative agreement, this Program Area has made the transition from primarily developing its databases of risk relevant behaviors associated with HIV exposure and transmission to utilizing these databases in developing innovative behavioral intervention strategies designed to reduce risk behaviors and change behaviors related to HIV exposure and transmission risk. With small exception, personnel and resources are devoted to analyzing data and developing intervention technologies to further our goals. These technologies will be tested and evaluated within the context of four research protocols. These will be explained in greater depth in the text. Also during this reporting period, data analysis has continued with ongoing behavioral protocols; this data is being used to design interventions for new protocols which are currently in initial phases of implementation. Other mission-related efforts of the Program Area that will be described include: 1) a survey of military obstetricians-gynecologists' affective reactions to clinical uncertainty and discussing HIV/AIDS during routine gynecologic care (RV89); 2) a study of neuropsychological functioning and job performance in military aviation personnel (RV62); and 3) a centralized STD database at Ft. Bragg, N.C. (RV56).

This Program Area report will delineate the specific research goals and objectives of this program area and will describe in detail the current and new protocols organized to achieve the mission. Significant findings and accomplishments are reported following each individual protocol/project discussion.

RESEARCH GOALS AND OBJECTIVES:

In order to achieve the Behavioral Prevention mission, a comprehensive research program has been set forth to identify the targets of and outcome measures for biopsychosocial interventions that will:

- (1) Reduce the rate of exposures to HIV among uninfected military medical beneficiaries.
- (2) Reduce the rate of HIV transmission by infected military medical beneficiaries.
- (3) Maximize the occupational longevity and productivity and

the psychosocial/neuropsychiatric functioning among infected military medical beneficiaries.

(4) Maximize compliance with medical treatments and experimental protocols among infected military medical beneficiaries and other populations as specified by the MMCARR.

Primary efforts initiated to establish the necessary prevalence estimates and databases to meet these objectives consisted of two large research studies, one for seronegative Army personnel (the U.S. Army-Wide HIV/AIDS Survey) and one for HIV-infected military medical beneficiaries in all military services, the Tri-Service HIV Biopsychosocial Study (RV26). Each of these will be discussed in detail below.

Army-Wide HIV/AIDS Survey (AWAS):

Overview:

Briefly, the U.S. Army-Wide HIV/AIDS Survey (AWAS) was administered anonymously to active duty Army personnel at 30 bases in the U.S. and Europe. The purpose of the survey was to assess knowledge, attitudes, and behaviors that would be important in predicting who in the future had increased odds of being exposed to HIV. The AWAS was a representative survey of active duty Army personnel stationed OCONUS and CONUS. This anonymous survey was served as a database for developing behavioral preventive interventions designed at lowering the active duty soldier's risk of being exposed to HIV and other STDs.

Goals and Objectives:

The AWAS was designed to estimate the prevalence of HIV exposure risk-relevant behaviors; to identify knowledge, attitudinal, sociocultural, behavioral, and situational factors associated with HIV exposure potential; and, in a second set of analyses, to identify factors associated with the occurrence of sexually transmitted diseases in an ethnically and geographically diverse, sexually active population.

Technical Approach:

The U.S. AWAS was administered anonymously to active duty Army personnel at 30 Army bases in the U.S. and Europe. The survey sample size was calculated to provide reliable estimates of attitudes, knowledge, and behaviors of U.S. Army personnel world-wide. It also was designed to be large enough to increase precision of statistical estimates from demographic subgroups within the Army. In addition, the sample was required to be large enough to accommodate an expected 75% response rate. The sampling plan was a multistage stratified probability cluster design. The

sample has statistical validity for, and is representative of the Army in 1991, after the Operation Desert Storm deactivation and return of units to their home bases.

Participants Enrolled:

18,072. The weighted sample ethnic demographic composition is representative of the U.S. Army in 1991.

Sites:

Selected CONUS and OCONUS Army installations

Significant Findings:

Analysis of this database continues. A detailed report of these findings are given in the Final Report for the HIV Research Project - DAMD 17-88-Z-8007.

Summary:

This data is being used as the primary basis for identifying foci for behavioral interventions designed for individuals at high risk for exposure to HIV.

RV26-"Tri-Service HIV Biopsychosocial Study"-

Overview:

The Tri-Service HIV Biopsychosocial Study is a comprehensive, multimethod study designed to assess behavior related to the risk of HIV-infected individuals transmitting the virus to uninfected individuals. The study had as its centerpiece a survey of transmission risk behaviors (the Seropositive Behavior Survey) administered anonymously to HIV-infected individuals seen at one of 5 military medical facilities. Other components of the study included: Psychosocial Questionnaires (PSQ), a psychiatric interview (the structured Clinical Interview for DSM-III), and a comprehensive neuropsychological assessment. In addition, a neurobehavioral addendum to the larger study was conducted with a small subsample of HIV positive individuals. Finally, as a recent addition to the psychosocial questionnaires, data were collected concerning expectations of and perceptions about treatment and therapeutic protocols as these psychosocial factors relate to adherence to and facilitation of clinical trials.

Objectives:

The purpose of the Tri-Service HIV Biopsychosocial Study was to develop a database which could serve as the scientific foundation for behavioral preventive interventions designed to reduce transmission risk behavior in HIV infected military medical beneficiaries. The objectives of the neuropsychology component were to: 1) develop predictors of job difficulties, job performance, and job satisfaction through longitudinal assessment of neuropsychological functioning; 2) test whether specific cognitive profiles can predict specific job difficulties; 3) develop a Phase I/II study to test the safety/feasibility of using cognitive profiles based on neuropsychological assessment to: a) predict job difficulties and b) define potential areas for interventions to enhance job performance and to prevent decline. Each component of the study is described below.

Technical Approach:

Seropositive Behavior Survey (SBS):

The SBS was a self-report survey of HIV transmission-relevant behaviors, knowledge, and attitudes. The SBS was administered anonymously to 1102 HIV infected U.S. military medical beneficiaries. The objective was to estimate the prevalence of transmission-relevant behaviors in HIV infected individuals in order to understand the factors underlying these transmission risk behaviors so that effective prevention programs might be developed. Data collection ended on 1 March 1993. Work with this instrument during the new cooperative agreement has focused on completing quality control of the database and performing statistical analyses on the complete database in order to inform the development of behavioral risk reduction interventions.

Patients Enrolled: 1102

Patient Visits: 1102

Sites:

Walter Reed Army Medical Center; Balboa Naval Hospital, San Diego; National Naval Medical Center, Bethesda; Wilford Hall Medical Center; Womack Army Medical Center.

Significant Findings:

Preliminary analyses based on 767 respondents indicated that 57% of the sample had penetrative intercourse with uninfected partners during the last six months. One-fourth of these uninfected partners were also military medical beneficiaries. Although 75% of seropositive individuals informed their sexual partners of their serostatus, only 66% informed their sexual partners before sex. Between 40%-60% of anonymous or one-time sexual partners and 15% of primary/regular sexual partners were

not informed at all by patients of their seropositive status. Ten percent of spouses and another 15% of primary/regular sexual partners were not informed until at least one month after notification of seroconversion.

A detailed report of these findings are given in the Final Report for the HIV Research Project - DAMD 17-88-Z-8007.

Psychosocial Questionnaires (PSQs) and Structured Clinical Interview for DSM III-R (SCID):

Technical Approach:

Two major data collection methods have been utilized: (1) the use of self-report, standardized questionnaires, and (2) a standardized psychiatric interview (Structured Clinical Interview for DSM-III-R [SCID]; Williams, Gibbon, First, Spitzer, Davies, Borus, Howes, Kane, Pope, Rounsaville, & Wittchen). Questionnaires and SCIDs have been administered at participants' consecutive staging evaluation visits at the major medical centers. The range of time between visits was approximately 5-24 months. However, in general, those participants still on active duty were seen at 6-8 month intervals and those on temporary or permanent retirement lists were seen at 12-18 month intervals.

Patients Enrolled:

A total of 1043 HIV-seropositive military medical beneficiaries (MMBs) completed the questionnaires at Time 1 for RV-26. Those completing Time 2 questionnaires number: 597 respondents; Time 3 = 223 respondents; Time 4 = 71 respondents; Time 5 = 12 respondents. The discontinued measures used in the early phases of the study were completed by 400-700 individuals, depending on the measure, and typically completed only at Time 1.

Perceptions of Treatment: AZT Questionnaires were completed by 850 individuals at Time 1, 324 at Time 2, 77 at Time 3, and 8 at Time 4. Therapeutic vaccine questionnaires were completed by 67 participants in the gp160 trials and 25 individuals in the gp160 + AZT trial. Follow-up questionnaires to Perceptions of Treatment are currently being collected.

SCIDs were completed at least one time by 870 individuals at Walter Reed Army Medical Center (WRAMC) and Wilford Hall Medical Center (WHMC). The psychiatric interview originally was to be administered at WRAMC, National Naval Medical Center (NNMC), and WHMC; however, after several years of pretesting and actual data collection, problems with the validity and reliability of the instrument led to the decision to discontinue its use.

Sites:

WRAMC, WHMC, NNMCM, NHSD

All data except SCID data were collected at the NNMCM, Bethesda, and Balboa Naval Hospital, San Diego. The SCID was never initiated at the Navy hospitals due to the scientific issues, mentioned previously. Collection of SCID data now has concluded at all sites.

Significant Findings:

Analyses of psychosocial data during these 6 months focused primarily on issues relevant to perceptions of treatment, specifically therapeutic vaccine trials. To date, 92 individuals completed questionnaires prior to their initiating participation in therapeutic vaccine protocols (either gp160 or gp160 + AZT) and 45 individuals received follow-up questionnaires approximately 8 months into a given trial. Preliminary analyses of the data indicate that early into the course of the double-blind trial of gp160, individuals do make assumptions about whether they are receiving the vaccine or a placebo. As compared to those who believed they are receiving the vaccine, those believing they are receiving the placebo are more likely to try another treatment while remaining in the trial or to drop out of the trial if they perceived decline in their health. Positive feelings about the trial related to more intentions to adhere to the trial procedures and less tolerance for non-adherence by other participants. Finally, over time, individuals appear less definite in their attitudes or behavioral intentions regarding trial participation.

A detailed report of these findings are given in the Final Report for the HIV Research Project - DAMD 17-88-Z-8007.

Neuropsychological Assessment:

The current objectives of the neuropsychological component of RV26 are to: 1) develop predictors of job difficulties; job performance, job satisfaction, and test whether specific cognitive profiles can predict specific job difficulties, through longitudinal assessment of neuropsychological functioning; and 2) develop a Phase I/II study to test the safety/feasibility of using cognitive profiles based on neuropsychological assessment to: a) predict job difficulties and b) define potential areas for interventions to enhance job performance and to prevent decline.

Technical approach:

Subjects have been seen at approximately six month intervals. Neuropsychological batteries alternate between visits. Battery A was administered at baseline and at odd-numbered visits. Battery B is administered at even-numbered visits. Half of the HIV negative participants began with Battery A, and half began with Battery B. On follow-up evaluations, administration alternates

between batteries, for a total of three visits (i.e., HIV negative subjects receive A-B-A or B-A-B).

A combination of standard and experimental neuropsychological measures were selected, based upon prior research at WRAMC and WHMC to: 1) provide assessment of all realms of cognitive and motor function, and 2) assess in depth those areas most likely to be affected at the early stages of HIV disease. Two alternating batteries were used, to maximize the number of measures administered while not increasing evaluation time further.

Patient enrollment:

Data collection for this component is completed. A total of 763 HIV positive and 148 HIV seronegative control subjects have been enrolled and seen for initial visits at three sites.

Patient visits:

Of the HIV positive individuals, 536 have been assessed twice, 275 three times, 120 four times, 25 five times, and 1 six times. Thirty seronegative controls have received second assessments and six have received a third assessment.

Sites:

Walter Reed Army Medical Center, Washington, DC; National Naval Medical Center, Bethesda, MD; and Wilford Hall Air Force Medical Center, San Antonio, TX.

Significant findings:

A number of findings have been reported in the period of 1 April through 30 September 1993. The effect of mood state on neuropsychological performance was examined by correlating results from neuropsychological measures with those from self-reported mood state measures. The magnitudes of statistically significant correlations were small indicating that, across the continuum of mood state, mood had little impact on neuropsychological performance. However, when analyses were limited to subjects at the extremes of mood state (few symptoms vs. many symptoms), both disease stage and mood state impacted on attention, response speed, and motor functions. Subjects at later stages or with many symptoms of anxiety or depression performed most poorly on neuropsychological measures. Thus, findings suggested that clinically significant, self-reported mood disorder may produce neuropsychological decrement, but these effects are independent of HIV disease stage.

Another set of analyses examined the types of neuropsychological difficulties observed. Factor analysis revealed six domains of functioning: coding/executive function, visuomotor/focussed attention, psychomotor speed,

language/dominant hemisphere skills, visual memory, and verbal memory. As compared to HIV negative subjects, HIV positive subjects were poorer on the encoding/executive function and psychomotor speed factors only, demonstrating impairment specificity at the early stages of HIV disease. Reaction time performance was examined in more detail, to understand underlying impairment. Again, HIV positive subjects were slower than HIV negative subjects on all measures. In addition, although HIV negative subjects improved their performance when given extra warning time before having to respond, HIV positive subjects showed no such improvement. Findings were taken to suggest that response slowing in HIV positive subjects may be due to difficulty 1) mobilizing attention initially and/or 2) initiating a response.

A final set of analyses explored self-reported occupational functioning and its relation to self-reported mood state and neuropsychological performance. As compared to HIV negative individuals, HIV positive individuals reported 1) slightly more general complaints of difficulties, but this was affected by employment status, 2) reduced productivity and fewer work demands, but less work difficulty and more responsibility, and 3) less job satisfaction. Those subjects with self-reported symptoms of distress reported more general complaints and less job satisfaction, but only HIV positive subjects with more distress reported more job difficulties. Finally, for HIV positive subjects, poorer neuropsychological functioning related most strongly to general complaints, but there was little relation to occupational performance or satisfaction. These findings suggested that HIV positive subjects were at risk for reduced job satisfaction and, to some degree, reductions in self-reported job performance. However, these changes related most strongly to mood and, when neuropsychological difficulties were reported, HIV positive subjects appeared to be able to compensate on the job. Thus, interventions that target psychosocial factors may be most likely to prevent reductions in job satisfaction or performance.

Summary of RV26: Tri-Service Biopsychosocial HIV Study

Preliminary data analyses from the Seropositive Behavior Study strongly suggest that, despite current HIV transmission reduction education programs currently implemented in the military, HIV infected individuals are continuing to engage in clinically significant levels of transmission risk-relevant behaviors. In response to these findings (and utilizing other data from the HIV Biopsychosocial Study) this Program Area is currently designing a Tri-service research intervention study, the goals of which are to develop and evaluate the safety and efficacy of four intervention technologies to reduce transmission risk-relevant behavior in military medical beneficiaries (see summary of RV82).

Data from the psychosocial questionnaires indicate that psychosocial factors may be important in facilitating adherence to

participation in vaccine trials and adherence to trial procedures.

During the period of transition to the cooperative agreement, the neuropsychological component has shifted its focus from collecting descriptive data to examining the relation between neuropsychological functioning and job functioning. Results of current data indicate that although commonly experienced symptoms of depression and anxiety do not appear to substantially affect neuropsychological performance, clinically significant levels of mood disturbance may. The impact of mood on neuropsychological functioning, however, appears to be independent of the effects of HIV disease stage. At early disease stages, HIV positive individuals manifest particular types of cognitive difficulties, characterized by slowed response speed and deficits in aspects of attention, initiation, and encoding skills. Deficits are not universal, however. Finally, there are indications that, by their own reports, some HIV positive individuals experience early changes in job performance and satisfaction. These changes relate most strongly to self-reported mood state, and HIV positive individuals appear able to compensate on the job for early, subtle neuropsychological difficulties.

RV26-"Neurobehavioral Changes In Early Human Immunodeficiency Virus Infection: Foundations for Study of Military Performance (An Addendum to the Tri-Service Biopsychosocial Study)"-

Overview:

The purpose of the addendum was to build a foundation for the measurement of possible HIV related changes in military performance.

Goals and Objectives:

This protocol addendum aims to:

- 1) demonstrate that selected computerized cognitive measures of performance will be sensitive to neurobehavioral changes associated with HIV and
- 2) link performance on these measures to self-reported job performance and satisfaction.

Technical Approach:

A subset of subjects from the main study were selected and recruited for participation. One group of individuals in the addendum was selected on the basis of having participated in an earlier neurobehavioral study at Walter Reed Army Medical Center, in order to understand the longer term effects of HIV. A second

group of individuals was selected on the basis having no pre-existing neurobehavioral disorder, in order of to understand more clearly the effects of HIV alone. Subjects were evaluated every 6 months in conjunction with regular RV-26 visits. In addition to RV-26 measures, subjects completed a brief neurological screening examination, MRI scan, lumbar puncture, computerized cognitive measures and self-report measures of occupational performance and satisfaction. Neurodiagnostic measures were included to determine whether non-specific neurological abnormalities due to HIV have any bearing on behavioral measures of performance and whether these neurological abnormalities should be used as the basis for job reclassification decisions.

Patient enrollment. Visits:

Based on scientific and logistic considerations, data collection was completed on 30 June 1993. Between 1 April and 30 June 1993, 8 evaluations were completed. At the time of study completion, 37 subjects had consented to participate; all but 7 actually completed evaluations. Thirty subjects completed one evaluation, 22 subjects completed two evaluations, and 12 subjects completed three evaluations.

Site: WRAMC

Significant Results:

Data from initial evaluations of subjects who completed lumbar puncture (N=20) have been analyzed. Subjects were divided into two groups, based upon whether cerebrospinal fluid culture was positive (CSFPOS, N=9) or negative (CSFNEG, N=11) for the presence of HIV. CSFPOS subjects had significantly higher levels of quinolinic acid, an endogenous neurotoxin previously shown to be increased in HIV positive individuals, and associated with significantly slower reaction times. Results suggested a relation between biological markers of central nervous system dysfunction (CSF HIV culture, quinolinic acid) and a behavioral measure of cognitive change. Additional analyses of follow-up visits are being planned.

RV56 - "Analysis of Sexually Transmitted Disease (STD) Patients at Ft. Bragg, NC."

Overview:

Ft. Bragg has a relatively high incidence of STDs and a well-established data collection procedures, and a captive soldier population. The baseline STD data provided by this study will be used to develop interventions to reduce HIV risk-related behaviors.

Goals and Objectives:

RV56 is designed to:

- 1) determine incidence of specific sexually transmitted diseases at Ft. Bragg, NC;
- 2) describe the epidemiological characteristics of individuals who acquire STDs; and
- 3) identify demographically and behaviorally determined groups that would be the targets of sexual history questionnaires and behavioral interventions in future protocols.

Technical approach:

All patient information collected by the STD clinic is entered into the database by clinic personnel. Information is consolidated monthly and analyzed for trends. Data is stratified demographically (e.g., age, ethnic group, gender, rank and unit) and clinically (e.g., type of infection, results of laboratory studies, treatments/medications etc.). Information is used to evaluate trends in specific STDs and will be used to monitor the effectiveness of various proposed behavioral interventions. RV56 will be expanded in the future (Addendum in progress) to provide more sophisticated laboratory analyses in order to strengthen outcome measures to assess behavioral interventions. This addendum will combine the Foundation research functions with the existing STD/HIV military components into a consolidated program to address specific clinical/laboratory and behavioral issues. Such an approach was in response to recommendations by the Armed Forces Epidemiology Board and by the World Health Organization's Global Program on AIDS, as well as being the current state-of-art in the field of STD/HIV.

Patients enrolled: 50,064

Patient Visits: All patients who are seen in the STD clinic have their clinical records recorded in the database.

SITES: EDC (STD) clinic, Womack Army Medical Center, Ft. Bragg, NC.

Significant Results:

Changes in STD trends have been noted over the last few years. These have been compared to civilian trends, and similarities have been noted. Some striking differences have emerged due to the effects of deployment on the military STD rates (e.g., post-deployment STD rates tripled). Such findings have supported the restructuring of the standard HIV/STD education classes

offered to active duty troops with a special focus on those troops that are deploying.

Summary:

RV56 and its addendum are expected to supply background information and supporting biological data necessary for the conduct of other Behavioral Prevention research intervention protocols. The database allows for the tracking of unit STD incidence rates and individual recidivism rates which will be needed as supporting outcome measures for the safety and efficacy study of behavioral interventions designed to reduce exposure risk behaviors scheduled to be conducted at Ft. Bragg.

RV62 - "Neurobehavioral Effects of Early Human Immunodeficiency Virus Infection: Foundations for Study of Military Aviator Performance"

Overview:

This protocol will serve as a pilot or small scale study that seeks to understand how HIV may affect aviation personnel performance.

Goals and Objectives:

Current goals of RV62 are to:

- 1) determine the prevalence of neurobehavioral deficits in HIV positive aviation personnel and progression of deficits over time;
- 2) clarify relations among neuropsychological changes, mood state, job satisfaction, and perceived changes in job performance;
- 3) establish a core battery of measures sensitive to early changes in performance, which may be applied to clinical screening of aviators; and
- 4) establish initial targets of intervention to minimize the effects of disease on performance and to maximize occupational longevity.

Technical Approach:

At least 15 HIV positive aviation personnel, including pilots, navigators, radar control operators, weapons systems controllers, and air traffic controllers, will be assessed for 3 years, and at least 15 demographically-matched HIV negative control subjects for one year. All subjects will be evaluated every 6 months. HIV

positive subjects will complete neurological examination, neurodiagnostic evaluation (lumbar puncture, MRI, ERP, SPECT), neuropsychological evaluation, computerized cognitive assessment, and standardized, validated self-report questionnaire measures of depression, anxiety, job performance, job satisfaction, and awareness of changes in function. This is a comprehensive evaluation, designed to detect abnormalities most likely to impact on performance, particularly changes in attention, response speed, motor skills, visuospatial skills, and learning/memory. Neurodiagnostic measures are included to determine whether non-specific neurological abnormalities associated with HIV have any bearing on behavioral measures of performance, and whether these neurological abnormalities should be used as the basis for job reclassification decisions. HIV negative subjects will receive all measures, except lumbar puncture. Yearly evaluations include all measures. Intervening 6 month evaluations include neurodiagnostic evaluation and a subset of neuropsychological measures.

Patient Enrollment:

Data collection began in August, 1993. Two HIV positive subjects and 1 HIV negative control subject have been enrolled and have completed baseline evaluation. Nine additional HIV positive subjects have been scheduled for baseline evaluation.

Patient Visits: 3

Site: National Naval Medical Center

Significant Results: Not Applicable

Summary:

Collaborative relationships have been established; patient assessment has begun. Additional HIV seronegative control subjects are being recruited with assistance from a number of sources, including Air Force Office of the Surgeon General, Air Force School of Aerospace Medicine, and Naval Aerospace and Operational Medical Institute.

Much of the work and data collected from the protocols described above are currently being utilized to design new protocols focused on behavioral interventions. These pending protocols are described in detail below:

RV82--Phase I/II Study of Safety, Behavior Responsiveness, and Efficacy of Behavioral Interventions in HIV Seropositive Military Medical Beneficiaries-

Overview:

RV82 will develop and test the safety and efficacy of four innovative intervention strategies designed to reduce HIV transmission risk behavior by HIV infected military medical beneficiaries. This protocol represents the kind of change in the focus of research efforts that the program has undertaken during the new cooperative agreement. The protocol has been approved by the Retroviral Clinical Research Committee (RC)2 and the interventions are currently being developed.

Goals and Objectives:

RV82 will develop and evaluate innovative behavioral intervention formats designed to reduce HIV transmission by military medical beneficiaries already infected with HIV. Further, it will provide information concerning the optimal dose and schedule of interventions for achieving cessation (or reduction) in HIV transmission risk relevant behaviors.

Technical Approach:

Based on the research findings from the Tri-service Biopsychosocial HIV Study (RV26), 5 key areas were identified as foci for intervention. These include:

- 1) facilitating the disclosure of one's serostatus, particularly before any sexual activity occurs;
- 2) increasing the practice of nonpenetrative sexual activity with HIV uninfected partners and substituting these activities for penetrative acts;
- 3) enhancing one's ability to initiate and complete discussion of safer sexual practices with potential sexual partners (including communication of a preference for abstinence);
- 4) increasing one's social network and repertoire of nonsexual activities;
- 5) finding more effective ways to deal with stress (this includes reducing drug and alcohol consumption, particularly in situations which might lead to sexual activities).

These five content areas will be addressed in each of the interventions. Pretest and outcome measures will be adapted from the Seropositive Behavior Survey. Those factors statistically

found to be critical in predicting transmission risk behavior will be assessed prior to the intervention, after the intervention, and at a 6 month follow-up period. In addition, factors hypothesized to be important in the change process such as readiness for change, ability and perception of one's ability to perform prescribed behaviors, and mood states (e.g., anxiety, depression) are included in the assessment measures. The four intervention formats are described below:

1) The interactive videodisc for seropositive individuals ("Challenges") produced for HIV infected military personnel by the Center for Interactive Media in Medicine (CIMM) at USUHS for Phase I intervention part of this study, the interactive videodisc will be assessed for safety in the context of RV72. Should that study find it to be safe intervention with appropriate and promising behavioral responsiveness, it will be incorporated into the later phases of RV82.

2) The audiotape intervention is a fifty minute audiotape which combines actualities by HIV+ individuals with narration. Its advantages include the ability to use HIV+ peers to whom patients can relate without compromising confidentiality, its relatively low development costs, its low technology requirements, its ease in distribution, and its ability to be easily revised, updated, or adapted for special segments of the population.

3) The Behavior Risk Group intervention is a three session set of "modules" that deal with the 5 areas previously described. It is led by two facilitators and lasts approximately 2 hours per session. In a highly interactive context, individuals participate in the group and complete exercises designed to heighten awareness of "automatic" scripts that often culminate in risky situations, and to learn new social scripts that can decrease transmission risk. The advantages of this intervention format include the interactive mode, the ability to modify content to meet the individual concerns of the group's participants, and the ability to practice behaviors and receive feedback from peers and trained facilitators. The potential disadvantages are the need to have facilitators trained to conduct the modules, the time involved by participants, and the ability to conduct this group only at installations with at least 4 or 5 HIV infected individuals (thereby eliminating use at smaller installations).

4) The Health Risk Appraisal (HRA) capitalizes on a format that is already used and familiar to military personnel. A specific HIV risk appraisal has been developed which mirrors the format of the HRA utilized by most preventive medicine services. The content for the HRA is based on the data determined by RV26 to be related to HIV transmission risk behavior. A computer profile of risk along with individualized messages for the respondent will be generated for each individual. Individualized counseling to elaborate and reinforce messages and to offer alternative behaviors will accompany the computer profile.

Individuals will be randomized to one of the four interventions or to the standard HIV Program being conducted at the individual's medical center. Education assessment will be conducted at baseline, post intervention assessment, and 6 month follow-up. Based on the findings from this Phase I/II study, the study will be expanded to include administration of combined interventions (e.g., an individual might receive the audiotape in the context of a group) and testing on a broader scale (Phase III).

Patients Enrolled:

None. Protocol is in the review process.

Sites:

Tentatively, HIV infected individuals will be recruited for this protocol from the D.C. Area Site (NNMC and WRAMC), and Balboa Naval Hospital in San Diego.

Significant Findings: None to date.

Summary:

Execution of RV82 will provide information about the safety and efficacy of four potentially efficacious behavioral intervention techniques for the cessation and reduction of HIV transmission risk relevant behaviors. The protocol has been submitted for final scientific review in November, 1993.

RV81-"Prevention of Exposure to HIV and Other Sexually Transmitted Diseases (STDs) in a Seronegative Military Population: A Comparative Study of the Safety and Efficacy of Intensive STD/HIV Prevention Interventions (Phase I/II)"

Overview:

RV-81 is an HIV exposure risk reduction preventive intervention directed at a high risk population (STD clinic patients). It represents another of the Behavioral Prevention Program Area's major shifts in direction towards interventions. Its focus on risk reduction behavior in those at high risk for HIV or other STD exposure is entirely consistent with the recommendations of the Armed Forces Epidemiological Board report in March, 1993.

Goals and Objectives:

The primary objective of this study is to examine the relative safety, feasibility, behavioral responsiveness, and effectiveness of several innovative preventive intervention strategies designed to reduce exposure to HIV and other STDs in a population at high risk for HIV infection (active duty military STD clinic patients). In addition, this protocol will evaluate the immediate impact of interventions on compliance with the standard STD recommendations (e.g., return for test of cure, partner notification), as well as risk related knowledge, attitudes, perceptions of risk, and behavioral intentions to change; and 2) describe demographic, attitudinal and behavioral characteristics of individuals resistant and/or susceptible to behavior change, in order to target programs more effectively.

Technical Approach:

This protocol will involve comparing standard STD clinic care with three experimental interventions: a videodisc preventive education format combined with counseling individuals, targeted behavioral intervention, and a health risk appraisal. The content of the videodisc intervention was designed expressly for HIV prevention in the military. The targeted behavioral intervention will focus on helping patients identify and change their behavior in situations associated with increased exposure risk (e.g., partners who pose obvious high risk, situations where excessive alcohol use takes place). The health risk appraisal will build on existing appraisal systems for general health and provide individualized feedback about current risk status and related behaviors which need change. The targets of intervention (HIV risk behaviors and associated factors such as alcohol abuse, attitudes about HIV prevention, etc.) were selected through analysis of the Army Wide AIDS Survey, which surveyed HIV exposure risk-relevant behaviors, attitudes, knowledge, and situations among a representative sample of over 18,000 soldiers world wide.

The study will utilize a short term pre/post design, in which baseline measures are obtained after diagnosis of a Center for Disease Control (CDC) reportable STD in the Womack Army Medical Center Evaluation and Diagnostic Clinic, and post test will be conducted at a two-week follow-up visit. Additional follow-up will be conducted at two months post-baseline, largely to test the feasibility of different follow-up strategies and the possibility of doing follow-up intervention ("booster") sessions in subsequent studies. RV-81 is viewed as an initial step in providing a large scale exposure preventive intervention to the STD population. Additional studies are planned to incorporate and refine the interventions which prove successful under this protocol.

Patients Enrolled:

None (Protocol was submitted for final Tri-service scientific review in November, 1993)

Site:

Womack Army Medical Center, Fort Bragg, NC.

Significant Findings: None to date

Summary:

This new protocol will permit comparison of innovative prevention techniques with a high risk population. It is anticipated that, as soon as the protocol is approved by the Tri-service and Fort Bragg human subjects reviews, it should be operational in early 1994.

RV72-"Using Interactive Media to Promote Responsible Sexual Behavior in HIV-Positive Personnel"-

Overview:

RV-72 is a preventive transmission risk reduction intervention protocol designed for HIV seropositive active duty military personnel. As such, it is an assessment of a specific innovative technology, the interactive videodisc. This protocol was submitted for scientific review in the Fall, 1993.

Goals and Objectives:

The protocol seeks to evaluate the immediate impact of the videodisc in terms of changes in knowledge, attitudes, and behavioral intentions. It also will evaluate the longer term impact of the video in terms of reported behavior and incidence of STDs. Finally, there will be an evaluation of the process aspects of the video in terms of knowledge acquisition and application of strategies taught in the video.

Technical Approach:

The protocol compares standard HIV medical center educational activities with the combination of those activities and exposure to interactive video materials which have been designed especially for HIV seropositive military personnel by the Center for Interactive Media in Medicine at USUHS. Pre and post video measures will be completed by those in the "video" condition. A composite instrument (i.e, eliminating redundancies) will be completed by those in the "no video" condition. All assessment instruments have been adapted from the Seropositive Behavior Survey, an instrument that was used to collect data on risk relevant knowledge, attitudes, and behavior from seropositive military medical beneficiaries.

Patients Enrolled:

None (Protocol has provisional Tri-service scientific approval pending completion of the outcome measure)

Sites:

Walter Reed Army Medical Center, Washington, DC; National Naval Medical Center, Bethesda, MD; Womack Army Medical Center, Fort Bragg, NC.

Significant Findings: None to date

Summary:

This new protocol will permit evaluation of innovative intervention technology with standard HIV education in a population at high risk for HIV transmission. The interactive character of the video allows viewers to guide some of the action, respond to questions posed by actors in the video, and receive feedback concerning their responses to video-initiated questions. It promotes more active learning than other media and has been found to be an acceptable modality in pretest focus groups. This approach is viewed as being important in helping personnel better assess their transmission risk.

RV76-"The Use of Interactive Media to Reduce the Risk of HIV Exposure in United States Air Force Basic Trainees"-

Overview:

This protocol is designed to evaluate the effectiveness of two different large-group interventions in reducing the risk of HIV exposure in seronegative military personnel (participants in Basic Military Training (BMT) at Lackland Air Force Base in San Antonio, Texas). The interventions include a group administration of an interactive videodisc (IAVD) and a traditional lecture utilizing comparable content.

Goals and Objectives:

RV76 will evaluate the effectiveness of existing intervention technologies in changing behavioral intentions and attitudes, and ultimately, in changing HIV exposure risk-relevant behaviors of a general military (Air Force) population.

Technical Approach:

Two different, large/group interventions -- an interactive videodisc versus a more traditional lecture -- are compared against a standard (no treatment) control condition. Participants will be assessed prior to exposure to the intervention, again after the intervention and at a 2 month follow-up. The purpose is to evaluate both the short and intermediate term effects of these preventive interventions in reducing specific risk factors for exposure to HIV. Focus groups were conducted with representative groups of basic trainees to solicit their feedback and to guide our modifications of the intervention. The assessment measure, which incorporates those items from the AWAS found to increase the odds of exposure to a STD/HIV, will assess changes in HIV related knowledge, attitudes, beliefs, and behaviors as well as in various biological markers such as rates of sexually transmitted diseases.

Patients Enrolled:

None

Patient Visits:

None

Sites:

Lackland Air Force Base, San Antonio, TX.

Significant Findings: None to date

Summary:

RV76 received final scientific approval as a single center study on November 25, 1992; plans concerning the reorganization delayed funding until mid-1993. The interventions and assessment measure have been developed. Over the next 2-3 months, a small-scale (N=240) pilot study will be conducted with United States Air Force security police and law enforcement trainees before proceeding with the full scale intervention with the much larger basic trainee group planned for early in 1994.

RV89-"Obstetricians-Gynecologists' (OB-GYN) Affective Reactions to Uncertainty and Discussing HIV/AIDS during Routine Gynecologic Care"-

Overview:

This study will measure the influence of affective reactions of OB-GYNs to clinical uncertainty, and patient, physician, and organizational characteristics, on the inclusion of HIV/AIDS educational prevention activities as a normal component of routine gynecologic care.

Goals and Objectives:

RV89 will seek to determine how affective factors, particularly anxiety due to uncertainty, may influence OB-GYN's HIV screening and patient education efforts.

Technical Approach

The research will compare the number of HIV-related educational activities respondents may select in response to two written case simulations (WCS) with their scores on the "Anxiety Due to Uncertainty" (ADU) subscale (Pearse et al., 1982) and responses to measures of patient, physician and organizational factors. Linear relationships between the number of activities selected and the factors that may affect selection will be examined first. Following this, interactions among ADU scores and patient, physician and organizational factors will be examined to determine whether ADU scores will vary as a function of these factors and influence the number of activities selected.

Approximately 150 Army OB-GYNs from posts nationwide will be asked to complete a 5-section, 53-item, self-administered questionnaire. Obstetricians-gynecologists were selected for the study because they: 1) serve as primary care providers and specialists to several million U.S. women; and 2) are in an excellent position to provide HIV/AIDS education as part of their regular clinical duties. The first four sections of the questionnaire provide information about the factors under study:

- (1) Demographics,
- (2) Prevention knowledge with respect to STDs/HIV and other prevention practices,
- (3) Attitudes, training and practice related to HIV prevention; and
- (4) the ADU subscale to measure respondents' anxiety due to clinical uncertainty.

The final section consists of WCS of two hypothetical patients, one considered at "high risk" for HIV, and the other considered at "low risk". Respondents will be asked to read each WCS, and then select any or all of 12 HIV related educational activities listed that they would perform in response to the WCS. Questions related to how comfortable and confident respondents feel in performing each activity are included in the 3-part response set.

Patients Enrolled: 0

Patient Visits: 0

Site: Not applicable

Summary:

RV89 was developed to address two important areas that had not yet been addressed by the Behavioral Prevention Program: women and primary health care providers. The study received approval from the Retroviral Clinical Research Committee and tentative scientific review approval in November, 1993. Results from the study can provide data to OTSG to develop effective educational and training programs which ultimately may further an objective of the Behavioral Prevention Program Area: to reduce the rate of exposures to HIV among uninfected military medical beneficiaries.

Finally, working with the Vaccines for Preventions Program Area, the Behavioral Prevention Program Area is developing the behavioral component that will accompany the vaccine trials in Thailand. These efforts will be conducted under the auspices of an addendum to RV69. The proposed addendum is described below.

RV69 addendum-"Behavioral Addendum: A Phase I Study of Safety and Immunogenicity of a Recombinant Envelope-Based Product in Human Immunodeficiency Virus (HIV) Seronegative Thai Volunteers: Behavioral Side Effects and Behavioral Factors in Study Recruitment, Participation, and Adherence"-

Overview:

The RV-69 Behavioral Addendum is designed to support recruitment, enrollment, adherence, and risk factor management for the Thailand seronegative vaccine trial. As such, it reflects the Behavioral Prevention Program Area's increased emphasis on preventive activities and its efforts to collaborate with other Program Areas.

Goals and Objectives:

To develop and evaluate methods for the recruitment, enrollment, and retention of protocol participants, as well as develop and evaluate behavioral counseling programs to prevent adverse behavioral side effects, and support protocol participation and adherence.

Technical Approach:

The protocol includes three substudies, each having a somewhat different focus and methodology:

Study 1: Participation Study:

This is a cross-sectional study and will use analysis of variance (ANOVA) to examine how different levels of willingness to participate are related to major variables such as social discrimination, benefits to self, and demographic variables such

as sex and education. Scales created in the pilot study will be examined in terms of their robustness in this study with respect to parameters such as internal consistency and external validity.

Study 2: Risk Behavior Study:

Risk behavior will be collected in a longitudinal manner and will be analyzed using repeated measures analysis of variance (ANOVA) techniques. The major questions of interest are the degree and direction of change over time, as well as factors associated with those changes over time. Factors such as mood, coping style, and social influences have been predictive of risk behavior in past research with Western populations. There is some indication from cross-sectional ethnographic analysis of Thailand populations that some of these variables may be useful predictors in the vaccine study population. Areas where risk behavior becomes frequent or does not change over time may be targets for intensified behavioral intervention. The content of contemporaneous risk behavior counseling will need to be considered in interpreting the output from this study.

Study 3: Adherence Study:

Adherence data will be collected longitudinally and analyzed in a repeated measures ANOVA format, where possible. If frequencies of noncompliance are low, then it will be necessary to employ nonparametric techniques. As in previous research, the general goal of the analysis will be to evaluate correlates of adherence, with this information used to change modifiable aspects of the vaccine program. Changes in program procedures will need to be noted over time to the extent that they may moderate change in behavior over time.

Patients Enrolled:

This Protocol was approved by MMCARR's (RC)² in October, 1993.

Patients Visits: None

Sites: Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Significant Findings: None

Summary:

The proposed protocol will provide data to support operational aspects of the joint USA/Royal Thai Army (RTA) vaccine trials in Thailand. It will provide information for recruitment of participants, as well as input for programs to discourage HIV risk behavior and noncompliance with study procedures.

CONCLUDING REMARKS:

The Behavioral Prevention Program Area has continued its outstanding performance in documenting the behavioral aspects of HIV exposure and transmission potential and initiating the design of effective behavioral interventions to prevent HIV exposure and transmission. This Program Area has also contributed its behavioral expertise to other Program Areas in the conduct of Vaccine Therapy and Drug and Gene Therapy protocols. Most notably, in May 1993, the Scientific Director of this Program Area was invited by the World Health Organization in Geneva, Switzerland to serve as a short-term consultant in leading a technical working group on analyzing the socio-behavioral aspects of HIV Vaccine Trials in developing countries, and preparing a document on this topic.

In addition to developing a number of new protocols to address MMCARR HIV prevention and intervention objectives over the last 6 months, the Behavioral Prevention Program has been extremely productive in terms of scientific publications and presentations. A complete listing of 17 published papers, 20 abstracts, and 2 scientific and military reports in which these data were presented during the report period of 1 April, 1993 to 30 September, 1993 follows the text of this section.

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**BEHAVIORAL PREVENTION PROGRAM
PRESENTATIONS, POSTERS, and PUBLISHED ABSTRACTS
(PY1/FY-93)**

MILITARY

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BEHAVIORAL PREVENTION PROGRAM
ACCEPTED ABSTRACTS: PRESENTATIONS, POSTERS
(PY1/FY-93)

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DRUG AND GENE THERAPY

**Program Area Coordinator: CAPT Douglas Mayers, MD, MC,
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Foundation Scientific Director: Daniel St. Louis, Ph.D.

**Assistant Department Chief: LTC Arthur Brown, MD, MC,
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Program Area Summary: This program area concentrates on exploring promising anti-HIV drugs for clinical efficacy trials. A recent new focus in this area is on the development of efficient gene delivery systems to be ultimately used in early stage treatment of HIV.

Human Use Protocols

Protocol #	Protocol Title (Abbreviated)	Principal Investigator
RV3	Early Rx AZT	Hawkes
RV28	Pharmacology Database	Cortese*
RV43	Prospective Emergence/AZT	Mayers
RV60	Alferon N (completed)	Skillman
RV65	Prospective Study of Oral U-87201E (Completed)	Mayers
RVOI6	WR6026 - Phase I (Completed)	Hendrix
RVOI8	Prophylaxis of MAC	Berman
RV79(P)	Codon 215 - ACTG 244	Mayers
RV88(P)	Protease Inhibitor	Mayers

LABORATORY WORK UNITS/PROJECTS

Project Title (Abbreviated)	Principal Investigator
Detection of HIV-1 Infected Cells by Flow Cytometry	Vahey

Detection of HIV-1 Cells..PCR and Flourescent
in situ hybridization

Vahey

Viral Burden - RNA and DNA
PCR analysis

Vahey

Molecular and Genetic characterization of
MoMuLV and HIV-1

Prophylactic and Immunotherapeutic Vaccine Strategies (1&2) St. Louis*

Assessing anti-HIV antiviral gene therapy strategies

St. Louis

Assessing anti-HIV antiviral strategies in hematopoeitic
stem cells

Mosca*

(P) = Pending Protocol

*Foundation Principal Investigator (PI)

OVERVIEW:

The HIV Drugs and Gene Therapy Program has two primary components. The first component, the HIV chemotherapy/chemoprophylaxis group, in collaboration with other government agencies and private industry, employs promising anti-HIV drugs for clinical efficacy trials in military HIV infected populations. In conjunction with the AIDS Clinical Trial Group (ACTG), this group has developed an HIV susceptibility to antiviral drug assay to examine both the potency of promising new antiviral drugs and to monitor the susceptibility to drug of virus derived from patients on therapy. The Cytopathology lab and the Flow Cytometry group are recent additions to this component and Program Area. This group has been refocused to determine and validate surrogate markers for disease progression and to determine if these markers can be used to monitor efficacy of drug or gene therapies administered to patients. Efforts within this group are assigned to the Chemotherapy/Chemoprophylaxis Work Unit and the Cellular Pathology Work Unit (Section II). The Laboratory and Clinical Research Protocols which comprise this unit are described in detail below, as well as significant findings and accomplishments.

Within the second component, the Gene Therapy group is working to develop efficient gene delivery systems capable of transducing and expressing genes encoding HIV and SIV antigens to immunize uninfected individuals or augment immune responses in infected individuals and anti-HIV/-SIV antivirals to treat early stage HIV infected personnel. This work unit is described in Section III of this Program Area report.

RESEARCH GOALS AND OBJECTIVES:

I. Chemotherapy /Chemoprophylaxis Work Unit

The major focus of the Chemotherapy/Chemoprophylaxis Work Unit is to conduct clinical trials with chemotherapeutic agents directed against HIV. The goal of these trials are to prevent progression of HIV disease in patients with early stage disease. This work unit will continue to evaluate promising antiviral drugs in an *in vitro* assay system and determine which drugs will be used in future clinical trials. Major objectives of this unit are:

- 1) Develop chemotherapeutic strategies to prevent progression of early stage HIV disease with an emphasis on combination chemotherapy.

- 2) Develop assay systems to monitor drug agents and determine the clinical significance of *in vitro* drug resistance.
- 3) Evaluate the molecular mechanisms of drug resistance and develop rapid assays for drug resistant HIV.
- 4) Target strategies to prevent the emergence of drug resistant HIV variants or treat patients with drug resistant strains, and evaluate agents for use in chemoprophylaxis of HIV transmission after high risk exposure.
- 5) Determine surrogate markers of disease progression and use these markers for following the course of treatment.

To achieve these objectives, there are several ongoing or planned chemotherapy trials (protocols):

RV43--"Prospective study of the emergence of Zidovudine (AZT) resistance in patients infected with the human immunodeficiency virus (HIV) who are treated with AZT"--

Clinical Site(s): Walter Reed Army Medical Center (WRAMC) National Naval Medical Center (NNMC)

Number of Patients Enrolled: 100

Description/Summary of Protocol:

A cohort of 100 patients with CD4 counts <400 cells/mm³ on zidovudine monotherapy is evaluated every three months for a period of 3 years. Patients have a screening history and physical exam along with T cell subsets, p24 antigen and drug level determination. HIV isolates at each time point are evaluated for susceptibility to AZT, ddI and ddC, along with syncytial phenotype. Multiple aliquots of plasma and peripheral blood mononuclear cells (PBMC) are stored from each time point for further studies. The objectives of the study are:

1. To determine the time course, frequency and clinical parameters associated with the development of AZT resistance in HIV isolated from patients on AZT.
2. To determine if there exists a level of AZT resistance, measured *in vitro*, which correlates with clinical deterioration in patients who are receiving AZT.
3. To develop a repository of frozen HIV infected PBMC with resistant virus for future studies into the molecular basis of dideoxynucleotide resistance.

Number of Patient Visits to Date: 654

Significant Findings:

- Several factors predict the early emergence of AZT resistant virus including:
 1. Low CD4 cell counts at initiation of AZT therapy
 2. P24 antigen positive status
 3. Presence of a syncytium inducing (SI) HIV isolate
 4. Plasma HIV RNA > 10⁵ copies/ml
- CD4 cell counts remain stable in patients with AZT-susceptible virus for periods as long as 4 years.
- CD4 cells decline by 120 cells/mm³ in the year preceding the emergence of *in vitro* AZT resistance (defined as IC₅₀ > 1μM AZT).
- Mutations in reverse transcriptase codon 215 can be detected by polymerase chain reaction in patient plasma or PBMC at a time when the patient's CD4 count remains near baseline from the start of therapy.
- After the emergence of a population of HIV with mutant codon 215, 50% of patients will drop their CD4 count by > 50% from baseline, and 25% will have CD4 cell counts < 50 cells/mm³.

CRV-7: *Prospective collection and banking of lymphocytes and clinical data on HIV infected individuals taking antiretroviral agents.*

Clinical Site: Fitzsimons Army Medical Center (FAMC)

Number of Patients Enrolled: 532

Description/Summary of Protocol:

This study is an extension of a previous phase II study of AZT therapy in 220 patients followed by investigators at Fitzsimons Army Medical Center and Denver Health and Hospital. Samples of patient sera and cells are collected every 6 months and stored in a central repository. The systematic collection of clinical data and specimens from

patients on prolonged antiretroviral therapy (periods exceeding 5 years) has made the repository at Fitzsimons an invaluable resource for drug resistance studies being conducted at WRAIR.

Significant Findings:

- A case control study of patients on AZT monotherapy demonstrated the association of AZT resistance with clinical decline. A manuscript of this study is in preparation.
- A natural history study of the entire cohort of patients showed that initiation of AZT therapy delays Walter Reed stage progression by approximately 500 to 800 days, but subsequent stage progression occurs at similar rates in untreated patients and patients progressing on therapy.
- A set of samples obtained from recent HIV seroconverters in the cohort is being validated for use in a retrospective study of the incidence of transmission of AZT resistant virus.

RV65--"Prospective open label study of the emergence of drug resistance in patients infected with HIV-1 taking oral U-87201E"--

Summary:

Six patients were given U-87201E to determine: the time course of development of resistance to U-87201E in patients who had AZT resistance (*in-vitro*); genotypic changes in HIV reverse transcriptase associated with resistance to U-87201E; the genotypic and phenotypic effects of treatment with U-87201E on the alterations of the HIV-1 virus population associated with *in vitro* AZT resistance; whether several passages of patient pre-drug HIV isolates in the presence of U-87201E will generate the resistant mutants that may subsequently emerge in the patients.

Significant Results:

All six patients were enrolled and started on U-87201E. Of the six patients started on medication, four developed a maculopapular skin rash between the 11th and 15th day in addition, one of the four had fevers, myalgias and mild LFT elevations; one patient developed renal toxicity and drug was held until renal biopsy was done. Before drug could be restarted, his HIV-1 virus was found to have developed resistance to U-87201E and per protocol the drug could not be continued; the last patient developed a lymphoma brain tumor

while on therapy and drug was stopped as he was having numerous neurological complications secondary to the tumor. The investigators wanted to re-challenge two patients that developed a rash with U-87201E, but by time approval was obtained, patients were either on other therapies or reluctant to enter the trial.

RVOI6-"Escalating Multiple-dose Safety and Tolerance of WR 6026 Hydrochloride in HIV Infected Patients"-

This protocol was conducted at Wilford Hall Medical Center (WHMC) in San Antonio, Texas. WR 6026 is a 8-aminoquinoline compound with promising activity against pneumocystis carinii. The study objectives were:

- 1) to determine the maximum tolerated dose of WR 6026 in HIV infected patients;
- 2) to determine whether any unexpected toxicities were caused by WR 6026 in HIV infected patients;
- 3) to determine whether there was additional toxicity when WR 6026 is given for 21 days rather than 14 days;
- 4) to further investigate the pharmacokinetics and pharmacodynamics of WR 6026

Significant Findings:

The clinical phase of this study was completed in July, 1993, with pharmacokinetic and safety data analysis in progress at the NIH. Sixteen of 20 enrolled patients received study drug at the WHMC site. The maximum tolerated dose was defined as 120mg with a significant number of subjects developing methemoglobinemia at 150mg. WHMC toxicities included two subjects developing a rash with no residual sequelae. An abstract was accepted and presented at the IXth International Conference of AIDS, Berlin 1993.

RVOI8-"A Double-Blind, Placebo Controlled, Parallel Group, Multi-center Study for the Prophylaxis of Mycobacterium Avium Complex with Azithromycin in HIV Infected Patients" -

This protocol is a twenty-four month prophylaxis study using a weekly dose of Azithromycin as possible protection against Mycobacterium Avium Complex (MAC). HIV infected patients with CD4 counts <100 were randomly assigned to placebo or azithromycin (1200 mg weekly) groups. Patients are evaluated monthly for the major endpoints: mycobacteremia, other bacterial infections, signs/symptoms of drug toxicity.

Significant Findings:

Over 70 patients at eight sites have been enrolled and some patients have already reached endpoints in this double blind study. The eight sites are:

- Eisenhower Army Medical Center, Ft. Gordon, Georgia
- Fitzsimons Army Medical Center, Aurora, Colorado
- Walter Reed Army Medical Center, Washington, DC
- Wilford Hall Medical Center, San Antonio, Texas
- National Naval Medical Center, Bethesda, Maryland
- Brooke Army Medical Center, Ft. Sam Houston, Texas
- Naval Hospital at San Diego, California
- Womack Army Medical Center, Ft. Bragg, North Carolina

Although this protocol continues to be a low priority protocol for the MMCARR, clinicians are in agreement to continue the current patients on protocol; no new patients will be recruited.

RV79-"A blinded, randomized trial comparing Zidovudine (ZDV) vs ZDV + ddI vs ZDV + ddI + Nevirapine in asymptomatic patients on ZDV monotherapy who develop a mutation at codon 215 of HIV reverse transcriptase in serum/plasma viral RNA"-

Clinical Site(s): National Naval Medical Center, Bethesda
 Balboa Naval Hospital, San Diego
 Walter Reed Army Medical Center
 Wilford Hall Medical Center

Total Number of Patients to be Enrolled: 150 DoD and 150 ACTG

Description/Summary of Protocol:

A cohort of 300 asymptomatic patients on ZDV monotherapy with CD4 counts 300-600 cells/mm³ will be followed for the emergence of ZDV resistance determined by detection of a mutation of reverse transcriptase at codon 215 in plasma viral RNA. After detection of the 215 mutation, patients will be randomized to ZDV, ZDV + ddI, or ZDV + ddI + Nevirapine and followed for subsequent CD4 decline. Primary objectives of the study are:

1. To validate that alteration of codon 215 of reverse transcriptase in plasma virus precedes the increase in viral burden and decline in CD4 count associated with clinical failure on Zidovudine monotherapy.

2. To determine whether alternative regimens of antiretroviral agents alter the course of viral burden increase and CD4 decline associated with clinical failure on Zidovudine monotherapy when treatment is changed on the basis of plasma RNA PCR results.

Additional (future) Number of Patient Visits Projected to Completion of Protocol: 2700 (DoD)

Significant Findings:

This is the first joint chemotherapy trial between the ACTG and the MMCARR. This study offers the possibility of rationalizing ZDV therapy by the use of plasma RNA PCR to detect emergence of ZDV resistant virus prior to modification of antiretroviral therapy. The study will prospectively relate emergence of ZDV resistance, viral burden and syncytial phenotype with CD4 decline to address the relationship of viral parameters to clinical decline. This study was designed based on the results from RV43 and should open in late 1993.

RV88- "A safety, tolerance, pharmacokinetic and pilot efficacy study of multiple doses of DMP 323 in HIV-infected patients with AZT active control"-

Clinical Site(s): National Naval Medical Center, Bethesda
 Balboa Naval Hospital, San Diego
 Walter Reed Army Medical Center
 Wilford Hall Medical Center

Total Number of Patients to be Enrolled: 48

Description/Summary of Protocol:

This is a double-blind, randomized, comparative agent controlled, multiple escalating dose trial of DMP323 (an oral HIV protease inhibitor) versus AZT in 48 patients with CD4 counts ≤ 500 cells/mm³ and p24AG ≥ 35 pg/ml. Patients will either receive DMP323 250mg TID, 500mg TID, 750mg TID, or AZT 200mg three times a day. One half of the patients on each arm will have a history of virus evaluated as resistant to AZT. Patients will be observed for up to 8 weeks and offered a 24 week open label treatment extension with DMP323. The objectives of this trial are:

1. To determine the pharmacokinetic, tolerability and safety profile of DMP 323 upon administration of

multiple ascending doses to HIV infected patients and in combination with AZT.

2. To determine doses of DMP 323 which produce measurable antiviral activity.
3. Prospectively survey for the emergence of *in vitro* resistance to DMP 323.

Significant Findings:

This study is on hold due to formulation problems with the drug. The company may switch to a second generation compound for use in a proposed trial in the summer-fall 1994.

II. Cellular Pathology Work Unit

The lack of reliable surrogate markers of HIV disease progression hinders the ability to assess the effectiveness of interventional agents such as chemotherapeutic drugs. The primary mission with this unit is to determine reliable surrogate markers and validate these markers in material derived from patients on chemotherapy. Two protocols have been designed to fulfill this mission. At the time of this report, these protocols were in the final stages of protocol approval and were slated to begin in the fall of 1993.

RV77-"Rectal Mucosal and Lymph Node Biopsy of Early Stage HIV-Infected Patients"-

RV78-"Pharyngeal and Lymph Node Biopsy of Early Stage HIV-Infected Patients"-

Background:

The ability to evaluate therapy in HIV-infected individuals has been hampered by the lack of a suitable animal model or *in vitro* assay capable of reflecting the complex interplay within a human biologic organ system. Additionally, in early disease, HIV staging systems do not accurately reflect prognostic significance of Walter Reed stage 1/2 patients.

Objectives:

Our laboratory will utilize human lymphoid follicular tissue as a model to refine early HIV staging and to monitor therapeutic trials for MMCARR investigators. The researchers plan to do this by identifying surrogate markers which are prognostic and capable of assessing intervention therapies. Both surrogate markers and viral burden will be quantitated by cell type and tissue compartment of lymphoid tissues.

Preliminary Findings to date:

The researchers presented the immunologic profile and viral distribution between two Walter Reed Stage II (WR II) matched nodes and a noninfected, hyperplastic lymph node. A unique feature of this study is the performance of quantitative image analysis (IA) on tissue sections in the evaluation of the immunophenotypic profile. A significant finding is the increase in CD8 positive cells within the germinal centers (GC) of infected nodes. There was a 9-fold increase in the CD8 effector cells over the noninfected hyperplastic node. Follicular areas, which serve as an HIV reservoir, were increased 27% over control tissue when measured by image analysis.

Lymph nodes from HIV positive, WR II patients with a pathologic diagnosis of Persistent Generalized Lymphadenopathy demonstrated differences in the pattern of HIV viral distribution. The variant patterns suggest an altered evolution of disease progression in stage-matched patients. Increases in individual cells positive for HIV expression were noted in the germinal centers and interfollicular areas of one node. This node also showed involutional changes of the GC. The node lacking involutional features showed a diffuse pattern of HIV expression throughout the GC with less individual cell positivity. This pattern of infection suggests an HIV distribution associated with dendritic reticulum cells.

This study indicates the need for more refined surrogate markers in early stage HIV disease. Monitoring CD8 infiltrates and viral burden within tissue compartments of the lymph node may serve as useful adjuvants to prognosticate disease progression and evaluate treatments of HIV infected patients.

"Development of PCR in situ Hybridization for the Detection of HIV pro-virus"-

Background:

Existent technology is unable to detect provirus in tissue sections. While *in situ* hybridization reflects the relative amount of replicating virus, as demonstrated by mRNA product, this does not accurately assess the amount of integrated HIV viral burden in lymphoid tissues. The development of PCR *in situ* hybridization will allow morphologic assessment of the localization of virus and the

relative amount of virus as a means to monitor patient response to therapy or natural progression of HIV disease.

Objectives:

The researchers will attempt to develop this technology for diagnostic assessment of provirus or other surrogate marker. The localization of the surrogate, will be evaluated to include provirus, by tissue compartment and cell type in human lymphoid elements.

Progress to date:

Background investigations started in March of 93, approval and equipment purchase in September 1993, with initial runs starting in Sept 93.

"Detection of HIV-1 Infected Cells by Flow Cytometry"-

Description of Project:

A sensitive Flow Cytometric technique has been developed by this Program Area enabling the detection of HIV-1 infected cells actively expressing viral mRNA and the ability to distinguish the phenotype of these cells. This technique requires labeling HIV-1 DNA with a nucleoside analogue containing digoxigenin, then hybridizing the labelled probe to fixed, permeabilized HIV-1 infected or uninfected cells, followed by detection of the hybridized probe using a fluorescent labelled, anti-digoxigenin monoclonal antibody. The Flow Cytometer is used to quantitate and sort the fluorescently labelled HIV-1 infected cells hybridizing the labeled probe. This *in situ* hybridization technique can be used in conjunction with fluorescently labelled antibodies directed against cell surface markers to identify the phenotype of the infected cells in PBMC or lymph nodes.

Objectives:

1. To develop Fluorescent *In Situ* Hybridization (FISH) and Flow Cytometry as a method of rapidly quantitating the number of HIV infected cells actively producing mRNA in:
 - a) patient whole blood,
 - b) patient tissue cell suspensions and
 - c) frozen patient samples

2. To develop an approach to identify the phenotype of the HIV-1 infected cells by employing Flow Cytometry FISH analysis and Fluorescent Activated Cell sorting (FACs) simultaneously.
3. To use both Flow Cytometry FISH analysis and FACs to quantitate the number of infected cells expressing viral mRNA's and to identify the phenotype of the infected cell during drug or gene therapy.

Significant Findings to Date:

1. Developed fixation and cell permeablization procedures for FISH. Developed unique cell staining procedure (Evans Blue) to detect positive hybridization.
2. Flow cytometry FISH analysis of cell cultures directly correlated with the standard slide method of *in situ* hybridization using alkaline phosphatase and NBT/BCIP.
3. Cell culture mixing experiments demonstrated that flow cytometry FISH analysis could detect 1 infected cell in a population of 1000 uninfected cells.
4. Flow Cytometry FISH assay was successfully used to quantitate the number of cells expressing HIV mRNA in a population of newly infected PHA stimulated PBMC's
5. Performed combination Flow Cytometry FISH assay and FACs analysis. Demonstrated that the CD4+ sorted cell population in PHA stimulated PBMC's had the greatest number of cells actively expressing viral mRNA.
6. Newly infected PHA stimulated PBMC's did not demonstrate any increases in mRNA signal by either increasing the viral contact time or increased culture time.

"Detection of HIV Infected Cells using Flow Cytometry- In Situ PCR and Fluorescent In Situ Hybridization"-

Description of Project:

Ashley Haase has recently demonstrated that levels of HIV mRNA and DNA in cells can be monitored by using fluorescent labelled probes after *in situ* PCR (Science, Vol.259). In collaboration with the Cellular Pathology Laboratory work unit, the researchers are currently developing a similar approach in identifying infected cells

containing HIV DNA and infected cells actively expressing HIV RNA in PBMC and in tissue compartments such as the lymph node. Using the same fixation and cell permeabilization procedures described before, PCR primers can be used to amplify genomic or subgenomic mRNA and/or HIV DNA. The PCR product will be detected by hybridization with specific fluorescent probes.

Objectives:

1. To develop *in situ* PCR, Fluorescent *In Situ* Hybridization (FISH) and Flow Cytometry as a method of rapidly quantitating the number of HIV infected cells and the levels of viral mRNA in:
 - 1) patient whole blood,
 - 2) patient tissue cell suspensions and
 - 3) frozen patient samples.
2. To develop an approach to identify the phenotype of the HIV-1 infected cells by employing Flow Cytometry Fluorescent *In Situ* Hybridization (FISH) analysis and Fluorescent Activated Cell sorting (FACs) simultaneously.
3. To use both Flow Cytometry FISH analysis and FACs to identify the phenotype of the infected cells and the cells expressing viral RNA during drug or gene therapy.

Significant Findings to Date:

1. The cell fixation and permeabilization parameters for *in situ* PCR are currently being assessed. Conditions must be established that ensure the cellular retention of the PCR products and hybridization probes.
2. Pilot studies on uninfected cells, A301, and chronically infected cells, ACH2, revealed that current procedural conditions show high levels of PCR products *in situ* in the infected cells, low levels in uninfected cells with minimal leakage of PCR products in the supernatants.

"Viral Burden - RNA and DNA PCR Analysis in Purified Sorted Cell Populations"-

Description of Project:

CD4 cell populations with different cell surface determinants appear to be infected at different rates during the course of disease (Aids Research and Human Retroviruses, Vol.7,1991). If this is the case, then it might be possible to measure the changes in infection in the different CD4 cell populations and use the data generated as a surrogate marker for disease progression or therapeutic intervention. In collaboration with the Intervention Assessment Program Area, the Flow Cytometer is being utilized to sort the different CD4 cell populations and to determine the viral burden in these cells by RNA and DNA PCR. A quantitative plate assay for PCR is also being developed to screen multiple samples rapidly and efficiently.

Objectives:

1. To develop Fluorescent Activated Cell sorting using the Flow Cytometer to rapidly and efficiently sort patient PBMC's and patient tissue cell suspensions in the various CD4+ cell populations.
2. To develop RNA and DNA PCR plate assay with material obtained from fixed cell sorts.
3. To validate this assay with patient material on drug or gene therapies.

Significant Findings to Date:

1. The researchers were able to highly purify fixed cells with contamination rates of less than 0.01%.
2. Stained uninfected cells (Leuko-PAK) were successfully sorted and found to be negative by PCR amplification when mixed with a HIV-1 chronically infected cell line (ACH2).
3. Quality controls for PCR amplification were developed using DNA derived from fixed infected ACH2 and uninfected cells and SK primers.
4. Results from pilot studies show that HIV infection in CD4+ cells increases with Walter Reed staging.
5. Preliminary experiments indicated that the SRA plate assay system for the detection of PCR products is useful in determining positive cell endpoints.

III. Gene Therapy Work Unit

The primary mission of this unit is to develop an efficient gene delivery system capable of transducing and expressing HIV immunogens to induce immune responses in uninfected personnel or augment immune responses in HIV infected personnel and anti-HIV antiviral agents to inhibit virus replication and preserve the immune system of HIV infected personnel. The following are the primary objectives of this program:

- 1) Develop efficient gene delivery systems capable of transducing and expressing genes encoding HIV and SIV antigens and anti- HIV/-SIV antivirals.
- 2) Evaluate efficacy, safety and toxicity of prophylactic and immunotherapeutic vaccine and antiviral strategies using gene delivery systems in mouse, SCID-Hu mouse and Rhesus macaque models.
- 3) Evaluate clinical efficacy of the gene therapy strategies in both early stage HIV infected and uninfected military populations.

To accomplish these objectives, the following projects are ongoing or proposed. Details of each are described below as well as significant findings or results.

"Molecular and genetic characterization of MoMuLV (Part 1) and HIV-1 (Part 2) based packaging cell lines and recombinant retroviral vectors"-

The researchers are developing an efficient gene delivery system capable of transducing and expressing HIV immunogens and anti-HIV antiviral agents to prevent HIV infection in uninfected individuals or to preserve the immune system of HIV infected populations.

Objectives:

1. Engineer cell lines to express either MoMuLV GAG/Pol and amphotropic envelope polypeptides or HIV structural and regulatory proteins and assess packaging efficiency of murine and HIV based retroviral vectors.
2. Assess efficiency of transduction, targetting and expression of retroviral vectors in various cell lines, primary CD4+ T cells and CD34 cells.

Significant Findings:

Part 1:

This laboratory has developed two prototype retroviral vectors, N2* and N2*SD/SA. Both vector types are designed so that they can easily be engineered to transduce a dominant selectable marker in addition to the segments of the HIV or SIV genome or anti-HIV antiviral coding sequences. The dominant selectable marker in the retroviral vector facilitates the identification of transduced cells by conferring resistance to cytotoxic drugs. Since the researchers have observed that some promoters used to direct expression of the dominant selectable marker down regulate the constitutive expression of genes directed by the 5' LTR, the vectors are designed to express the dominant selectable marker either from a spliced mRNA or from a bicistronic mRNA the containing EMCV ribosome binding site upstream from this gene. Finally, to optimize expression of the recombinant provirus in specific cell types, the enhancer sequences located in the LTRs can be replaced with other viral or cellular enhancer elements with known specificity. Alternative retroviral vector designs also constructed by Gilboa include the expression cassette in the U3 region of the LTR. positioning of the gene in this position of the vector results in more consistent expression of the transduced gene. In an effort to reduce the frequency of generation of replication competent virus in packaging cell lines, the researchers are currently developing packaging cell lines which are non murine in origin (African Green Monkey Kidney Vero cells and a Dog cell line, D17) using a split genome GAG/Pol and amphotropic envelope expression vectors. (Neither Vero cell nor the D17 cells harbor any known endogenous retrovirus.) The levels of viral protein expression in these transfected putative packaging cell lines will soon be characterized by radioimmuno-precipitation with rabbit anti Moloney GAG, GAG/POL and ENV antibodies generated by ABI using either of both purified inactivated amphotropic virus and a recombinant vaccinia vector expressing the amphotropic ENV gene. Furthermore, clones derived from the transfection will be assayed for their ability to produce high titer recombinant retrovirus, free from replication competent virus.

Part 2

This laboratory has previously developed prototype HIV based packaging cell lines and retroviral vectors. Introduction of HIV-based retroviral vectors into the packaging cells yielded packaged vectors capable of transducing a marker gene to the CD4+ T cell line SupT1. The highest titer documented to date was greater than 10^2 transducing particles per ml of supernatant. In most cases, the HIV based vector integrated in the SupT1 genome without undergoing structural rearrangements. Analysis of HIV vector-specific transcripts in transduced SupT1 cells revealed high levels of transcripts originating from the internal promoter within the vector. While no full length HIV promoter-directed transcripts were observed, a low level of vector-specific transcription consistent in size with spliced transcripts arising from the HIV promoter was detected. Characterization of these potentially spliced transcripts is ongoing. Furthermore, no replication competent virus has been detected in any transducing particle preparations to date. A manuscript describing these results is in preparation.

Ongoing research involves characterization of gene expression in transfected packaging cells. Although analysis of transcription patterns in these cells is not yet complete, preliminary analysis of protein expression suggests that processing of viral precursor proteins is impaired. Concurrently, the researchers are developing more sensitive assays to detect both transducing particles and replication competent virus.

*"Prophylactic and Immunotherapeutic Vaccine Strategies,
Part 1 and 2"*

Hypothesis:

To date, the immune mechanisms that might contribute to the prevention or reduction of the pathological effects of HIV infection in the military population are poorly understood. Early stage military personnel infected with HIV show potent T cell proliferative and cytotoxic T cell responses specific to HIV. Moreover, these individuals have developed high titer anti-HIV antibody responses capable of neutralizing cell free viral transmission in culture. Despite the apparent potency of these responses; the immune mechanisms induced in infected individuals are not sufficient to clear the virus and thus prevent

disease progression. However, the immune response induced in HIV infected personnel may significantly contribute to controlling infection before manifestation of AIDS. This program area is focused on the development of an expression and delivery system capable of expressing all or some of the HIV and SIV antigens in transduced cells *in vivo*, in the absence of virus replication. The product developed in this program must be an effective means of activating virus specific Class I and Class II restricted immune responses. Additionally, the product must be safe, nontoxic and be effective after one or perhaps two administrations.

Part 1

Description:

Develop plasmid expression vectors capable of expressing all the SIV or HIV structural and regulatory proteins. These expression vectors will be used to assess direct intramuscular DNA injection as an approach to prophylactic or immunotherapeutic vaccine strategies in the Rhesus macaques model system.

Objectives:

- 1) Immunize Rhesus macaques by intramuscular injections with purified plasmid expression vectors capable of synthesizing SIV or HIV GAG/Pol and ENV proteins in cells that take up the DNA.
- 2) Assess immune response in immunized monkeys - T cell proliferation assays, cytotoxic T lymphocyte assays and specific Ab responses.
- 3) Assess possible change in immune response when cytokine expression vectors are co-injected with the viral structural protein expression vectors.
- 4) Boost immunized monkeys with whole inactivated virus and assess changes in immune response.
- 5) Challenge animals with homologous low dose of virus and assess changes in immune response.
- 6) Assess viral burden challenged animals by QC PCR.
- 7) Develop mouse model system to assess new vaccine strategies.

Significant Findings:

In 1991, John Wolfe showed that direct intramuscular injection of a plasmid encoding the E. coli Lac Z gene resulted in the expression of β galactosidase in myotubes along the injection tract. This surprising report suggested that direct intramuscular injection of DNA expression vectors might be a viable approach to developing novel vaccine strategies. In collaboration with the Vaccines for Prevention Program Area, the researchers set out to test whether direct intramuscular DNA injection of an SIV based plasmid expression vector could lead to specific anti-SIV cellular and humoral immune responses in Rhesus macaque. Three Rhesus macaques were injected four times at 7 day intervals with 200ug of a purified plasmid expression vector encoding SIV-ENV239. Sera from animals bled at weekly intervals for a two month period developed no detectable SIV-ENV specific antibody responses. Since neither T cells proliferation assays nor cytotoxic T lymphocyte assays were developed at this time, neither were performed.

These three monkeys were boosted with a fifth intramuscular injection of the DNA expression vector 2 weeks prior to challenge with 10 ID₅₀ of SIV 251. Sera drawn from the animals prior to challenge again showed no detectable antibody response. However, T cell proliferation assays performed in the presence of IL-2 and antigen on the PBMC derived from each animal revealed that each animal did indeed react to the injection protocol. Animals 1710, 0711 and 1306 developed stimulation indices (SI) of 4.3, 12 and 21.3 respectively. Control animals routinely show SI of approximately 0.7. Upon challenge with SIV 251, monkeys 0711 and 1306 developed anamnestic response to the SIV envelope protein (see attached figure). Monkey 1710 responded poorly to challenge and never developed high titer antibodies. Levels of p28 during the acute infection were barely detectable in animal 1306 while p28 levels 6 ng/ml in animals 1710 and 0711. Typically p28 levels drop after the acute phase of infection presumably due to anti-SIV antibody responses.

Although none of the animals appeared to experience any toxic effects from the injection of the DNA, their sera were not monitored for anti-DNA activity. Animal 1710 has since died of SIV associated complications.

Future Plan and Workscope:

The researchers propose to immunize rhesus macaque with an expression vector encoding all of the SIV structural and regulatory proteins. In an effort to augment the immune responses, these animals could be co-injected with hu-IL-2 and hu-GMCSF expression vectors. The fate of the injected DNA will be monitored by *in situ* PCR and by quantitative PCR techniques. The data generated from this study will be useful for both the Federal Drug Administration and the RAC approval of potential future human use. Both committees will require that the researchers show that the DNA injected into the muscle tissue does to integrate into other somatic or germline tissue. The researchers also propose that some above animals be boosted with whole inactivated virus. If animals are primed to antigen, then boosting with inactivated virus should lead to more potent anti-SIV antibody responses. Control animals will be injected with a nonspecific DNA expression vector called CMV/Bgal and boosted with purified B-galactosidase protein. All immune parameters will be monitored carefully in these animals. CD4 and CD8 cell numbers and viral burden will be monitored periodically to estimate vaccine efficacy. If virus replication is detected in vaccinated animals, then animals will kept to observe if vaccination has increased the life expectancy of the immunized animals.

In addition to this study, the researchers propose the development of a mouse model system. Since immune mechanisms are well characterized in mice, it is possible to use this model as a test bed for new ideas. To develop this model the researchers intend to establish a collaboration with Kelvin Lee at Naval Medical Research Institute. The mouse model will also be useful in fulfilling the RAC requirements for safety and toxicity testing.

Part 2

Description:

Develop murine and HIV-1 retroviral vectors capable of expressing all the SIV or HIV structural and regulatory proteins. These recombinant retroviral vectors will be used to immunize infected or uninfected Rhesus macaques in an effort to assess this prophylactic and immunotherapeutic vaccine strategy.

Objectives:

- 1) Immunize Rhesus macaques by intraperitoneal injections with autologous primary skin fibroblasts transduced with MoMuLV retroviral vectors capable of expressing SIV or HIV GAG/Pol and ENV protein products.
- 2) Assess immune response in immunized monkeys - T cell proliferation assays, cytotoxic T lymphocyte assays and specific Ab responses (ELISA, Western Blots and Viral Neutralization Assays).
- 3) Assess possible changes in immune response when cytokine expression vectors are co-transduced with the viral structural protein expression vectors in the skin fibroblasts.
- 4) Boost immunized monkeys with whole inactivated virus or subunit protein and assess changes in immune response.
- 5) Challenge animals with homologous low dose of virus and assess changes in immune response (see above).
- 6) Assess viral burden in PBMC and lymph nodes in challenged animals by p24 assays, QC PCR, *in situ* hybridization and other pathology studies.
- 7) Develop retroviral vectors as direct injectables.

Significant Findings:

The researchers previously reported the initial demonstration in mice of the feasibility of using retroviral vector mediated gene transfer as a means of delivering genes encoding HIV antigens to induce HIV specific immune responses. Mice immunized with autologous retrovirus transduced cells developed CD8+ Class I restricted immune responses specific to HIV-ENV capable of killing specific target cells *in vitro* and *in vivo* despite the low levels of envelope expression in the transduced cells. The specificity of killing appeared to be in part directed against determinants within the variable loop, V3, of HIV-IIIb gp120. Memory CTL specific to envelope were observed in animals immunized 9 months prior to testing. This result suggests that immunization with transduced cells leads to at least some memory responses. Moreover, the induction of neutralizing antibody responses specific to gp160 in this study suggests

that the transduced gp160 was also presented to B and T helper cells in the context of Class II MHC. The mechanism of Class II presentation in this context or the role of T helper cells in the induction of cytotoxic T lymphocytes was not evaluated.

Several retroviral vectors were constructed that were capable of expressing either the HIV envelope protein or the SIV envelope protein (see section (i) MoMuLV based Retroviral Vectors for description of the vector design.) Each construct is currently being analyzed for both virus titer and its ability to express the envelope gene in the transduced cells.

Publications:

1. St. Louis, D. and I. M. Verma. 1988. An alternate approach to somatic cell gene therapy. Proc. Natl. Acad. Sci. USA 85 (9), pp. 3150-3154.
2. Barber, J.R., D.J. Jolly, D. St. Louis and J.F. Warner. 1990. Utilization of retroviral vectors for the potential treatment of Acquired Immunodeficiency Syndrome. Ann. Rev. Aids Res. Vol. I, pp. 329-355.
3. Warner, J.F., C-G. Anderson, L. Laube, D.J. Jolly, K. Townsend, S. Chada and D. St. Louis. 1991. Induction of HIV-specific CTL and antibody responses in mice using retroviral vector-transduced cells. AIDS Res. and Human Retroviruses. 7, 645-655.

Abstracts:

1. St. Louis, D., Townsend, K., Anderson, C-G., Jolly, D., and Warner, J.: Retroviral Approaches to an HIV-1 Vaccine, Second Annual Meeting of the National Cooperative Vaccine Development Groups for AIDS, (1989), Fort Lauderdale.
2. St. Louis, D., Townsend, K., Chada, S., Anderson, C-G., Laube, L. and Warner, J.: Induction of HIV specific immune responses in mice and monkeys using retroviral vector transduced cells, Seventh International Conference on AIDS, (1991), Florence.

Future Plan and Workscope:

In collaboration with Vaccines for Prevention Program Area, Rhesus macaques will be immunized 4 times (either subcutaneously or intraperitoneally) at 14 day intervals with 10^7 primary autologous fibroblasts transduced with a retroviral vector encoding SIV ENV-239. Control animals will be injected with cells transduced with a retroviral vector capable of expressing the dominant selectable marker, the

neomycin phosphotransferase gene. After each immunization, peripheral blood lymphocytes will be assayed for T cell proliferative responses and cytotoxic T cell responses specific to gp160. Serum samples collected at weekly intervals will be assayed for gp160 reactivity by ELISA and by Western blotting techniques. Sera found to have SIV gp160 reactivity will be monitored for their ability to neutralize SIV 239 in culture. One week after the last immunization, lymphocytes isolated from draining lymph nodes will be assayed for T cell proliferative and CTL responses. Immunized animals will be challenged with a low titre of SIV 239. All immune parameters will be monitored carefully in these animals. CD4 and CD8 cell numbers and viral burden will be monitored periodically to estimate vaccine efficacy. If virus replication is detected in vaccinated animals, then animals will be kept to observe if vaccination has increased the life expectancy of the immunized animals. In addition, autologous skin fibroblasts will be co-transduced with retroviral vectors encoding either IL-2 or GMCSF and vectors encoding SIV ENV-239. It is likely that cytokine secretion by the cells expressing SIV gp-160 will augment anti-gp160 immune responses in immunized animals.

"Assessing anti-HIV antiviral gene therapy strategies in Mature CD4+ T-Cells Susceptible to HIV Expression and Replication"

Description:

This project explores mechanisms used by HIV to create tailored expression of therapeutic agents which are designed to inhibit HIV expression and replication. By arming HIV susceptible cells with effective antiviral genetic constructions the researchers expect to stalemate HIV dissemination in HIV infected individuals. By maintaining normal cellular function in uninfected cells while suppressing HIV expression in cells harboring HIV, infected individuals should be maintained in an early-stage, asymptomatic state, allowing them to continue their life in spite of harboring HIV.

Research Objectives:

1. Efficiently deliver anti-HIV antiviral HIV susceptible cells using retroviral vectors

2. Assess antiviral activity of various anti-HIV genetic constructions in tissue cultured cells and primary cells.
3. Test promising anti-HIV antiviral strategy in an animal model system and in early-stage HIV-infected individuals.

Significant Results:

With respect to the question of HIV-based vectors being repackaged in HIV-infected cells, the researchers have demonstrated that HIV-infected cells could be used as "factories" to manufacture HIV-like particles for delivery of anti-HIV genetic constructions to neighboring cells within an infected individual.

Relevant Publications:

Bednarik, DP, Mosca, JD, Raj, NBK, and Pitha, PM.
Inhibition of human immunodeficiency virus (HIV) replication by HIV-transactivated alpha2-interferon.
Proc. Natl. Acad. Sci. USA. 86: 4958-4962, 1989.

Mosca, JD, d'Arcy, L, Ritchey, DW, and Burke, DS
Expression of anti-HIV compounds directed from the HIV-LTR:
A potential gene therapy for HIV-infected individuals.
UCLA Keystone Symposium, 1992.

Mosca, JD, Kim, J, Perera, P, Yu, Z, Ritchey, D, d'Arcy, L, Kaushal, I, Xu, J, Vahey, M, St. Louis, D, Redfield, R, and Burke, D.
Determining if an HIV-based retroviral vector system will target HIV-infected cells.
VIII International Conference on AIDS, Amsterdam, Netherlands, 1992.

Carroll, R, Lin, JT, Kim, J, Mosca, JD, Vahey, M, Burke, DS, and St. Louis, DC.
Construction and characterization of HIV-1 retroviral vectors and replication-defective HIV-1 packaging cell lines.
IX International Conference on AIDS, Berlin, Germany, 1993.

Kim, J, Mosca, JD, Vahey, M, McLinden, RJ, Burke, DS, Redfield, RR
Consequences of human immunodeficiency virus type 1 superinfection of chronically-infected cells.
AIDS Res. and Hum. Retr. (In Press)

Mosca, JD. Kim, J, Perera, P, Kaushal, S, Vahey, M, Yu, Z, Ritchey, DW, Xu, J, St. Louis, DC, Redfield, RR, and Burke, DS.

Human Immunodeficiency Virus Type 1 can Superinfect HIV-Infected Cells.

J. Virol. 1993 (In preparation).

"Assessing anti-HIV antiviral strategies in pluripotent hematopoietic stem cells"-

Description:

This project exploits the proliferation and differentiation potential of hematopoietic stem cells and their progenitors. The introduction of genetic material into one stem cell is equivalent to targeting thousands of mature monocytes and lymphocytes. Once armed with expression systems where anti-HIV genetic products are made in response to HIV infection, these mature cells will limit further HIV dissemination and will provide a repertoire of functional mature blood cells to sustain an HIV infected individual.

Research Objectives:

1. Efficiently deliver anti-HIV antivirals to primitive hematopoietic stem cells.
2. Assess antiviral activity of various anti-HIV genetic constructions in retroviral vectors in tissue culture stem cell equivalence and in mature CD4+ cells derived from differentiated primary CD34 cells.
3. To test promising anti-HIV antiviral strategy in an animal model system and in early-stage HIV infected individuals.

Significant Results:

- Adhering CD34+ cells to CD34 coated antibody flasks at the time of infection with a retroviral vector appears to enhance reporter gene expression.
- Addition of inducers to infected CD34+ cultures, (dexamethasone to MuLV infected and TNFalpha to HIV infected) increases the number of expressing cells and the kinetics of expression to earlier

time, suggests that the transduction efficiency observed in CD34+ cultures is an underestimation of the number of cells transduced by the retrovirus

- Stromal cells (Lof11-10) reconstitution in hu-BM-SCID experiments protects the mice from gamma-irradiated mediated death, suggesting that Lof11-10 cells (through the production of human cytokines) may aid in the ability of CD34+ cells to engraft the hu-BM-SCID mice

Relevant Publications:

Mosca JD, Kaushal, S, Gartner, S, La Russa, V, Lui, Y, Yu, Z, Ritchey, D, Xu, J, Perera, P, Kim, J, St. Louis, D, Vahey, M, Redfield, R, and Burke, D.

HIV-1 expression in stromal cells does not affect cytokines production.

UCLA/UCI AIDS Symposium, Palm Springs, CA 1993.

Mosca JD, Hall E, Kaushal S, LaRussa V, Kessler S, Burke DS, et al. MuLV Infection and Differentiation of Primitive Human CD34+ Hematopoietic Cells in Liquid Cultures and in SCID Mice. IXth International Conference on AIDS, Berlin, Germany, 1993. Oral Presentation.

Kaushal S., VanCott T, Burke DS, and Mosca JD.

Possible requirement of additional factors in association with CD4 binding for HIV infection.

Annual Meeting of the Laboratory of Tumor Cell Biology, Bethesda, MD, 1993.

Mosca JD, Hall E, Kaushal S, LaRussa V, Kessler S, Gartner S, Kim J, St. Louis DC, Mayers D, Perera LP, Yu Z, Ritchey DW, Xu J, and Burke DS.

Developing a Microenvironment for the Differentiation of Primitive CD34+ Hematopoietic Cells in hu-BM-SCID.

The First National Conference on Human Retroviruses and Related Infections, Washington, DC, 1993.

La Russa VF, Cutting MA, Kaushal S, Leiby D, Toro L, Mosca JD, Kessler SW, and Reid T.

Generation of Stromal Cell Colonies from CD34+ Cells.

Annual Meeting of the American Society of Hematology, St. Louis, MO, 1993.

La Russa VF, Mosca JD, Kaushal S, Cutting MA, Kessler SW, and Reid T.

CD34+ Stromal Cell Precursors are Possible Targets for HIV.

Annual Meeting of the American Society of Hematology, St. Louis, MO, 1993.

CONCLUDING REMARKS:

This Program Area responded quickly to the new HIV Research Program priorities, initiating high MMCARR priority clinical trials, such as the Codon 215 Protocol. This protocol represents a major successful collaborative planning effort between WRAIR and the NIH. In addition, laboratory efforts within other pre-existing mission areas were folded into this Program Area to incorporate and focus scientific talents to better support the current mission of the HIV Research Program. This Program Area also continues to work with all Program Areas to offer expertise in key laboratory and clinical trial efforts.

VACCINE THERAPY

Program Area Coordinator: COL (P) Robert R. Redfield, MD, MC, US ARMY

Foundation Scientific Director: To be determined

Assistant Department Chief: LTC Deborah Birx, MD, MC, US Army

Program Area Summary: The overall goal of the Vaccine Therapy Program Area is the development, evaluation and demonstration of efficacy of HIV specific vaccines for both treatment and potential prevention of HIV infection and disease.

HUMAN USE PROTOCOLS

Protocol No.	Protocol Title (Abbreviated)	Principal Investigator
RV21A	Recombinant gp160 Phase I	Redfield
RV21B	Recombinant gp160 Phase II	Redfield
RV21C	Civilian Sites gp160 Phase II	Redfield
RV51	gp120/Genentech	Redfield
RV57	gp160/AZT	Redfield
RV92(P)	Recombinant gp160 Phase III	Birx

LABORATORY WORK UNITS

Project Titles(abbreviated)	Principal Investigator
Humoral, cellular immunogenicity	Birx
Charact. of conformational antibody reactivity by spectrotyping	Loomis*
Fine mapping of serum conform. antibody react. to V3	Loomis
Purification of human antibody react. to conserved envelop regions	Polonis
Charact. of heterologous and autologous env AB in	Polonis

vaccine volunteers	
Characterization of autologous neutralization	Polonis
Humoral fine mapping	Van Cott
Humoral immune response during seroconversion	Van Cott
Spectrotype, rabies, and tetanus reactivity in vaccine pts.	Loomis
Genotypic crossreactogenicity of human antibody	Loomis
Development of HIV envelope-specific cytotoxic T cell clones	Ratto
Determination of differential gp 160 env. access.	VanCott
Charac. of gp120 sCD4 binding interaction	VanCott
Correlation of antibody binding with functional properties	VanCott
Expression of HIV-env in transformed B Cells	Kim
PCR of TCR in antigen-specific T cells	Sitz
Charact. of naive and memory phenotyping in vaccine patients	Sitz
Characterization of phenotype changes	Sitz
Neutralization characteristics of purified antibody	Polonis
Charact. of sCD4-induced conform. changes in gp120	VanCott
Autologous V3 peptide serology	VanCott
International Serotyping	VanCott
Application of spectrotyping to animal models	Loomis
Immunogenicity of proteosomes	Lowell
T cell recognition mapping in vaccine patients	Sitz
Charact. of recombinant and purified gp120 prep.	VanCott
PEPSCAN model for alternative product formulation	Loomis
Humoral responses of vaccinated rabbits to conformational epitopes	VanCott

Molecular genetics of vaccine patients	Michael
Variation in env. in HIV-1(IIIB) infected lab workers	Michael
Regulation of Negative strand Transcription in HIV-1	Michael
Negative-strand Transcription in HIV-1	Michael
Charact. of viral heterogeneity, viral load, adaptive immunity HIV perinatal transmission	Robb
Charact. of biologic properties of primary viral isolates	Polonis
Differential expression of HIV-1 genotypes	Michael
Differential expression of HIV-1 genotypes in blood	Michael
Viral variation and viral burden	Robb
Purification and biochem. characterization of ASO-1	Loomis
Measurement of HIV viral burden in pediatric PBMC and plasma	Robb

* Foundation Principal Investigators

OVERVIEW

The overall objective of the Vaccine Therapy Program Area is the development, evaluation and demonstration of efficacy of HIV specific vaccines for both treatment and potential prevention of HIV infection and disease. The scientific strategy of Vaccine Therapy is comprehensive yet highly focused on the interdependent, concurrent basic science investigation and applied clinical evaluation of candidate vaccine and immune base products, both narrowly focused in the area of HIV immunoregulation. Such a dynamic interactive program between basic science and applied clinical research allows each component to contribute to the other's, thereby optimizing the pace of both the basic science and the clinical application of this important area of research. The mission is centered around the scientific investigation of human HIV immunity (with emphasis on cellular immune mechanisms) and the mechanics of HIV replication in the human host. The viral expression *in vivo* and the characterization of the dynamic interaction between immune mechanisms and viral expression is shedding light on human HIV immunoregulation.

A key component within this Program Area is the utilization of volunteers in first generation vaccine therapeutic trials, with a primary focus on the untreated and baseline patient samples. The parallel study of HIV immunoregulation and new product development (designed to define and modify HIV immunity as well as HIV replication and its regulatory *in vivo*) represents a unique strength of the Vaccine Therapy Program Area. Additionally, these early trials facilitate technology development by increasing the scientific knowledge base which can then be applied to prioritize or design second and third generation candidate products for both treatment and prevention of HIV. The clinical evaluation of these new products will provide the technology and knowledge base required for the understanding of effective HIV immunoregulation and mechanism of viral clearance. This is a stepwise process (of increased basic scientific knowledge gained from product application and evaluation, leading to new product and new technology development) which will ultimately define the effective HIV immunoregulation mechanisms responsible for HIV control and clearance. Candidate products and regimens will be optimized to induce and maintain these responses; with success, the desired result would be that HIV infection would no longer be a threat to the US military or our Nation.

RESEARCH GOALS AND OBJECTIVES:

As a consequence of the HIV Research Program reorganization and shifting priorities, the research effort conducted within this Program Area was significantly curtailed during the reporting period. This has required substantial modification of efforts previously conducted based on the Hagerstown planning document and the Memphis Vaccine Development for Treatment and Prevention planning meeting. The reallocation of budget and personnel previously within this Program Area to other Program Areas combined with the required deprogramming of previously approved program components has resulted in an overall programmatic de-emphasis of the research efforts conducted by Vaccine Therapy. An summary of the required modifications, based upon the formal recommendations of the MMCARR Director's review meeting include:

- 1) de-program previously approved MMCARR clinical research protocols:
 - Pediatric natural history (RV13)
 - Core diagnostic (children) (RV16)
 - Perinatal Infection (RV41)
 - rgp160 vaccine therapy in pregnant women (RV54)
 - rgp160 vaccine therapy in HIV infected infants and children (RV74)
 - Chiron rgp120 Phase I vaccine therapy trial (RV71)
 - Genentech MN rgp120 vaccine therapy trial (RV75)
- 2) discontinue Lab Work Units:
 - characterization of HIV env biosynthesis in T cells and macrophages
 - phenotyping of uninfected donor PBMC
- 3) modify percent effort for specific laboratory work units
- 4) de-program BAA units entitled:
 - early T-pre-cursor thymocytes as potential targets for HIV infection
 - pathobiology of HIV in human monocyte-macrophage
 - antigen markers for clinical manifestation and prevention of HIV
 - protein of HIV cross-react with self-antigen
 - use of CD4/gp120 interactions in development of anti-HIV drugs and vaccines

- antibody to the RNA dependent DNA polymerase of HIV
 - transdominant rev and protease mutant proteins of HIV/SIV as anti viral agents
 - use of synthetic peptides and anti-idiotypes for controlling HIV
 - development of an RNA assay to assess HIV-1 latency
- 5) transfer lab work units to other program areas:
- use of signal transduction in HIV infected patients to Drug and Gene Therapy
 - *in vivo* stimulation of CD4 cells in immunosuppressed primate model Drug and Gene Therapy
- 6) co-ordinate Thai research protocols with Vaccines for Prevention:
- rgp160 Phase I vaccine therapy trial in Thai volunteers
 - rgp160 Phase I vaccine trial in seronegative Thai volunteers
 - lab work unit international serotyping
- 7) co-ordinate lab units with the Drug and Gene Therapy Program Area:
- development of replicating retroviral vaccine
 - development and characterization of retroviral vector HIV-based vaccines
 - immunogenicity of pCEPenv naked DNA

Although these actions have required an acute reduction in personnel and the narrowing of the research scope, the five major technical objectives which had been previously identified within the Vaccine Therapy Program Area remain constant. These are as follows:

1. Continued development and application of new technology to measure and characterize HIV specific immune responses;
2. Develop and apply new technology to measure and characterize HIV load and expression *in vivo*;
3. Define mechanisms responsible for effective post-infection HIV immunoregulation;

4. Develop the required technology and scientific infrastructure to define the protective immune response and aid in the successful execution of preventive vaccine trials.
5. Develop and evaluate candidate HIV vaccine in a step wise fashion for efficacy in the treatment and prevention of HIV infection.

Details of the progress and the work which comprises each major objective are delineated below, categorized within the appropriate objective area. Accomplishments are also detailed.

Objective 1 - Develop and apply new technology to measure and characterize HIV specific immune responses:

Specific milestones achieved to meet the prescribed objective have included:

- Development of one of the premier human HIV immunology laboratories to support and execute the laboratory component of retroviral research. Development of this comprehensive HIV immunology laboratory was an enormous feat and a prerequisite to the successful fulfillment of the primary objective of this Program Area: the development and demonstration of safe and efficacious HIV vaccine for treatment and prevention of HIV infection and disease.
- Development of the technology to characterize human HIV epitope-specific humoral and cellular immune responses; these techniques have been applied to the evaluation of volunteers with HIV infection.
- Development of new successful technologies to characterize HIV envelope antibodies. These have included:
 - quantitative peptide ELISA,
 - fusion protein immunoblotting quantitated by phosphoimaging techniques,
 - linear epitope mapping by Geysen pin peptide analysis,
 - real time biospecific analysis techniques capable of assessing antibody binding kinetics (association and dissociation constants) and the impact of distinct molecular conformation on binding, antibody spectrotyping to characterize antibodies to conformational epitopes, and

- FACS analysis of antibodies to viral products expressed on the surface of chronically or acutely infected cells.
 - Functional assay to characterize neutralization capacity have been developed including a PBMC autologous neutralization assay.
- Development of technology to characterize cellular immune responses has also been successful. These have included:

- T cell proliferative response to HIV proteins,
- fine mapping of T cell anti-envelope responses and cytotoxic T cell assays.
- development of specific human T cell lines and subcloning of epitope specific T cells to explore their specific function and potential HIV immunoregulatory properties.

- Application of the *in vitro* assays required to characterize and assess human immunological state. These included:

- T cell proliferative assays to mitogen and recall antigens(such as candida and tetanus),
- assay to quantitate control antibodies against tetanus
- assays to measure cytokine production (sIL-2r, TNF, IL-6),
- assays to measure natural killer cell activity.

This comprehensive approach to the evaluation of an HIV-infected immune system has allowed this Program Area to extensively describe and categorize the extent of immunologic dysfunction associated with asymptomatic HIV infection.

- Previously, early and mid stage HIV infected patients were postulated to have significant immunological dysfunction, which was supported by *in vitro* analysis of immunological function; Vaccine Therapy demonstrated instead that these patients maintain *in vivo* functional integrity. HIV infected volunteers demonstrate the capacity to mount a new primary response to administered control vaccines (rabies vaccine) and demonstrate a normal booster response to recall antigen (tetanus toxiod vaccine). This observation documents the immunological resilience of early stage HIV patients and fails to confirm previous suggestions of significant irreversible immunological end organ damage in early stage disease.

- Standardized the use of delayed hypersensitivity skin testing in the evaluation of HIV infection and the independent prognostic value demonstrated. This has important implications both in the medical management of HIV infection and clinical evaluation of potential therapeutic agents as a validated surrogate marker of disease progression.

Objective 2: Develop and apply new technology to measure and characterize HIV load and expression in vivo

During this period there also has been substantial progress in the development of technology to measure HIV replication and expression in vivo. Recent accomplishments include:

- Evaluation of HIV expression in vivo demonstrated the absence of viral latency in patients with HIV infection. This observation confirms that host directed immune responses which occur as a consequence of natural infection do not result in viral clearance.

- Developed and applied quantitative Polymerase Chain Reaction (PCR) based assays measuring HIV proviral copy number RNA copy number and RNA transcript analysis.

- Developed and applied techniques to sequence viral LTR's and analyze their transcriptional activity. Additional strategies to sequence envelope genes have been developed and applied. These techniques provide critical tools for further dissection of HIV immunoregulation when combined with the comprehensive immunologic evaluation.

- Characterized partially adaptive (memory) immunity directed against HIV in the setting of chronic infection.

- Discovered that in natural infection the anti-envelope humoral and cellular immune responses are restricted. This observation has important implications for vaccine development.

- Confirmed by subsequent evidence that post-infection vaccine can induce broad anti-envelope recognition that the restricted response is not predominantly caused by a genetic restriction, but is a consequence of antigen processing and presentation.

- Suggested a role of T cell immune recognition through the characterization of cellular immunity directed against

envelop in long term survivors. The characterization of the development of adaptive immunity in laboratory workers infected with HIV confirms the previous hypothesis that the immunological restriction noted in the setting of natural infection was not a consequence of *in vitro* antigens used but rather *in vivo* restriction of immune recognition.

- Revealed a novel and potentially extremely important observation with studies of HIV transcription. Both in cells lines and in infected patients, HIV transcription is not restricted to the production of positive stranded mRNA. Negative stranded transcription occurs. Sequence analysis confirms the presence of multiple open reading frames, thereby potentially increasing the coding capacity of the HIV genome. Additional studies demonstrate a unique promoter for negative stranded transcription which can be down regulated by tat. These observations may have important diagnostic and therapeutic implications.

Objective 3: Definition of mechanisms responsible for effective post-infection immunoregulation

Update:

- Characterization of volunteers adaptive anti-HIV immune response is ongoing. It is likely that the ability to determine which, if any, immune responses are associated with effective immunoregulatory will occur as a direct consequence of the current multi-centered DOD sponsored Phase II rgp160 vaccine trial. The inability to compare various products' performance, particularly in terms of their impact on the occurrence of validated hard clinical endpoints, has limited the pace of future progress.

- The inability to begin Phase I evaluations as programmed in FY93 has also hindered this important effort. The inability to develop new initiatives of additional immune based products for specific immune reconstitution of late stage patients has limited the pace of progress.

Objective 4: Develop the required technology and scientific infrastructure to define the protective immune response and successfully execute preventive vaccine trials.

While the pace of vaccine development for treatment is accelerating, measurable progress in primary vaccine development for prevention has been limited. This has occurred primarily due to the lack of the scientific

delineation of candidate protective immune responses and the lack of scientifically agreed upon selection criteria for product development and selection for efficacy trials. However, progress has continued:

- Progress has been made in the identification of a field trial site. A cooperative relationship towards the goal of preventive vaccine development with the Thailand military research unit has been established. The incidence of HIV infection has been defined in the candidate volunteer populations. Two initial vaccine protocols (RV 68 and RV69 pages 55-56) were developed to facilitate technology transfer, infrastructure building and the building of a longer term scientific collaboration between the US and Thailand military in the area of vaccine development for treatment and prevention. Local infrastructure development is currently in its final stages; these efforts are now coordinated within the Vaccines for Prevention Program Area.

- Specific assays have been developed to facilitate trial execution in Thailand. A battery of unique DTH antigens have been selected, and a series of recall antigens for in vitro T cell assays have been selected and utilized to screen Thai reactivity. A series of humoral and cellular assays have been developed that can immunotype the viruses present in Thailand to allow dissection of type vs. group anti-HIV responses and define their role in HIV treatment and vaccine development for prevention. Specific assays have been developed that can separate vaccinated volunteers from HIV-infected individuals in preparation for preventive vaccine trials. This Program Area is poised to support the preventive vaccine effort planned for Thailand in cooperation with the Vaccines for Prevention Program Area.

Objective 5: Develop and evaluate candidate HIV vaccines for safety immunogenicity and efficacy in the treatment and prevention of HIV infection.

During this time period, Phase I clinical evaluations of several candidate HIV recombinant envelope derived vaccines were completed in patients with early HIV infection. Most notably, the feasibility of post-infection vaccination using different product to safely broaden the host anti-humoral and cellular immune responses was scientifically documented. Descriptions of each current Vaccine protocol, and significant findings to date, are detailed below:

RV21 A-"Active Immunization of HIV infected patients with Recombinant gp160 HIV Protein: Phase I Study of Immunotherapy, Immunogenicity and Toxicity"-

The initial protocol was designed to evaluate the feasibility of post HIV infection vaccination with HIV viral products utilizing a recombinant rgp160 vaccine. This Phase I safety and immunogenicity trial began in April, 1989, was completed in November 1990 and was published in the New England Journal of Medicine in June 1991. The continuation trial was designed to assess the long term immunogenicity and safety of this product and was implemented in light of the programmatic decision to pursue a five year Phase II/III efficacy trial with rgp160. The continuation trial began in November 1990, was modified by addendum in May 1992 and again in May of 1993. As of September, 1993, each volunteer was receiving 160ug of rgp160 every other month.

SIGNIFICANT FINDINGS:

- first to demonstrate the feasibility of concept of vaccine therapy of HIV infection. Subsequently, 5 independent groups have confirmed vaccine therapy published findings.

- refined schedule for post infection vaccination. Data provided by this trial continues to be exploited by multiple companies and investigators currently involved in therapeutic vaccine development facilitating the optimization of immunization schedule.

- demonstrated immunologic resilience of early and mid stage HIV infection volunteers.

- provided critical information related to long term immunogenicity and duration of vaccine induced immune responses and facilitated optimal execution of ongoing Phase II trial (eg. extension protocol provide critical information related to vaccination booster schedule applied to modify phase 2 trial from q 4 month to q 2 month boosters prior to expansion)

- provided critical information related to long term safety and serves as a sentinel for the possibility of safety issues related to long term hyper-immunization with gp160/alum.

- continued to facilitate the development of novel assays developed to assess the human adaptive anti-HIV immune responses and evaluation for

application to Phase II/III efficacy trial and prophylactic vaccine development program.

-continued to facilitate the development of novel assays designed to assess *in vivo* HIV replication kinetics which can subsequently be applied to drug development, gene therapy and prophylactic vaccine development program areas.

-provided opportunity to assess *in vivo* interrelationship between induction of specific adaptive anti-HIV immune responses and viral variation (currently under development).

-demonstrated unique anti-HIV cellular responses. Combined with the CHO, expressed products provide an immunologic profile to all future treatment and prevention employing envelope-based products.

RV51-"A Phase I Study of the Safety and Immunogenicity of IIIB rgp120/HIV Vaccine in HIV-1 Seropositive Adult Volunteers"-

This Phase I protocol was completed during this reporting period. The initial trial was a Phase I open label dose finding trial (100ug, 300ug, 600ug) in 19 volunteers followed by a blinded randomized component (300ug versus placebo) in 25 volunteers. Volunteers were vaccinated at 0,1,4,8,16 weeks with follow up evaluations every 2 weeks through 24 weeks. This trial opened in November 1990 following confirmation of immunogenicity of 300ug and 600ug rgp120 dosage (8/92); subsequently randomized arm enrollment opened September 1992 and closed December 1992. Extension addendum to evaluate variation in boosting schedule in terms of immunogenicity and safety began in April 1992 and was completed in September 1993. 41 of the original volunteers re-enrolled and completed this portion of the trial. Protocol extension to administer 600ug every other month is to begin in November of 1993.

SIGNIFICANT FINDINGS:

-first to demonstrate the immunogenicity and safety of a CHO cell expression HIV envelope product in volunteers with early stage HIV infection.

-first to document dosage response of this product. All subsequent investigators have taken advantage of

the results of this trial to optimize trial design using CHO expression Genentech vaccine products in both seronegative and seropositive trials.

-product found to have a unique dose response profile which has lead to extensive evaluation of the character of the CHO product.

-significant percentage of product found to be denatured.

-primary immune response linked to "denatured" portion of molecule.

-diminished cellular response has led company to explore adjuvant techniques.

-development of *in vitro* assay techniques required to assess T cell anti-HIV responses utilizing CHO cell expressed rgp120. Currently utilized by sponsor in all other prophylactic and therapeutic clinical trials.

-development and application of novel techniques to measure humoral response directed against "conformationally intact" rgp120 (sero-spectrotyping and biospecific interaction analysis).

-comparative immunogenicity of CHO cell derived rgp120, and baculoviral derived rgp160 demonstrated a unique immunologic profile currently under intense investigation.

-no evidence of continued expansion of V3 reactivity post 9 injections to "group specificity" as suggested by prior baboon studies.

RV 57-"Active Immunization of AZT Treated HIV Infected Patients with Recombinant gp160 HIV Protein: Phase I/II Study of Immunogenicity, Toxicity, and Effect on In Vivo Immunoregulation"-

This is a Phase I/II multi-center, open label feasibility trial of rgp160 in patients with HIV infection (Walter Reed Stages 1-5) and currently receiving AZT. Specific objectives include:

- 1) assess the immunogenicity and safety of rgp160 in patients with more advanced HIV disease and in patients with early disease receiving AZT; and

2) determine parameters predictive of post infection immune responsiveness. Patients are stratified by T cell intervals. Each volunteer will receive 160ug of rgp160 on days 0,7 and at months 1,2,4,6,10. Trial duration is 12 months (not inclusive of preevaluation).

SIGNIFICANT FINDINGS:

-This trial opened for enrollment November 1992; enrollment was completed within 6 months. Currently 64 volunteers are on trial and 53 post initial vaccination. Duration of follow up ranges up to 10 months.

-Data obtained from this trial will provide important information related to effect of AZT on immunogenicity and safety profile of rgp160. Preliminary analysis suggest suboptimal immunogenicity (<50%), associated with CD4 cell intervals < 300.

-In addition, this trial will provide systematic information related to immunogenicity and safety in HIV infected populations with more advanced disease.

-Provides platform for additional human adjuvant studies. To date, 8 adjuvants have been studied extensively in small animals in collaboration with Dr. Britta Wahren.

-Verified unique relationship between adjuvant and antigen combinations, requiring verification of each combination.

-The sponsors are agreed to utilize this population to study these adjuvants which can then be exploited in future therapeutic and prophylactic strategies, addendum currently in preparation.

RV21B-"Active Immunization of Early Patients with Recombinant gp160 HIV Protein: Phase II Study of Toxicity, Immunogenicity, in vivo Immunoregulation and Clinical Efficacy" -

This protocol is a Phase II, multi-center, double blinded, placebo controlled trial designed to evaluate the clinical efficacy by surrogate markers of post infection vaccination with rgp160 in the treatment of HIV infection and to validate adaptive anti-HIV immune responses in terms of *in vivo* HIV expression and clinical progression. Enrollment began in 11/90

initially limited to 140 volunteers. Following confirmation of safety and immunogenicity within this initial group, complete enrollment was approved spring 1992 and completed enrollment (608 volunteers) were on schedule in the Fall of 1992. Initial efficacy analysis by surrogate markers is scheduled for December 1993 and final analysis scheduled for the end of 1995.

SIGNIFICANT FINDINGS:

This is an ongoing double blinded trial; only limited information is available to date:

- Developed network of DOD, NIH and civilian sites for efficient trial execution. Currently, 601 volunteers are on trial and being evaluated at 2 month intervals. 311 volunteers are beyond 6 months, 134 volunteers beyond 18 months (range of follow up 3 - 27 months). Protocol execution continues with less than 1% missed visits.

- Confirmation of immunologic resilience of patients with early HIV infection in terms of primary response to novel antigen (rabies) and secondary response to recall antigen (Tetanus toxoid).

- Confirmation of natural history of adaptive immune responses.

- Demonstrated highly restricted T/B cell response directed against envelope.

- Demonstrated lack of expansion of T/B cell repertoire during natural infection.

- Confirmation of immunogenicity of rgp160 in setting of HIV infection (first 140 patients).

- Confirmation of short term safety of rgp160 in setting of HIV infection.

- Independent of outcome in terms of clinical efficacy this cohort of patients will provide the opportunity to validate adaptive immune responses directed against HIV in terms of *in vivo* HIV regulation and clinical disease progression in both the treatment and placebo arms. Specifically, cohorts of placebo patients stratified by clinical course in blinded arms will be extensively evaluated.

These Phase I and Phase II protocols of candidate therapeutic vaccines have demonstrated the feasibility of post infection vaccination with HIV specific immunogens. The primary focus of this objective remains the evaluation and definition of the variables which impact the immunogenicity of candidate vaccine products with intense focus of the qualitative impact on the induction of functional adaptive HIV immune responses. In light of the deprogramming of feasibility population-proposed Phase I trials designed to validate and define immunoregulatory parameters associated with transmission and disease progression, researchers within this Program Area propose to develop new Phase I protocols designed to evaluate and define the variables which impact the immunogenicity of candidate vaccine products. An area of intense concentration will be the assessment of these products to produce a qualitative impact on the induction of functional adaptive HIV immune responses. Major categories which may impact on human immunogenicity of candidate vaccine products include:

- population receiving vaccination;
- route, dose and schedule of immunogen;
- formulation of vaccine; and
- antigen source.

The development and execution of new Phase I trials will also provide additional scientific opportunity to meet previously defined program area objectives 1 - 3 and could also facilitate prophylactic product development.

CONCLUDING REMARKS

Despite the constraints imposed as a consequence of the reorganization, Vaccine Therapy leadership and personnel remain committed to the pursuit of scientific excellence and will continue to contribute its part to the overall success of the MMCARR HIV research program towards the accomplishment of its stated goal: to reduce the mortality, morbidity and HIV rate of US military personnel caused by this presently deadly pathogen. The scientific productivity of this Program Area can be objectively measured by the publishing of 20 manuscripts during this 6 month period. Additional notable accomplishments have been highlighted under the respective research objectives. A brief review includes:

- Continued development and application of new technology to measure and characterize HIV specific immune responses;

- Continued development and application of new technology to measure and characterize HIV expression and replication kinetics;
- Discovery and characterization of bi-directional transcription in HIV replication;
- Established the long term safety of rgp120 and rgp160 in volunteers with chronic HIV infection;
- Continued the ongoing execution a large scale multi-centered cooperative vaccine therapeutic trial (with a less than 1% missed rate) to assess the clinical utility of the approach and validate effective immunoregulatory responses in post infection control and clearance.

As with all Program Areas, considerable time, thought and effort has been invested by the scientists and researchers to realign the science within this Program Area to best serve the mission of the HIV Research Program.

The newly revised five year strategy emphasizes the detailed characterization of adaptive HIV immune responses directed against HIV associated with natural infection (utilizing placebo controlled arms) and those induced by candidate vaccines, including the validation of the *in vivo* relevance in each response in terms of anti-viral activity, and the clinical relationship to viral transmissibility, disease progression and survival. Over the next two years characterization of surrogate markers (both immune and viral based) will be assessed and validated in terms of disease progression.

As described above, the feasibility of several candidate vaccine products to modify the host directed immune response has been demonstrated. Vaccine Therapy plans to continue the previous outlined course of the Department of Retroviral Research and hopes to expand Phase I evaluation of diverse products to determine the impact of product variables (viral strain, viral component, protein structure, cell production system used, expression system used, adjuvant, formulation) on human immunogenicity and safety and will apply this information toward efforts in the Vaccines for Prevention Program Area. If additional funding support is provided, it is anticipated that four new products starts could be initiated in 1994, targeting viral genotype, cells production and adjuvant formulation as priority variables. The limitation of new Phase I starts will be rate limiting to the ability of Vaccine Therapy to accomplish its defined objectives.

By the completion of 1995, the first clinical efficacy trial of vaccine therapy will be completed and analyzed. This milestone will provide an important opportunity that, if coupled with planned additional Phase I products, the resulting information would allow a rational decision analysis to be pursued to determine priority selection of additional products for advanced development or new product development. In addition, blinded immunoregulatory data is currently being collected; this data will allow the validation of current and newly developed assays noted in objective 1 and 2. This data will also provide the needed documentation of the *in vivo* relevance of these assays and will allow accomplishment of objective 3 and steer future product development related to objectives 4 and 5.

It had also been planned for this Program Area to design and prepare for potential execution a clinical trial to assess the efficacy of therapeutic vaccination as a direct prevention strategy beginning in 1994. A prerequisite for final product selection for such a trial is demonstration of clinical efficacy in terms of an *in vivo* reduction of HIV (or improved control of replication) and/or clinical efficacy in terms of delayed disease progression or improved survival. Protocol development and population definition is necessary now in order to minimize unnecessary delays in the evaluation of this potential HIV prevention strategy for US Armed Forces deployed to highly endemic areas of the world such as Haiti, Africa, or South East Asia. Unfortunately this effort has also been deferred as a consequence of the HIV Research Program imposed fiscal restrictions and will require additional funding initiatives.

Over the next several years continued focused basic science research will be conducted within the area of HIV immunoregulation. Selected application of this new technology will be targeted for advanced development. Ongoing efforts in this area have been summarized above. To guarantee continued focus and programmatic priorities, these efforts will be reassessed yearly in terms of their contribution to one of the 5 major program Area objectives. This review and refinement will determine how to optimize the application of knowledge gained to this clinical evaluation program. Additionally, a yearly review will maximize the utility of gathered information in order to design new research basic science efforts required for the continued advancement of this highly focused investigation into HIV immunoregulation and its clinical applications.

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INTERVENTION ASSESSMENT

Program Area Coordinator: Maryanne Vahey, Ph.D.

Foundation Scientific Director: Kenneth Wagner D.O.

Assistant Department Chief: To Be Determined

PROGRAM AREA SUMMARY: The objectives of the Intervention Assessment Program Area concentrate on the development and evaluation of methods for rapid diagnosis and quantitation of HIV as well as the exploration of studies of methods to assess interventions. Surveillance of HIV incidence and disease progression in military populations are also areas of focus, emphasizing longitudinal natural history studies. With the recent reorganization, this program area now represents a coordination of clinical and field trials with prescribed laboratory efforts to accomplish these goals.

PROTOCOLS FOR HUMAN STUDIES

Protocol # Protocol Title (Abbreviated) Principal Investigator

RV 1	Natural History	Wagner*
RV 2	Core Diagnostics	Wagner*
RV 5	Dental Natural History**	Konzelman*
RV 18	Dermatology Natural History***	Smith
RV 22	Retrospective Review	Oster
RV 44	Syphilis	Johnson
RV 64	Cardiac Studies	Deering
RV 67	Staph Aureus#	Decker

LABORATORY WORK UNITS

(Project Titles abbreviated)

I. Clinical and Field Trial Support

Tissue Biopsy Protocols RV77, 78

RV 21 Phase II

Thailand Natural History/Surveillance RV91

II. Technology Development

Refinement of current LH PCR technology

Development of a fluorescent based liquid hybrid. assay

Develop. of a high throughput, plate based fluor. assay

III. Surveillance of Tri-Service HIV Incidence and Disease Progression

Characterization of the natural history of HIV

Immunologic features of HIV disease

Virologic features of HIV disease

Characterization of demographic properties of HIV

Clinical Trial Modeling and analyses

*Foundation Principal Investigator (PI)

**Separately funded through NIH/NIDR

***Separately funded through NIH/NIAMS - funding terminated 1992, will continue pending availability of resources

#Separately funded through pharmaceutical firms

RESEARCH GOALS AND OBJECTIVES

This program area has three primary objectives:

- Provide quantitative PCR viral load analyses for the support of clinical and field trials of intervention/prevention strategies.
- Develop highly sensitive viral quantitation methods in support of surveillance of vaccinees in prevention and intervention trials.
- Establish and define patterns and magnitude of clinical and laboratory markers in the natural history of HIV disease that may be predictive of outcome and indicative of performance of intervention/prevention strategies.

A laboratory component and a natural history component comprise research initiatives in this area. Although this Program Area represents a coordinated effort, the two primary components will be discussed separately.

I. LABORATORY COMPONENT:

The laboratory component of this program area has been organized into three finely focused work units:

1. Clinical and Field Trial Support.
2. Development of New Techniques to Accurately Quantitate Viral Genes in Blood and Tissues.
3. Surveillance of HIV Incidence and Disease Tri-Service Progression within US Military Populations.

Work Unit One: Clinical and Field Trial Support

This primary work unit will support the clinical and field trials of the MMCARR.

Background:

A large body of literature suggests that plasma is most reflective of changes in the recent characteristics of the viral load, especially in the application of the

antiviral drug treatment with AZT. Algorithms for the conduct of Phase II trials suggest that the assessment of cellular DNA and plasma RNA is essential at well defined time points. Some assessment of the tissue viral load is also relevant in a limited number of sub-study cohorts to obtain the overall picture of HIV disease progression.

The goals of this work unit include:

- Provide state of the art quantitative polymerase chain reaction (PCR) viral load analysis in support of MMCARR clinical and field trials of intervention strategies.

- Provide highly sensitive quantitative PCR analysis in support of surveillance for prevention trials. Specimens will include plasma, cells, serum and tissue and will entail both DNA and RT-PCR.

Work conducted to meet these goals is organized into projects which are executed concurrently with certain active human use protocols. These projects are discussed below:

RV 43 AZT Antiviral Trials and RV 79 - Codon 215 - Laboratory research component-

These two protocols are currently active in the Drug and Gene Therapy Program Area. Detailed descriptions of these protocols can be found on page 98. This project has processed samples from RV 43 for viral load determination and plans to process 3-5,000 samples per year for plasma RNA quantitation (for RV 43/79) and will carry out work in conjunction with RV 79 to pursue detection of resistance mutation at RT 215 marker.

Significant Results from RV 43 laboratory work:

Laboratory work resulted in finding that viral load in patients receiving AZT is indicative of patient outcome.(abstract)

Demonstration that 1.5 to 2 log changes in plasma viral load is correlated with development of resistance to nucleoside analogues.

RV 77 and 78 tissue/biopsy protocols - laboratory research analysis

This project is conducted in collaboration with two tissue biopsy protocols active in the Drug and Gene Therapy Program Area and receives tissue collected under the auspices of these two human use protocols. Tissue analyses are performed with a focus on the derivation and execution for quantitation of viral RNA and DNA in tissue.

Significant Results:

The first samples from RV 78 were processed in September, 1993 and will be made available for PCR analysis in early December 1993.

RV 21 Phase II Studies (Vaccine Therapy)-

This project is conducted in collaboration with the Vaccine Therapy Program Area, specifically the gp160 Phase II Trials. Detailed information on this Vaccine protocol may be found on page 137. This project quantitates DNA and RNA viral load in 2-3,000 samples per year of plasma and PBMC.

Significant Results:

This project has been in progress for over 5 years and recently provided the first description of quantitation of virus load in patients on an immunotherapy trial (Manuscript submitted to AIDS & Human Retrovirus and is currently in review).

RV 91 Thailand Natural History/Surveillance (Vaccines for Prevention)-

This project will be conducted in tandem with a pending Natural History/Surveillance Study in Thailand (RV91). Detailed information on the human use protocol can be found on page 56. Once this protocol has met all approvals, this laboratory project will initially study 100 samples with the goal to determine the utility of North American amplification scheme and will provide quantitative assessment of viral load in PBMC and plasma.

Significant Results:

Preliminary laboratory work, in advance of the protocol, has resulted in the validation of North American Primer Scheme which is amenable to application on Thai Samples.

Work Unit Two: Technology Development-

Development of New Techniques to Accurately Quantitate Viral Genes in Blood and Tissues

State of the art assay development that will ultimately support applications in the clinical and field trial work unit comprise the efforts within this work unit. Twenty percent of this program area's laboratory effort will be devoted to assay refinement and technical validation. Specific goals include:

- Develop and validate (technically and clinically) procedures that determine the pattern and magnitude of viral load in HIV infected persons in the natural history setting as well as in the course of intervention or as a component of a sensitive surveillance program for preventive strategies.

- Provide a resource for the development of customized strategies for the quantitation of virus as required by Vaccines for Prevention, Drug and Gene Therapy and Vaccine Therapy Program Areas.

Background:

The current assay system for the quantitation of virus from clinical specimens has been extensively validated in terms of its technical performance. The assay system has the greatest working dynamic range of any assay of its type currently available. Reproducibility, accuracy and sensitivity are all state of the art and have been assessed in blinded head to head studies of assays used in 6 leading laboratories as part of an ACTG effort to sample all currently available procedures. The liquid hybridization (LH) assay employed by the MMCARR is rapid, of moderate throughput, but still requires individual tube set up and the use of isotopes. Development of a third generation assay would provide high throughput and use of fluorescent probes in place of isotopes. Details of work and plans within this unit follow:

Refinement of current LH PCR Technology-

Significant progress has been made in the refinement of current LH PCR technology to meet specifications of clinical and field trials (new primer schemes and chemistries). (Patent submitted on November 17, 1993). During 1993, this technology has been validated. A review of the validation is outlined below along with specific attributes of the technology.

•Technical Validation:

- dynamic range of five to six logs.
- accuracy - 100% on positive and negative clinical samples.
- precision - within 5% across the range.

•Performance Validation: in comparison with 12 laboratories in an ACTG blinded assessment of 6 state of the art methodologies, determined as:

- best accuracy and precision.
- greatest dynamic range.
- best conservation of clinical specimen.
- only fully automated data management system.
- second in rate of sample throughput.

•Unique features of LH-PCR Methodology:

- Rapid: 24 hour turn around time.
- Sensitive:
 - Accurate to 2 copies/100K cells and to 10 copies/ml plasma.
 - Dynamic range of five (DNA) to six (RNA) logs.
- Specific: Single band PCR product detected by internal probe.
- Micro-scale:
 - Requires only 10ul DNA, RNA, from 1 million PBMC.
 - Requires only 50ul plasma and serum.
- Universal
 - Tested and applicable to cells, tissue, plasma and serum.
 - Tested and applicable to North American and Thai isolates.
- Quantitative: External cloned template standardization.

Additionally, in this project area, a PCR assay for the detection of the 215 mutation, a marker of viral resistance to antivirals, was developed in collaboration with SRA.

Development of a fluorescent based liquid hybridization assay-

This project plans to develop a field deployable non-isotopic assay for the quantitation of HIV in clinical specimens.

Significant Results:

Work has not been initiated as of this report date.

Development of a high throughput, plate based fluorescent assay-

This project is in the development phase. A 96 well plate based fluorescent assay will be developed to support high volume trials, such as Phase III clinical trials. This assay will detect gag HIV DNA and rRNA sequences.

Significant Results:

None to date, at the time of this report.

Work Unit Three: Surveillance of Tri-Service HIV Incidence and Disease Progression Within US Military Populations

Background:

Characterization of the innate pattern and magnitude of viral load and other potential clinical markers is fundamental to the assessment of the alteration of such parameters as a result of interventions. Detailed analysis of patterns in viral load in the blood and tissue of HIV infected individuals in the natural history setting can potentially yield information on the progression of disease. Analyses of the features of early stage disease over one year intervals may elucidate patterns of the viral burden and other markers that would predict disease course for 5-10 subsequent years.

Detailed studies of confirmed seroconvertors during the initial acute syndrome have been attempted by several investigators. Dr. Shaw, whose efforts in this area are supported by a Division of Retrovirology grant, points out that such studies are limited by the difficulty in logistically locating and following, on a daily basis, enough patients to make meaningful conclusions as to the inherent nature of the syndrome. Furthermore, patients who are identified for such studies are usually those who present with severe and unusual symptoms and may not truly represent the majority of patients in terms of acute syndrome or disease course. Perhaps as a result of such factors, preliminary studies of a limited number of patients indicate a disappointing relationship between features of acute syndrome and disease outcome. Most patients characterized have similar viral loads overall which fall within about 4-6 weeks of seroconversion.

While the MMCARR may have a greater chance of locating and productively following acute seroconvertors at appropriate time points, there may be other cohorts of patients, more readily available, in which data characterizing features of HIV disease that may act as prognostic of disease course, could be attained.

Dr. Robert van Gemen, a colleague of Dr. Jaap Goudsmit, shared some unpublished data with the Program Area coordinator on a pilot study of three years duration on a cohort of 30 early stage (Walter Reed Stage 1) HIV infected, intervention naive, patients. Plasma viral load was followed for 6 month intervals over the study period. Results indicated that of 21 patients, the majority, exhibited no change in viral load over the study period (defined using the NASBA technique of viral detection as less than 0.1 log over the study period). Nine patients exhibited a 1.0 log or greater decline in viral load and were classified as slow progressors by criteria of a reduced slope of CD4 decline over the period when compared to the group of 21 patients.

In a workshop held by the NIAID on 31 August, 1993, Dr. Michael Busch, Irwin Memorial Blood Center, San Francisco and Dr. Donald Francis, Genentech, presented unpublished data on pilot studies on small numbers of patients (35-55). The results presented indicated that the pattern of viral load in early stage HIV infected, intervention naive, patients followed at 6 month intervals over a two year period is characterized by three plateaus in viral DNA in PBMC. The three plateaus range between 0.25 log and 1.5 logs in DNA viral load and are associated with differences in progression rate as defined by the slope of CD4 decline over the study period. The three plateaus were described as: slow

progressors, rapid progressors and non-progressors (distinct from slow progressors).

Dr. John Hamilton, Duke University, and Dr. Richard Koup, Aaron Diamond AIDS Research Center, NY, presented preliminary data on plasma viral load levels and PBMC RNA levels in small, early stage, intervention naive cohorts followed at 6 month intervals for 24 months. The data suggested that the pattern and the magnitude of the plateau of viral load established over the two year period is indicative of disease progression as defined by rate of decline in CD4 cell number over the study period.

These preliminary observations suggest that the features of viral load established over a 12 month period in early stage disease may be indicative of disease course. Collation of the many types of data already assembled on the RV 1 and RV 2 cohort by investigators at WHMC, WRAIR and NNMCC, and the initiation of viral load analyses and other studies on defined populations of such patient material offers an excellent opportunity for the MMCARR to achieve a meaningful analysis of the natural history and progression of HIV disease.

Goals:

The third work unit, comprising 40% of effort, will utilize RV 1 and RV 2 as a basis on which to formulate hypotheses for analytical, as opposed to descriptive, studies of the natural history of HIV disease progression within the paradigm of defining what clinical and virological features of the initial events in the establishment of HIV disease (the 12-24 months following seroconversion) are prognostic of the ultimate disease course and outcome. Specific goals and projects proposed within this work unit are outlined below:

Characterization of the Natural History of HIV Disease Incidence and Progression-

Plans:

This project will utilize the specimen and clinical data bases residing in the RV1 and RV2 repositories. Areas of interest in this project are the first 12-24 months following seroconversion and the establishment of HIV disease as correlates of outcome.

Derivation of the patterns and magnitude of clinical and laboratory markers in the natural history of HIV Disease-

Plans:

This project will seek to facilitate interpretation of the performance of prevention and intervention strategies and will work to identify the potential of markers to prognosticate disease course and outcome.

Exploration of Immunologic features of HIV Disease establishment and progression-

Plans:

This project will focus on the characterization of the cellular immune response in rapidly progressing and non-progressing HIV infected persons in a Natural History setting. Plans include:

- Execute longitudinal analysis of phenotypic and functional T Cell characteristics.
 - Execute longitudinal analysis of MHC-unrestricted cytotoxic lymphocyte characteristics.
 - Determine the relationships to the immunological features of early stage disease to clinical endpoints.
-

Exploration of Virological features of HIV Disease establishment and progression

Plans:

This project will focus on the characterization of the innate pattern and magnitude of viral load in the blood, and tissue in the natural history of HIV disease. Specific goals include:

- Determine the phenotypic characterization and patterns of viral load T cells in rapidly progressing and non-progressing HIV infected persons.
- Execute characterization of viral load and viral genotype and phenotype in clinical isolates of rapidly progressing and non-progressing HIV infected persons.

--Determine the relationships of virologic features of early stage disease to clinical endpoints.

Characterization of specific demographic populations: age, sex, racial parameters-

Plans/Activity:

This ongoing effort will pursue characteristics of specific demographic populations.

Significant Results:

The RV-1 database recently demonstrated that in this US Military natural history cohort there were a minimum of differences in disease progression among ethnic groups.

Clinical Trial Modeling and Analysis

Plans/Activity:

This project will study the derivation of clinical parameters from the Natural History Protocols, RV1 and RV2, relevant to the conduct and analysis of trials. Specifically, this project will:

--address mechanisms for the retrieval of clinical endpoints in patients lost to followup in RV 1.

--determine the effect of the expanded AIDS case definition on the prevalence of AIDS in RV1.

--design multivariate models for predicting progression to AIDS and survival. (Manuscript submitted for publication by Dr. Blatt at WHMC in November of 1993).

Significant Results:

In progress.

II. Descriptive clinical studies:

Coordination of clinical data generated from the Natural History and Core Diagnostic Protocols (RV1 and RV2) with the

aforementioned laboratory efforts provide further resource information and valuable longitudinal data for all HIV research Program Areas.

Updates of these two cornerstone protocols are provided below:

RV-1 Natural History of HIV Infection in military infected medical beneficiaries"

and

RV-2 Core Diagnostics Protocol (Adults)

Background:

- HIV infection is a chronic disease with a median time from infection to disease of about 10 years. The predictive markers and cofactors associated with disease progression have not been defined. An understanding of factors that promote or retard disease progression is crucial to designing more effective therapies. Predictive markers may be surrogates of key clinical outcomes, permitting more rapid assessment of the efficacy of experimental therapies.

- The natural history of HIV infection has not been completely defined. Furthermore, the clinical course of HIV disease has been changing with the introduction of new therapies and the demographics and risk profiles of HIV-infected persons. Understanding HIV natural history, interpreting the impact of therapeutic interventions, and understanding disease pathogenesis are critical for evaluating therapeutic developments and interventions.

- In the absence of fully predictive animal models, both clinical-based and population-based observational studies will be needed to continue to provide the appropriate, well-characterized clinical and laboratory samples required for basic research into the pathogenesis of HIV infection.

- Population-based research is essential to evaluate the efficacy and effectiveness of intervention strategies designed to decrease or eliminate HIV transmission. Strategies that can be evaluated in population-based clinical trials include prophylactic vaccines, other biomedical interventions (e.g., topical microbicides), and educational/behavioral interventions.

- The spectrum of HIV-associated disease continues to evolve. Natural History studies are needed to identify new problems (e.g., drug-resistant tuberculosis) and help focus

the intervention-oriented research agenda on the areas of greatest urgency and/or need.

The RV1 & RV2 protocols are part of a Tri-Service effort to address these issues. Participating medical centers include Walter Reed Army Medical Center (WRAMC), Washington D.C.; Brooke Army Medical Center (BAMC), San Antonio, Texas; Wilford Hall Air Force Medical Center (WHMC), Lackland AFB, Texas; and National Naval Medical Center (NNMC), Bethesda, Maryland.

RV-1 is the primary natural history study and is now into its fifth year of data collection. This protocol has collected data on 1,930 active patients (385 patients have died, 268 have been lost to followup and 106 patients have DoD benefits). Information is collected during each staging visit for the HIV infected patient and maintained in a database. The information is then utilized for multiple purposes and protocols. Unique aspects of the database include documented clinical and laboratory data, stored serum and cells, significant number of seroconverters, and a radically mixed population with equal access to health care which serves as a reference source for other research Program Areas and protocols.

RV2 - "Core Protocol for HIV Developmental Diagnostics (Adults)" -

Protocol Description:

The purpose is to develop and evaluate new and/or improved laboratory methods for establishing the diagnosis of HIV infection and for determining the stage of illness. Various methods are utilized and developed to detect replicating HIV virus, HIV antigens and HIV nucleic acids. Methods include virus culture, antigen capture immunoassay, and polymerase chain reaction (PCR) amplification of HIV DNA.

Samples to date:

As of May 1993, 2,651 patient samples were received. All specimens were processed to provide sera, plasma and cells for analysis which consisted of 3,183 co-cultures for HIV. PCR was performed on 460 samples for HIV and 91 for HTLV. Radioimmunoprecipitation was performed on 1,046 HIV and 418 HTLV sera samples. An acid dissociative technique was developed for the performance of 1,932 p24 antigen assays by ELISA. 280 assays for anti-p24 antibody were conducted. All samples are retained in a repository for future use.

The Natural History database is currently being utilized to test hypotheses critical to the understanding of HIV. One of the major quests is finding surrogate markers that predict clinical outcome. Several studies have found that CD4 counts are not predictive of outcome, especially if patients are on anti-retroviral agents such as Zidovudine. Markers that are being considered include viral phenotype (conversion to a more virulent variant), Zidovudine resistance and viral burden as measured by quantitative PCR. The answer to these questions require a well described clinical database and saved serum and white cells, which the RV1/RV2 protocols provide.

New clinical findings occur routinely within the HIV disease process. An example of this is the recently reported association of lactic acidosis and fatty liver with Zidovudine usage. Principal Investigators are presently assessing the RV1/RV2 database, looking for this association, as requested by the FDA. Other clinical studies are being done which may or may not corroborate the present literature. Additional areas where RV1/2 were recently utilized:

- To validate and revise the Walter Reed Staging System.
- To develop a panel of seroconverters to look at SI/NSI phenotype, drug resistance and genetic subtypes.
- To select patients for evaluation of surrogate markers of rapid/slow progression.
- To screen patients for entry into RV 79 and other planned protocols.
- To demonstrate in association of Hypereosinophila and HIV-1 disease.
- To compare 1987 AIDS definition with 1993.
- Data utilized for multiple presentations and lectures by MMCARR researchers.
- Database and clinical labs recently utilized for search in nucleoside induced mitochondrial dysfunction.
- Data analysis has demonstrated that, in the U.S. Military HIV-1 infected population a minimum of differences exist among races concerning progression of the disease.
- Assessed the prevalence of lymphomas in the RV-1 populations.

- Completed Humoral immune response to gp 160 epitopes in rapid and nonprogressors.
- Completed Humoral immune response to the V3 region of gp 160 in rapid vs nonprogressors.
- Completed Effect of chronic hepatitis B antigenemia on progression of HIV disease.
- Completed Legionnaires' Disease in HIV-infected patients.
- Completed False positive syphilis serology and relationship to anticardiolipin antibodies.
- Completed Prognostic value of Oral Hairy Leukoplakia (OHL).
- Completed Evaluation of baseline variables as predictors of progression to AIDS and death.

Future areas of focus for the RV1 and RV2 protocols will include:

- Selection of patients to validate surrogate markers.
- Validate disease progression models and further delineate the natural history of HIV.
- Select patients for entry into interventional protocols.
- Place emphasis on seroconverters to look for viral resistance, SI/NSI, genetic diversity, and differences in progression.

Other Natural History protocols also contribute to expanding our knowledge of the course of HIV-1. Descriptions and results of these protocols can be found in the text below:

RV 5 - "The Natural History of the Oral Manifestations of HIV Infection in a US Military Population "-

This protocol is conducted within an interagency agreement between the National Institute of Dental Research and the USAMRDC. A technical and cost proposal was recently submitted for the proposed extension of the Interagency Agreement.

Protocol Description:

The purpose of this protocol ("RV-5") is to determine the prevalence, incidence and risk factors for oral

manifestations of HIV infection with relation to the degree of immunodeficiency. This study was designed to develop longitudinal data based upon biannual examinations. It is unique in that the patients represent a wide range of early to late stage HIV-1 infection. The protocol was initiated in April 1989 at the Walter Reed Army Medical Center. Volunteers received a comprehensive oral and dental examination at study entry and every six months thereafter. The evaluation included clinical examinations for periodontal diseases, dental caries, and oral mucosa conditions. Samples of saliva and subgingival dental plaque were collected at each visit for microbial and biochemical assays, and a questionnaire on oral health related behavior was administered.

Significant Findings to date:

This protocol met its enrollment goal of 1,000 patients in the Spring of 1993. Patient follow-up at six month intervals has continued since that time. This protocol has had approximately 3,000 protocol patient visits total. Data has been analyzed in relation to the subjects' medical condition and immune status. A preliminary analysis of over 1,000 patients determined that the prevalence of HIV-related oral mucosal lesions was 32 percent at baseline and 44 percent after 6 months of follow-up. About 30 percent of those who were initially free of mucosal pathologies developed lesions within six months. Oral candidiasis was the condition that developed most frequently, with 70 percent of incident cases being of the erythematous form. Prevalence of mucosal diseases was clearly associated with depleted CD4 counts. This protocol has resulted in 12 abstracts, 37 presentations, 5 publications and 4 Book Chapters.

On April 14, 1993 an expert team was assembled to review the progress of RV-5 to date. Dr. Phillip Swango, Principal Investigator for the study, reviewed data for the team, as well as the resulting research productivity. Final recommendations included continuation of the protocol for a minimum of 12 additional months to continue to capture essential longitudinal data.

Program Plans for 12 month extension:

Continuation of this unique longitudinal study is critical to the overall success and value of the data collected thus far. Key areas of focus during this extension will include, but not be limited to,

the assessment of periodontal changes relative to Walter Reed Staging and CD4/CD8 counts, as well as other co-factors, such as smoking. Assessment of soft tissue oral mucosal lesions and salivary gland parameters will be two additional major areas of study; changes will also be noted with advancing disease stage and declining CD4/CD8 counts. Ancillary studies will be conducted to include the longitudinal monitoring of additional oral conditions.

RV 18 - "Dermatology Natural History Study"-

An Interagency Agreement between the NIAMS and USAMRDC was terminated and the final report for this protocol was submitted in September of 1993. Although the clinical portion of this study is complete, the pathological specimens review and further evaluation of the data collected will continue involving microscopic slide review of skin biopsies performed during the conduct of this protocol.

Protocol Description:

Cutaneous disease is common in patients infected with HIV-1. The aim of this study was to see if the researchers could identify cutaneous markers of disease and of disease progression as measured by Walter Reed stages (WR). For 42 months, 912 HIV-1 patients were followed in all Walter Reed Stages. All patients had an extensive past and present medical history taken as well as a complete physical examination, periodic follow-up visits, and appropriate diagnostic procedures.

Significant Findings:

Increasing dryness of the skin (65%) and seborrheic dermatitis (46%) were early findings in a large percentage of patients in WR1, and both conditions increased in incidence and severity with disease progression. Tinea infections (61%), condyloma acuminata (16%), and verruca (19%) were seen early, but with disease progression, (although there was no clear increased prevalence), these infections became more diffuse and resistant to treatment. Flares in acne vulgaris (30%) and folliculitis (20%) showed a peak in early and mid stage disease with a decreased occurrence in late stage disease. Herpes simplex infections (14%), oral candidiasis (13%), molluscum contagiosum (6%), *S. aureus* infections (5%), and oral hairy leukoplakia (15%) showed a marked increase in

occurrence with advanced disease. Conditions which had a statistically significant association with disease progression as measured by a change of stage included: drug eruptions, seborrheic dermatitis, oral candidiasis, oral hairy leukoplakia, molluscum contagiosum, herpes-zoster, and hyperpigmentation (nail, oral, skin).

Final Summary:

This study showed the most frequent and persistent cutaneous disorders were asteatosis (with or without asteatotic eczema) and seborrheic dermatitis. Conditions which were associated with a change in WR stage included drug eruptions, seborrheic dermatitis, oral candidiasis, oral hairy leukoplakia, molluscum contagiosum, herpes-zoster, and hyperpigmentation (nail, oral, skin). In addition to Kaposi s sarcoma (KS), patients with HIV-1 disease have increased potential to develop both cutaneous epidithelial and probably melanocytic malignancies. Epithelial tumors were seen in patients in all Walter Reed stages of disease.

This protocol has resulted in 25 publications, 2 book chapters, 18 abstracts and 20 presentations.

RV 22 - *"The Clinical Presentation of HIV Infected Patients at Walter Reed Army Medical Center"* -

Protocol Description:

This protocol evaluates clinical and laboratory data on the first 400 adults seen in the WRAMC clinic who are infected with HIV-1 by retrospectively reviewing their records.

Significant Findings;

CD4 counts decrease with time in an exponential fashion. Life is prolonged with AZT +/- or pneumocystis prophylaxis. With these therapies CD4 cell counts do not correlate with prognosis. Other prognostic markers are needed in these patients. To date 172 charts have been reviewed.

RV44 - "Effect of HIV infection on the initial manifestations and response to the treatment of syphilis; a CDC-sponsored collaborative study"-

Protocol Description:

This protocol is a collaborative effort with the CDC and Centers at WRAMC, NNMCM, Philadelphia, Baltimore, Brooklyn, San Francisco and Houston. This study was conducted to assess whether HIV infected patients are at increased risk of failing treatment for early syphilis and to assess the effect of an enhanced therapy regimen. It is a multi-center, randomized, double-blind, treatment trial comparing usual (Penicillin G benzathine 2.4 mu I.M.) with enhanced (usual plus amoxicillin 6 g/d and probenecid 1.5 g/d for 14 days).

Results to date:

Of 330 subjects (56 HIV+ and 274 HIV-), 179 have been followed for 3 months, 11 for 6 months. HIV+ and HIV- subjects were of similar age, race, and syphilis stage. For those consenting to lumbar puncture, HIV+ subjects were more likely to have CSF WBC $>5/\text{mm}^3$ (7/20 vs 6/44), but not a reactive VDRL-CSF or CSF protein $>50 \text{ mg/dl}$. Therapy was well-tolerated, though 18% of enhanced vs 9% of usual therapy subjects reported diarrhea. HIV+ and HIV- subjects did not differ in clinical response to enhanced or to usual therapy. Initial serological titers were higher for HIV+ subjects (geometric mean 1:79 vs 1:55) but declined similarly (mean decline in 2-fold dilutions at 3 and 6 months, 2.7 and 3.3 for HIV+ vs 2.7 and 3.1 for HIV- subjects). A trend toward greater serological response was seen for enhanced vs usual therapy (mean titer declines at 3 and 6 months, 2.9 and 3.6 for enhanced vs 2.5 and 2.7 for usual therapy; 2-fold titer decrease and titer $>1:64$ at 6 months occurred in 5% of enhanced vs 15% of usual therapy subjects, $p=0.09$).

Conclusions:

Clinical response to treatment with either regimen was similar for HIV+ and HIV- subjects; no clinical failures were observed. These data suggest a trend toward greater serological response with enhanced therapy. If confirmed, this would suggest that usual therapy is not optimal and would add to concerns about the adequacy of current therapy for patients immunocompromised by HIV infection.

RV 64 - "Evaluation of Cardiac Function in Patients with HIV Infection" -

Total Number of Research Volunteers Enrolled:
250 HIV Positive Volunteers and 100 Control Subjects

Summary of Protocol:

Cardiac dysfunction is a common, but often unsuspected, complication of HIV-1 infection, contributing to morbidity and mortality. As many as one-half of ARC/AIDS patients develop myocarditis, ventricular dysfunction, conduction defects, or autonomic nervous disorders. No study has prospectively followed a large, early stage, HIV-infected population to determine the time of onset of these cardiac abnormalities in a time dependent analysis. This study is designed to

- (1) define the extent of cardiac dysfunction in early stage HIV infection,
- (2) to identify the best noninvasive method of detecting cardiac involvement and the optimal time to implement testing, and
- (3) to determine the prognostic significance of a given cardiac abnormality. This study should provide information for the military clinician regarding the appropriate timing and modality for screening active duty personnel for significant cardiac dysfunction. Additionally, identification of early stage cardiac dysfunction may suggest interventions to control or reverse progression of such dysfunction to maintain or improve force readiness.

Significant Findings:

No data analyses have been completed since the essential control cohort was just recently completed. The researchers have received the first 250 echocardiograms of HIV-infected persons. Thirty percent of which demonstrate echocardiographic abnormalities including 31% in the early stage (WR 1/2) subset.

RV 67 - "A Placebo controlled double-blinded study of the elimination of Staphylococcus aureus carriage in HIV infected patients with topical antimicrobial agents" -

Description:

The purpose of this protocol is to determine the efficacy of topical antimicrobial agents, mupirocin calcium ointment and chlorhexidine gluconate 4% foam in the eradication of *S. aureus* Nasal and skin carriage in HIV seropositive patients.

Significant Results:

No data analysis has been completed as patients are still being encoded into the study. To date, 63 patients have been screened and 15 randomized and treated. The intent in the study is to identify 100 patients who are stable *staphylococcus aureus* carriers.

Preliminary studies performed at the NNMC HIV research site demonstrated an increased *s. aureus* nasal carriage rate in HIV-infected subjects compared to non-HIV infected controls and increasing rates of recovery of *s. aureus* from the other skin sites. This increase in *s. aureus* colonization occurred at equal rates in all Walter Reed stages of HIV-1 disease. The researchers also noted an increasing problem with both deep soft tissue *s. aureus* infections and *s. aureus* sepsis. The relationship between carriage of *s. aureus* and subsequent development of infection serves as a basis for this study.

CONCLUDING REMARKS

The Intervention Assessment Program Area has provided a wealth of laboratory, clinical and natural history data which has facilitated the goals and objectives of the HIV Research Program. Data residing within RV-1 and RV-2 have recently proved to be of significant value to both the NIH and the FDA, in consideration of the issue of surrogate markers as well as the recent survey for nucleoside induced mitochondrial dysfunction. Information collected and technology developed in this program area will continue to benefit the HIV Research Program and ultimately the national HIV research effort. In brief review, major accomplishments since April, 1993, for the newest Program Area include:

1. Development of a quantitative PCR assay for DNA and RNA for which a patent was submitted.

2. MMCARR PCR assays have been included in head to head comparison with 12 ACTG laboratories covering 6 state-of-the-art methodologies.
3. Demonstration that North American PCR scheme is amenable to application on Thai samples.
4. Development of a PCR based 215 mutation detection system for use in support of chemotherapy trials.
5. Demonstration that 1.5 to 2 log changes in plasma viral load are correlated with development of resistance to nucleoside analogues (with Drug and Gene Therapy Program Area).
6. First encouraging news that viral load is correlated with outcome (with Drug and Gene Therapy Program Area).
7. First description of quantitation of virus load in patients on an immunotherapy trial (with Vaccine Therapy Program area).
8. Demonstration in the US Military HIV-1 natural history study that a minimum of differences exist between different races concerning progression of disease.
9. "Informal" acknowledgement from the NIH and the FDA of the tremendous value of RV1/2 and its ability to help address the issue of surrogate markers as well as other emergent issues.