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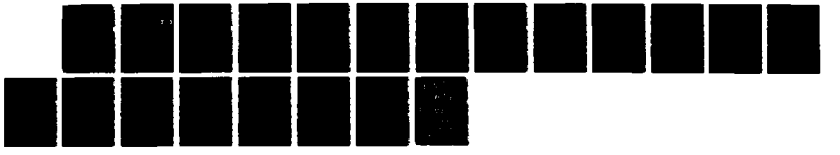

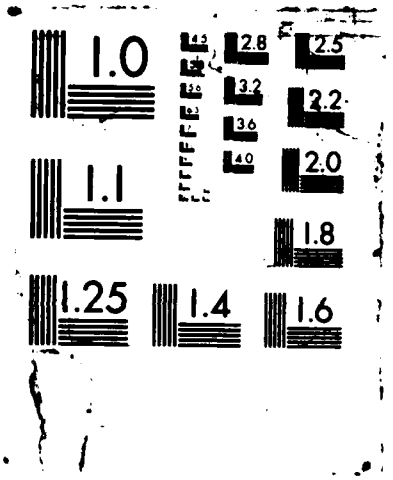
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Gp120 binding to CD4 requires beta-glycanase sensitive carbohydrate chains to retain specific binding ability. A 28Kd fragment of Gp120 has been identified with complete retention of CD4 binding capacity.

Human monocytes can be readily infected with HIV. In the absence of anti-HIV antibodies, phagocytic or non-phagocytic monocytes are equally sensitive. Anti-HIV antibodies at certain high dilutions may enhance HIV proliferation in human monocytes although exact conditions causing such enhancement are not yet defined.

Certain human HIV-seropositive individuals produce high titered antibodies which display molecular mimicry with CD4 and bind to Gp120. Such antibodies may tentatively be linked to good prognosis. Key words: Human Immunodeficiency Virus, Immunosuppression, Immunity

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# POTENTIAL BENEFITS AND HAZARDS OF HUMORAL IMMUNE REACTIONS AGAINST HTLV-III

ANNUAL REPORT

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SUMMARY

The aim of the present project is to define components within the humoral immune response against HIV which may act in a beneficial or detrimental manner for the host. Once such components have been identified, the long term goal is to establish optimal conditions for enlarging the positive and eliminating the negative reactions.

The project is primarily analyzing early events of HIV infection, that is the steps where the virus particles are interacting with the target cells. Defined radioassays allowing measurements of these reactions have been established and recombinant techniques have been applied to obtain viral envelope protein and cellular receptor protein in adequate quantities. Sera from HIV seropositive humans as well as immunized animals have been used throughout. Monocytes from normal donors as well as cell line monocytes have been used as defined subpopulations as targets for HIV in the presence or absence of anti-HIV antibodies. Fragmentation of viral envelope proteins have been performed allowing the localization of the cellular receptor-binding domain. A search for unique anti-HIV antibodies in infected people has been done looking for anti-envelope protein antibodies displaying in part molecular mimicry with the cellular receptor for HIV. Such antibodies have been found.

We conclude that it is possible to define regions within the HIV envelope proteins against which antibodies are expected

to be of positive value. Likewise, presence of anti-Gp120 antibodies where such antibodies in part may look like CD4 present themselves as interesting candidates for antibodies possible being linked with good prognosis. When phagocytic human monocytes are used as targets for HIV, certain sera from HIV-seropositive individuals will at high dilutions induce significant viral enhancement, that is incresed HIV proliferation. At low dilutions, the same sera inhibit HIV. It remains to be established using monoclonal antibodies whether there exist unique, beneficial or detrimental antibodies at the qualitative level.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

The investigators have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

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BODY OF THE REPORT

Defining structures on Gp120 responsible for CD4 binding capacity

Humoral antibodies reacting with the CD4-binding region of Gp120 from HIV should be group specific and blocking in simplistic terms. Evidence that such structures are present on a discrete region on Gp120 come from several studies involving introduced genetic changes in the envelope protein coding sequences (Laskey, Kowalski et al). Changes in the carboxyterminal region of Gp120 could thus be linked to a decrease in binding capacity of the mutated Gp120 to CD4. Such data indicate that changes in indicated spots are linked to a reduction in receptor binding capacity but this may be through direct relevance to the binding site or via allosteric changes. No group has reported on the production of fragments of Gp120 with retained CD4 capacity, which would be a way to directly demonstrate the localization of the conserved region on all HIV isolates so far tested (the region determining the CD4 binding part of Gp120).

We have carried out an initial screening of the requirements for the binding of Gp120 to CD4 prior to initiation of the present project. The most relevant point of information was the total requirement for retention of certain carbohydrate chains on Gp120 in order for CD4 binding capacity to be retained. We could thus conclude that only properly

glycosylated peptides from Gp120 are to be expected to be able to bind to CD4. In support for such a concept are the failures of any analyzed peptides derived from recombinant DNA work using prokaryotic systems involving Gp120 where no detectable binding to CD4 has been found. The claims that peptide T, an octapeptide derived from a sequence in one particular HIV isolate, can bind to CD4 have not been reconfirmed by a large number of groups including ourselves. In the present period we have thus started an analysis of the Gp120, correctly glycosylated, from the point of view of generating proteolytic fragments with CD4 binding. Using a battery of defined proteases and varying experimental conditions we have been able to create using V8 protease from S. aureus fragments from Gp120 with completely retained capacity to bind CD4. Table 1 summarizes the results obtained. In short, the minimum fragment so far obtained is around 28Kd in size, that is more than 75% of the envelope protein can be removed without affecting the CD4 binding capacity. As the average percentage of carbohydrates in Gp120 is around 50% it is likely that the peptide part in this fragment may be around 14Kd. Preliminary amino acid sequence data indicate that the 28Kd fragment is localized within the carboxyterminal part of Gp120, but the exact localization will be known shortly. In parallel we have already initiated experiments where defined DNA sequences in the Gp120 carboxyterminal region are inserted in eukaryotic expression systems to further narrow down the exact region responsible for CD4 binding ability.

Availability of such defined, correctly glycosylated peptides will be of great value in defining optimal antibodies in relation to blocking of HIV-CD4 interactions. It is also possible that deletion of certain normal neighbouring regions may allow a more focused antibody response in relation to group specific neutralizing antibodies.

Human monocytes as targets for HIV: Impact of phagocytic behaviour and presence of anti-HIV antibodies.

It is now well established that HIV can grow well in the human monocyte family of cells. The major route of entry under normal conditions is via the CD4 receptor, which is present on the surface of the great majority of these cells. The established fact that anti-viral antibodies in certain diseases may allow the virus to grow at enhanced rates in monocytes via immune complex mediated phagocytosis make it an important issue to analyze HIV in this context.

We have, before the initiation of this project, initiated a system to obtain normal human monocytes with defined phenotypes, alternatively obtaining clones from human monocytoid cell lines with stable, distinct phenotypes. Such clones have been shown to display highly select behaviour in relation to HIV infection, where the single most strongly correlation parameter to HIV susceptibility was the CD4

density on the cell surface. During the present we have in summary obtained the following results:

a) It is possible to define surface markers on human monocytes with positive or negative correlations in a seemingly close to absolute manner to phagocytic capacity or antibody-dependent cell mediated cytotoxicity (ADCC) and the M3 antigenic marker (Table 2). In a first series of studies we could show that in the absence of antibodies $M3^+$ phagocytic monocytes and $M3^-$ non-phagocytic monocytes are equally sensitive to HIV infection; that is, the general tendency for phagocytosis is not linked to a change in HIV sensitivity. The data also show that in contrast to vishna virus, there is no significant change in human monocytes according to their stage of maturation.

We have next done a preliminary series of studies exploring the sensitivity of infection of human monocytes and T cells from peripheral blood of normal donors in short term experiments excluding absolute (polybrene) and relative (Il-2) artefactual conditions from the in vitro system. Data in Table 3 indicate that under such conditions using our line of HTLV-IIIB isolate human monocytes, in particular if selected for adherance properties are at least as susceptible towards HIV infection as the T cell population. Within the T cell population there is a tendency for large T cells (activated ?) from normal blood to support HIV infection in a better manner than medium or small sized T lymphocytes from the same donor. Our data are thus in line

with the concept that under normal sexual transmission of the disease, monocytes may play the dominating role, that is being the first cell type infected. The data from our own studies and others that monocytes may also house viable HIV particles in a manner distinct from T cells in vivo and in vitro may also make monocytes from the infected donor and important factor during viral transmission. Along these lines we will carry out a series of studies where HIV carrying monocytes are killed by various immune protocols involving anti-HIV and other antibodies to see whether killing under such conditions is detrimental, that is enhancing spread of otherwise intracellular viral particles.

The central question whether antibodies against HIV will cause enhanced viral proliferation in human monocytes or monocytoid cell lines has been approached after the establishment of "normal" conditions for HIV infection in the absence of antibodies. Different concentrations of HIV particles have been tested for infectivity using 10-fold dilutions of sera from HIV infected people, making the dilutions in normal human serum to maintain identical concentrations of serum throughout. The degree of infectivity has been followed using two independent parameters: Triplicate reverse transcriptase measurements at each dilution step and time points or/and double blind readings as to the degree of cytopathic effects recorded. In parallel to using human monocytes as targets, other human cell types with Fc receptors for IgG/allowing enhanced

glueing to the cell surface of IgG-coated HIV particles/but devoid of phagocytic activity have also been tried. Likewise, murine macrophages with defined capacity to take up IgG-coated particles in the absence of a CD4-dependent binding (the murine counterpart to CD4, L3T4 does not display measurable affinity for Gp120 in our studies) have also been tested for capacity to support HIV in the presence of various concentrations of serum from HIV-seropositive individuals. In no experiments has polybrene been included.

The results, which still should be considered as preliminary due to the limited number of sera and experiments performed, are exemplified in Table 4. In one experiment it is shown that serum from HIV seropositive individual at no dilution caused any significant growth of HIV in Fc receptor positive, non-phagocytic, $CD4^-$ cells like our lines of K562 and Raji. Not shown are likewise negative data using the murine macrophages. However, a significant tendency for enhanced rate of appearance of HIV was noted at certain high dilutions of some HIV seropositive sera using as targets either fresh PBL:s from normal donors of cloned monocytoid cell line U937 cells as targets. At higher concentrations even when using the phagocytic clones of U937 HIV seropositive sera do normally inhibit HIV replication. Again stressing the preliminary nature of these results they do strongly support the view that under limiting conditions anti-HIV antibodies may have the paradoxical capacity to allow an enhanced proliferation of HIV in phagocytic

monocytes. What now will be done in these systems is to a) Obtain stable CD4 loss mutants within the phagocytic U937 clones. Clones obtained so far have not expressed stable losses, thus making interpretations of results fuzzy. b) Analyze in a stringent manner the enhancing elements present in certain HIV seropositive sera, that is shown their biochemical nature and antigen-binding specificity profile. c) Monoclonal antibodies against Gp120 and defined peptides of this protein are available from murine and human sources. Such antibodies will be used to delineate whether there are antibodies which will only have a possible enhancing nature, alternatively display a favourable blocking versus enhancing ratio in their functional behaviour. In some of these experiments using human serum antibodies as anti-HIV source the use of the 28kD Gp120 derived fragment in an immunosorbant column should provide an interesting tool to enrich for potentially primarily beneficial, blocking antibodies. Experiments designed to also explore the role of complement factors in possible enhancing situations will also be explored as these have been found to be of significance in other viral diseases linked to viral enhancement.

A search for human anti-HIV antibodies displaying molecular mimicry with the cellular receptor for HIV, CD4.

The fact that HIV is using an important molecule in the immune system, CD4, as its route of entry into the cells may

also have profound consequences for the specificity of the humoral antibody response against certain regions of Gp120. It is thus possible that certain anti-Gp120 antibodies may display molecular mimicry for the region of CD4 that binds to Gp120. If so, such antibodies may either be most efficient in blocking HIV attachment to CD4, alternatively they may through such mimicry deplete the normal, positive function of CD4 from $CD4^+$ cells, that is function as an immunosuppressive reagent. Anti-idiotypic antibodies against microbial antigens have also been shown to function as substitute vaccines in several experimental systems involving both viral, bacterial and parasitic disorders. It may thus be possible to use anti-CD4 antibodies to induce possible group specific neutralizing anti-HIV antibodies. Recent data from another group support such a possibility.

In our own approach we initially screened a battery of murine mouse anti-CD4 monoclonal antibodies for their capacity to block Gp120 binding to CD4. The best monoclonal, T4.2, was then used in a radioiodinated form to bind to $CD4^+$ cells using as potential inhibitors sera from normal or HIV seropositive individuals. Screening in such a manner 3 individuals (all HIV seropositive) were identified, whose serum in a selective manner did inhibit the binding of T4.2 to CD4. A series of experiments as summarized in Table 5 were next performed with serum from the individual with the highest blocking titer in his serum. In summary, the blocking material turned out to be IgG antibodies, reacting

with T4.2 antibodies and not with other anti-CD4 monoclonals thus behaving like natural "anti-idiotypic" antibodies for T4.2 and mimicking the determinant on CD4 that T4.2 reacts with. Most interesting, however, was the finding that tese human antibodies in fact did also display a dual function, namely a select binding to Gp120 from HIV. It is thus clear that humans infected with HIV may occassionally (around 5%) produce antibodies which in part may mimic CD4 and with Gp120 binding capacity. Attempts to prove if these human antibodies have a negative impact of normal CD4-dependent T cell proliferation in vitro failed to document such potentially immunosuppressive capacity. The antibodies were also inefficient neutralizing antibodies in realtion to HIV in vitro. When trying to correlate the rare occurrance of these kind of antibodies with clinical prognosis we have so far found that an individual seropositive for HIV in his earliest sample/early 1983/ and with a high titer of this unique anti-HIV antibody is displaying normal immune functions and has not moved towards disease using the Walter Reed staging system over the last 4 years. It is thus possible that such antibodies may constitute a marker for good prognosis in HIV infected individuals. What is here being done is to attempt to immortalize the antibody producing cells from this individual to obtain human monoclonal antibodies for further studies as to impact on HIV proliferation in relation to T and monocyte cultures.

Table 1. Creation of proteolytic fragments from Gp120 using V8 protease. Retention of CD4 binding capacity.

| | Molecular weight as assessed using SDS-PAGE (Kd) | | | | | |
|---|---|---|---|---|---|---|
| | 120 | 95 | 65 | 55 | 28 | 15 |
| Untreated | + | - | - | - | - | - |
| V8 protease | ± | + | + | + | + | + |
| CD4 binding | + | + | - | - | + | - |

Binding to CD4 specific as shown by blocking with anti-CD4 antibodies. CD4 binding requires retention of carbohydrate chains resistant to end F treatment but sensitive to beta-glycanase treatment (1).

Table 2. Phagocytic and non-phagocytic human monocytes are equally susceptible to HIV in the absence of anti-HIV antibodies.

| Normal blood monocytes | Phagocytosis | ADCC | HIV-susceptibility |
|---|---|---|---|
| $M3^+$ | 38 % | 1% | +++ |
| $M3^-$ | 1% | 28% | +++ |

Phagocytosis and ADCC determined using IgG-coated $^{51}Cr$-labelled chicken erythrocytes. % showing specific isotope uptake or release resp. HIV susceptibility = graded virus concentration, +++=similar sensitivity as HUT78, a susceptible human T cell line. M3 marker determined using the monoclonal OKM3 antibody. Sorting for $M3^+$ or $M3^-$ monocytes obtained using FACS.

Table 3. Relative capacity of peripheral blood mononuclear cell subsets to support HIV replication.

| Cell population | Mean reverse transcriptase (range) | |
|---|---|---|
| Unseparated PBL:s | 13.5 | (9.8 - 20.7) |
| Large T cells | 18.3 | (10.1 - 30.3) |
| Medium T cells | 6.8 | (4.5 - 9.8) |
| Small T cells | 8.7 | (7.2 - 11.2) |
| Adherant cells | 44.9 | (34.4 - 52.0) |
| $M3^+$ monocytes | 12.9 | (9.4 - 19.0) |
| $M3^-$ monocytes | 11.9 | (9.3 - 14.3) |

Reverse transcriptase at day 5 after infection. Range=Three different cultures. T cells separated on Ficoll-Isopaque. All cells from normal blood donor. HIV=HTLV-IIIB.

Table 4. Influence of HIV seropositive sera on HIV replication in various cell types.

Exp 1.

| Cell types | Virus dose | HIV serum dilution | Reverse transcriptase Day 6 | Day 13 |
|---|---|---|---|---|
| PBL | 1 | normal serum | 11.5 | 4.0 |
| -"- | 1 | 10% | 11.0 | 4.6 |
| -"- | 1 | 1% | 11.4 | 9.6 |
| -"- | 1 | 0.1% | 59.1 | all dead |
| -"- | 1:10 | normal serum | <1 | 4.4 |
| -"- | 1:10 | 10% | <1 | 7.7 |
| -"- | 1:10 | 1% | <1 | 21.6 |
| -"- | 1:10 | 0.1% | 1.4 | all dead |
| K562 | 1 | normal serum | 2.2 | <1 |
| -"- | 1 | 10% | <1 | <1 |
| -"- | 1 | 1% | 1.1 | <1 |
| -"- | 1 | 0.1% | 1.4 | <1 |
| Raji | 1 | normal serum | 2.8 | <1 |
| -"- | 1 | 10% | 4.3 | 3.1 |
| -"- | 1 | 1% | 3.1 | 1.0 |
| -"- | 1 | 0.1% | 5.2 | 1.4 |

Exp 2.

| Cell type | HIV serum dilution | Reverse transcriptase (CPE) 4 | 7 | 11 | 14 | 17 | 21 |
|---|---|---|---|---|---|---|---|
| U937, clone 2 | normal serum | <1 | 2$^{\pm}$ | 21$^{\pm}$ | 16$^{\pm}$++ | 34++ | 45++ |
| -"- | 1:5 | <1- | <1$^{\pm}$ | <1$^{\pm}$ | <1$^{\pm}$ | 1- | <1- |
| -"- | 1:25 | <1- | <1$^{\pm}$ | 10$^{\pm}$ | 18++ | 41++ | 65++ |
| -"- | 1:125 | <1- | 2+ | 15+ | 12++ | 131++ | 109++ |
| -"- | 1:625 | <1$^{\pm}$ | 3+ | 20+ | 15++ | 90++ | 140++ |
| -"- | 1:3125 | <1$^{\pm}$ | 9+ | 25+ | 16++ | 56++ | 106++ |
| -"- | 1:15625 | <1- | 6+ | 14+ | 4++ | 98++ | 89++ |

K562 and Raji=Fc receptor for IgG +, CD4$^{-}$, non-phagocytic. U927, clone 2= CD4$^{+}$, phagocytic, Fc receptor +. Exp 2 contained complement. All tests in same concentration of human serum. CPE=cytopathic effect.

Table 5. Certain human Gp120-HIV antibodies react with anti-CD4 antibodies.

a. Inhibition of T4.2 monoclonal antibodies binding to CD4 by human HIV positive sera.

| Serology | Clinical status | Number of individuals (+/tested) |
|---|---|---|
| $HIV^+$ | Asymptomatic | 1/24 (pos.sera=H306) |
| $HIV^+$ | PGL/AIDS | 0/12 |
| $HIV^+$ | Unknown (probably asymptomatic) | 2/22 |
| $HIV^-$ | Healthy | 0/25 (pool IgG also -) |

b. Specificity patterns of H306 inhibitory molecules

1. Will block only T4.2 antibodies and not 6 other murine anti-CD4 antibodies from binding to CD4. High titer= can be diluted 1:100.
2. Does not react itself with CD4 (=not auto-anti-CD4 antibodies).
3. Reacts with F(ab) region of T4.2 exclusively ("anti-idiotypic").
4. Consists of IgG molecules (can be purified on protein A Sepharose).
5. When purified on a T4.2-Sepharose immunosorbant H306 antibodies will react exclusively with Gp120 and Gp160 from HIV.
6. Will not block CD4 dependent T cell proliferations in vitro.
7. Is an inefficient HIV blocker in vitro in short term experiments.

Conclusion: The serum from a healthy, $HIV^+$ individual, H306, was found to over the entire observation period (1983-1987) contain high titered antibodies with a unique capacity to block a murine anti-CD4 antibody, T4.2, to bind to CD4. The H306 antibodies were also found to display another feature mimicking CD4, that is when purified on T4.2-Sepharose the bound and eluted antibodies react exclusively with Gp120-HIV.

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