ALLOIMMUNITY, AUTOIMMUNITY, AND AIDS

By

MICHAEL DAVID GRANT

B.Sc., The University of British Columbia, 1978

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Microbiology)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

August 1989

© Michael David Grant, 1989
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Microbiology

The University of British Columbia
Vancouver, Canada

Abstract

The sensitivity of the human immune system to the retrovirus HIV is difficult to explain on the basis of viral cytopathicity. AIDS develops often long after initial HIV infection in spite of a vigorous and sustained immune response against the virus which effectively contains viral replication. Although more virulent strains of HIV can be isolated from immunodeficient persons, there is no evidence that these strains predominate in vivo or that a large increase in production of infectious virus accompanies progression to disease. There is as yet no satisfactory mechanism to explain the immunosuppression, T4 cell depletion, autoimmunity, and immunodeficiency associated with HIV infection.

An hypothesis that AIDS results from immune responses to HIV gp 120 and to allogeneic cells stems from recent developments in the symmetrical network theory. It is proposed that T-cell selection processes produce a T-cell idiotypic network "centrepole," which bears an internal image of self MHC class II within T-cell receptors. This network centrepole interacts with the anti-class II helper cell repertoire to stabilize both its own expression and expression of the helper T-cell repertoire. The idiotypes involved in this mutual stabilization are related to certain idiotypes present on allogeneic lymphocytes and to the envelope glycoprotein of HIV gp120.

Through its relationship to CD4 protein, and hence class II MHC, HIV gp120 is thought to mimic the T-cell network centrepole. Anti-host class II receptors on allogeneic lymphocytes induce antibodies which also mimic the proposed centrepole. The immune response to gp120 is directed against the centrepole and against the antibodies (anti-anti-class II) induced by the receptors of allogeneic lymphocytes. The hypothesis evaluated in this thesis project is that the immune responses described above synergize in an attack on both the centrepole and the
helper cell repertoire, resulting in aberrant immune regulation, autoimmunity, and eventually, AIDS.

Sera from persons with AIDS or at risk of AIDS were examined for antibodies implicated in the above scheme. Anti-anti-CD4/anti-gp120 antibodies (putative anti-centrepole) were found in only a small minority of subjects and did not correlate with disease, while anti-anti-class II antibodies were almost never detected. Anti-MHC class I antibodies, reflecting alloimmunity, were associated with HIV infection and to some extent with disease progression. Autoantibodies against denatured collagen, reflecting autoimmunity, were found in almost all AIDS patients. The prevalence of these autoantibodies increases in HIV infection and with disease expression. Antibodies against denatured collagen show an interesting distribution which suggests they are related to the idiotypic determinants involved in the pathogenesis of AIDS and other similar immune disorders. The specificity of these antibodies suggests they arise through immunoregulatory defects induced through idiotypic network interactions.
Table of Contents

Abstract
Table of Contents
List of Tables
List of Figures
List of Abbreviations
Acknowledgement
Introduction
Materials and Methods
Results
Discussion
Bibliography

Abstract ii.
Table of Contents iv.
List of Tables v.
List of Figures vi.
List of Abbreviations viii.
Acknowledgement ix.
Introduction 1.
Materials and Methods 16.
Results 21.
Discussion 54.
Bibliography 69.
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Serum IgG Levels and Correlation Coefficients.</td>
<td>30.</td>
</tr>
<tr>
<td>II.</td>
<td>Mean ELISA OD values of group ± sem.</td>
<td>31.</td>
</tr>
<tr>
<td>III.</td>
<td>Significance Values.</td>
<td>33.</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anti-anti-CD4 ELISA values of homosexual, hemophiliac, and control sera.</td>
<td>22.</td>
</tr>
<tr>
<td>2.</td>
<td>Inhibition of anti-anti-CD4 ELISA with HIV gp 120.</td>
<td>23.</td>
</tr>
<tr>
<td>3.</td>
<td>Anti-anti-CD8 ELISA values of homosexual, hemophiliac, and control sera.</td>
<td>25.</td>
</tr>
<tr>
<td>5.</td>
<td>Anti-anti-gp 120 ELISA values of homosexual, hemophiliac, and control sera.</td>
<td>27.</td>
</tr>
<tr>
<td>6.</td>
<td>Anti-gelatin ELISA values of homosexual, hemophiliac, and control sera.</td>
<td>29.</td>
</tr>
<tr>
<td>7.</td>
<td>Inhibition of anti-gp 120 ELISA activity of AIDS sera with sera from autoimmune HIV- homosexuals and controls.</td>
<td>36.</td>
</tr>
<tr>
<td>8.</td>
<td>Titration of anti-gelatin ELISA activity.</td>
<td>38.</td>
</tr>
<tr>
<td>10.</td>
<td>Inhibition of anti-gelatin ELISA with human placental collagens.</td>
<td>40.</td>
</tr>
<tr>
<td>11.</td>
<td>Inhibition of anti-gelatin ELISA with fibronectin.</td>
<td>42.</td>
</tr>
<tr>
<td>12.</td>
<td>Immunoblot analysis of anti-collagen reactivity.</td>
<td>44.</td>
</tr>
</tbody>
</table>
Figure | Title                                                                 | Page |
---|---|---|
13. | Correlation of anti-gelatin ELISA values with serum IgG levels.     | 45.  |
15. | Inhibition of anti-gelatin ELISA with C1q.                           | 48.  |
16. | Immunoblot reactivity of purified anti-gelatin antibodies.           | 49.  |
17. | Non-specific binding of antibodies to C1q immobilized on nitrocellulose. | 50.  |
18. | Non-reactivity of purified anti-gelatin antibodies with the isolated collagen-like sequences of C1q. | 51.  |
19. | Augmentation of anti-C1q reactivity by IgG capture of C1q.           | 53.  |
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ach</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>AZT</td>
<td>azidothymidine</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>GVHD</td>
<td>graft versus host disease</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>kd</td>
<td>kilodaltons</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
</tr>
<tr>
<td>SRBC</td>
<td>sheep red blood cells</td>
</tr>
<tr>
<td>Tris</td>
<td>tris hydroxymethyl aminomethane</td>
</tr>
</tbody>
</table>
Acknowledgement

I gratefully acknowledge the support, encouragement, and guidance of Dr. Geoffrey Hoffmann, whose determination and insight made this project possible. Suggestions and evaluation provided by committee members Dr. F. Tufaro and Dr. J. Chantler have been invaluable towards completion of this project as has the cooperation and assistance of the management and staff of the B. C. Provincial Health Lab.

I thank Dr. Michael Weaver, Dr. Christos Tsoukas, and the B. C. Provincial Health Lab for providing serum samples and thank the NHRDP for financial support during the second year of my Master's program.
Introduction

Shortly after recognition of a novel acquired immunodeficiency syndrome (AIDS), a new human retrovirus (human immunodeficiency virus-1, HIV-1) was isolated from lymphocytes of affected individuals (1, 2). With the optimization of culture conditions and development of viral antibody and nucleic acid detection tests, HIV infection can now be demonstrated in essentially all AIDS patients. A clear association between HIV and AIDS is established, as is HIV's tropism for the T4 lymphocytes which disappear during progression to AIDS.

HIV uses the CD4 protein, expressed on the surface of lymphocytes, monocytes, dendritic cells and glial cells, as a receptor, and is cytopathic for some CD4+ cell lines under some in vitro conditions (3). Cell death in vitro occurs through expropriation of cellular metabolic machinery, cytolysis associated with the release of viral particles, or by fusion of cellular membranes (syncytia formation) (4-6). Infection and syncytia formation are inhibited in vitro by antibodies against epitopes of CD4 or HIV gp120 involved in the CD4/gp120 interaction (7, 8).

A number of regulatory genes found in HIV imply a complex mechanism of life cycle regulation. Transcriptional and translational enhancer and inhibitor elements complement the usual retroviral genes encoding structural, envelope, and catalytic proteins (9). Depending on the presence of proteins capable of interacting with HIV enhancer sequences, HIV may grow slowly, rapidly, or remain latent (10).

Although investigators generally agree on HIV as the cause of AIDS, the pathogenesis of AIDS is not simply related to the biology of HIV infection. Biochemical evidence does not support a direct role for HIV in the T4 lymphocyte depletion which characterizes AIDS. In situ hybridization and polymerase chain reaction studies reveal only 1/1000 to 1/10,000 T-lymphocytes infected with HIV at
any stage of disease (11, 12). There is also little evidence of in vivo cell fusion, especially of T-lymphocytes (13). A number of indirect mechanisms for HIV induced T4 depletion have been proposed, but no firm basis for this depletion and resultant immunodeficiency has been clinically or experimentally established. Some immunodeficiency arises even before depletion of T4 cells (14).

Infection with HIV occurs primarily via anal sex or parenteral exposure. In order to replicate and establish infection, HIV must contact permissive CD4+ cells found mainly in the blood and lymph. During acute infection, lymphadenopathy and other symptoms similar to those of mononucleosis often develop and there can be considerable production of virus (15, 16). At this stage, no significant depletion of T4 lymphocytes occurs, but the T4/T8 ratio decreases due to increased numbers of CD8+ lymphocytes (17). Symptoms accompanying primary infection usually resolve as a specific immune response develops, but HIV establishes a persistent, mainly latent infection. Despite the apparent success of the immune system in combattng HIV, some insidious form of damage to the immune system actually occurs from this point onwards.

Coincident with the development of HIV neutralizing antibodies, the absolute number of T4 lymphocytes begins to fall in HIV infected individuals. As the T4 number continues to fall, autoimmunity, neurological symptoms, immunodeficiency, malignancies, and opportunistic infections develop (18). There is no clear evidence that a rise in infectious virus titres precipitates these events, but increased serum levels of p24, the HIV core protein, tend to foreshadow or parallel clinical deterioration. Serum levels of β-2 microglobulin and curiously, neopterin, a marker of T4 lymphocyte activation, also tend to foreshadow AIDS onset (19). Paradoxically, infectious HIV becomes most difficult to detect in the final stages of AIDS (20).
The protracted course from infection with HIV to AIDS suggests slow persistent growth such as occurs with other animal lentiviruses. Success for these viruses depends on evasion of the immune system by antigenic variation, latency and reactivation, and or immunosuppression induced by a variety of mechanisms. HIV exhibits all these characteristics, but perhaps the most important in the pathogenesis of AIDS is the mechanism of immunosuppression. In relation to other human immunosuppressive viruses (eg. measles, cytomegalovirus, Epstein-Barr virus) HIV is present at extremely low levels (21). Since some immunodeficiency is seen prior to T4 cell depletion, an indirect mechanism of immunosuppression appears to operate at least early in infection. The nature of this mechanism is unknown, but may involve secreted viral proteins, induced or reduced secretion of cellular regulatory proteins, interference with antigen presentation, disruption of substituent T4 lymphocyte subsets, idiotypic network interactions, or some combination of the above.

The immunodeficiency of human HIV infection is especially mysterious in light of present animal models of infection. Chimpanzees are the only animal so far experimentally infected with HIV and although infectious virus is produced, no evidence of immunodeficiency is observed (22). Failure of HIV to induce disease in chimpanzees and the indirect mechanism of immunosuppression and T4 cell depletion in human HIV infection raises the possibility that HIV infection alone is insufficient to cause AIDS.

This possibility is also supported by the predominance of AIDS within particular risk groups. An important question is whether this predominance reflects only risk of exposure or whether it reflects risk group specific cofactors active in disease progression. In North America, AIDS is largely confined to blood product recipients, intravenous drug abusers, and male homosexuals (23). In Africa, the presumed continent of origin of HIV, infection and AIDS occur equally
in males and females. The primary means of HIV transmission appear to be exposure to a contaminated blood supply and heterosexual intercourse between carriers of other sexually transmitted diseases (24). Epidemiological studies on cohorts of HIV infected homosexuals in North America reveal no correlation between rate of progression to AIDS and exposure to other sexually transmitted diseases (25). Therefore, investigators acknowledge risk factors for HIV infection, but assume a similar prognosis for everyone once infection with HIV occurs. This assumption is, however, yet to be substantiated by epidemiological studies of cohorts of HIV infected persons not within the known risk groups. Despite agreement on overall average rates of progression to AIDS, there is tremendous individual variation in the clinical course of disease.

A number of proposed cofactors in AIDS pathogenesis relate to enhanced viral replication. These possible cofactors include immune stimulation, immunosuppression, virulence increasing mutations, and coinfection with pathogens capable of activating HIV replication, or with other strains or variants of HIV. The significance of any of these cofactors rests on the demonstration that increased viral replication causes the T4 depletion which characterizes AIDS.

The predominance of homosexual AIDS cases in North America is believed to reflect the route of introduction of HIV to the continent and extremely rapid early spread of the virus via anal sex and promiscuity. A corollary of this belief is that once the virus enters the general population, over time the proportion of heterosexual AIDS cases will gradually increase. So far, this is happening only within the intravenous drug abusing population, and it is still unclear to what degree HIV is distributed outside of the recognized risk groups. An important question remains whether the lack of AIDS cases outside the primary risk groups reflects low risk of infection or whether factors usually responsible for exposure to HIV also entail increased susceptibility to the effects of HIV.
HIV infection usually occurs concurrently with exposure to allogeneic proteins, including MHC antigens, lymphocyte receptors, and in some cases viable cells. Exposure to these agents induces an immune response which is theoretically directed against the immune response to the envelope glycoprotein of HIV, gp120 (26). It has been proposed that these immune responses include a response directed against immune system regulatory elements and that synergy between anti-HIV gp120 immunity and complementary immunity induced by allogeneic cells induces destabilization of the immune system idiotypic network. Some homosexuals, hemophiliacs, drug addicts, and transplant recipients show evidence of disturbed immune regulation in the absence of infection with HIV (27). Conceivably, this evolves from exposure to allogeneic cells and the immune response to allogeneic cells impels the pathogenicity of HIV through complementarity to anti-gp120 idiotypes.

There is considerable indirect evidence implicating the immune response in the pathogenesis of AIDS. AIDS develops despite humoral and cell mediated responses against HIV. Although profound immunodeficiency is the hallmark of AIDS, most patients show signs of prolonged immune system activation. Hypertrophy of lymphoid organs and lymphocyte hyperplasia occur early in HIV disease while hypoplasia and atrophy are late features (28). Despite the poor response of B and T-lymphocytes from HIV infected persons to mitogenic stimulation in vitro, polyclonal activation of B-lymphocytes is an early and sustained feature of HIV infection (29). Soluble factors which suppress the proliferation of T-lymphocytes are found in the serum of AIDS patients, yet activation of T-lymphocytes is necessary for HIV replication in these cells (30, 31).

Skin test anergy, first to new, and later to recall antigens, demonstrates the deficiency in cell mediated immunity associated with progressive HIV disease (32). Humoral responses to new antigens also decrease following HIV infection (33).
Infected T-cells display a selective defect in signal transduction through the CD3 protein and reduced production of, and response to interleukin-2. (34,35) Even uninfected T-cells from HIV infected persons respond subnormally in T-cell colony formation assays (36). HIV infected monocytes are defective in antigen presentation and release less interleukin-1 upon stimulation than uninfected monocytes (37, 38). Natural killer cell function and antibody dependent cell mediated cytotoxicity may also decrease in HIV infection (39, 40).

The initial fall in T4 lymphocytes was reported to occur specifically within the 2H4+ suppressor inducer subset (41, 42). There is a dramatic fall in this subset in severe burn victims who suffer immunodeficiency and this subset is also deficient in persons with systemic lupus erythematosus (SLE) (43, 44). A fall in the activity of this subset would likely amplify any direct effect of HIV on B-cell activation since, in vitro, 2H4+ T4 cells act via CD8+ T-cells to lessen immunoglobulin production by mitogen stimulated B-cells (45). Conceivably, this subset of T4 cells operates in vivo to suppress the production of autoantibodies, and depletion of this subset could precipitate autoimmune phenomena and immune dysregulation associated with HIV infection. Hence the loss of T4 cells could result in both a poor response to foreign antigens and increased autoimmunity. Although no biochemical basis for the selective depletion of this T4 subset has been defined, it may be that the migratory characteristics conferred by the cell surface proteins defining the specific T-cell subsets are involved.

Despite the progressive immunodeficiency of HIV infection, autoimmunity is a common and constant feature of HIV disease (46). Shortly after infection, serum immunoglobulin levels increase and circulating immune complexes and autoantibodies arise (47). These features tend to persist throughout HIV infection. Decreased hemolytic complement activity and depletion of specific complement components consistent with chronic complement activation also occur (48).
Similar serological and clinical features present in systemic lupus erythematosus, lepromatous leprosy, and graft versus host disease (GVHD). This suggests that these immune disorders reflect pathogenic features of HIV infection, specifically aberrant immune system regulation (49-51).

IgM and IgG autoantibodies against red blood cells, platelets, T and B lymphocytes, nuclear bodies, cardiolipin, and collagen occur in AIDS patients (52-56). Thrombocytopenia develops due to excessive clearance of platelets coated with immune complexes or antibodies against a 25 kilodalton protein in the platelet membrane (53, 57). Immune complexes eluted from platelet membranes of HIV infected persons are composed of anti-viral antibodies and anti-idiotypic antibodies, rather than anti-viral antibodies and viral antigens (58). The antibody nature of the immune complexes and the huge amounts of anti-gp120 purified from the serum of HIV infected thrombocytopenics (200-400 µg/mL) (58) strongly suggest the involvement of idiotypic network interactions in the induction of thrombocytopenia. Other clinical signs of autoimmunity include vasculitis, nephritis, encephalitis, peripheral neuropathy, and arthritis. Cutaneous and gastrointestinal manifestations also may result from autoimmunity (59).

Autoimmunity associated with HIV infection is treated with prednisone or even by splenectomy, and several independent groups have noted overall biological improvement in patients after splenectomy (60, 61). Nearly half of HIV infected persons treated with cyclosporin A responded with large increases in numbers of circulating T4 cells and all showed resolution of lymphadenopathy (62). Immunoglobulin therapy, used to treat thrombocytopenia and Rh factor incompatibility, was an early treatment for AIDS which produced some benefit (63). More recently, infusion of AIDS patients with serum from healthy HIV infected persons or with autologous plasma and lysed lymphocytes has produced clinical improvements such as weight gain, increased T4 count, and decreased incidence of
opportunistic infections (64, 65). Alpha-interferon has been effective in the treatment of patients with kaposi's sarcoma (66, 67). Although some treatments result in lower circulating levels of HIV antigens, the effects cannot be attributed solely to inhibition of viral replication. Clinical improvements may reflect direct effects on the immune system idiotypic network, and though so far temporary, suggest that damage is preventable or reversible if appropriate treatment is initiated at an early enough stage of disease.

Treatment of AIDS with azidothymidine (AZT), an inhibitor of reverse transcriptase, is now a common procedure. AZT prevents new infection by blocking provirus production and may to some extent also inhibit virus reactivation. AZT decreases the level of circulating viral antigen, reduces the incidence of opportunistic infections, and often dramatically relieves neurological symptoms. However the benefits of AZT are short-lived (68). There is no prolonged elevation of T4 levels or restoration of immune function and the toxic effects of AZT often become overwhelming within six months. Recently, AZT resistant strains of HIV have been observed after AZT treatment (69). Trials are beginning which employ AZT at earlier stages of HIV infection, but early results have produced little cause for optimism. There is definitely a pressing need for more effective, less toxic therapy.

A number of investigators have speculated on autoimmune pathogenic mechanisms of AIDS. It was first suggested that T-cells binding viral antigens could become bystander targets of the antiviral immune response (70). Other theories consider aberrant or anti-idiotypic immune responses resulting in immunity against MHC class II proteins or CD4 protein (71, 72). Immunological cross-reactivity has been demonstrated between HIV envelope glycoprotein and class II proteins (73, 74) and the concept of an anti-idiotypic response against the CD4 protein is consistent with observed immune responses against other virus receptor
proteins (75). Genetic predisposition to immune hyperactivity and autoimmunity hastened progression to AIDS in one cohort of HIV infected hemophiliacs (76). This also suggests the action of an (auto)immune component in AIDS pathogenesis. The most detailed autoimmune theory of AIDS takes into account the distribution of AIDS within specific groups and postulates that two immune responses are necessary to cause AIDS (77). This theory assumes a role for risk group specific cofactors and provides an explanation for the failure of HIV to induce AIDS in chimpanzees.

In this theory of AIDS pathogenesis, exposure to allogeneic cells is a critical cofactor for progression to disease. The theory is based on current knowledge of T-cell selection mechanisms and on complementarity observed between HIV gp120 and the CD4 protein. Class II restricted T-cells are selected in the thymus on the basis of weak complementarity between the T-cell receptor and self class II antigens. According to the network hypothesis, T-cells which regulate the activity of class II restricted T-cells will have receptors complementary to the anti-class II receptors. Selective pressure should impel convergence of regulatory T-cell receptors to shapes which can interact with the maximum number of anti-class II receptors. The dominant shape selected would be an internal image of class II, which mimicks class II proteins but is unique since it is encoded by different genes and not necessarily constrained to bind short peptides. An internally focussing T-cell idiotypic network results, providing mutual stabilization between diverse anti-class II T-cells and stringently selected regulatory T-cells with receptors mimicking class II proteins.

The important specificities in this scheme are self MHC class II, anti-class II T-cell receptors, and anti-anti-class II T-cell receptors. Normal regulation of the T-cell network is presumably maintained by the interaction of T-cells bearing the relevant specificities. In a murine model of autoimmune disease, anti-anti-self
class II antibodies precede the appearance of a number of specific autoantibodies (78). These anti-anti-class II antibodies could infringe on the postulated T-cell regulatory network by virtue of their specificity and induce disregulation and the production of autoantibodies.

Anti-anti-self class II antibodies can be induced in mice by immunization with allogeneic lymphocytes (79). Multiple alloimmunizations occur in blood product recipients, intravenous drug abusers, and homosexuals, therefore it is likely that people within these groups at high risk for infection with HIV also produce anti-anti-self antibodies. There is evidence for immunoregulatory disruption in persons within these groups even in the absence of HIV infection. Conceivably, disregulation occurs by the same process as in the autoimmune mouse model, and relates to anti-anti-self antibodies.

HIV gp120 induces antibodies which may be complementary to anti-anti-self class II antibodies. Gp120 is complementary to the CD4 protein which has class II proteins as natural ligands (80, 81). Regulatory T-cell receptors, HIV gp120, and the antibodies induced by allogeneic cells are all anti-anti-class II and MHC class II mimicking. Antibodies against gp120 could react with the regulatory T-cell receptors and the anti-anti-self antibodies. The anti-anti-self antibodies are potentially against receptors on CD4+ T-cells. The response to gp120 and the response to allogeneic cells are both potentially disruptive and are symmetrically directed against one another. Instead of mutual stabilization of the T-cell regulatory network by anti-MHC receptors and anti-anti-MHC receptors, mutual destruction by two complementary sets of antibodies could result. Therefore, synergy between two symmetrically opposed immune responses is proposed to provoke network destabilization, immunological abnormalities, and eventually, AIDS.
Several paradoxical aspects of AIDS are explained by this theory. Nearly all persons infected with HIV produce antibodies against gp120 which neutralize HIV in vitro. In vivo, however, these antibodies provide no lasting protection and may actually enhance the infectivity of HIV (82). The failure of HIV to induce AIDS in chimpanzees is expected in the absence of their exposure to allogeneic lymphocytes, and the extremely low viral titres observed in human AIDS patients are not inconsistent with this theory of pathogenesis. A consequence of this theory, if it is correct, is that people exposed to allogeneic lymphocytes who are vaccinated with HIV envelope glycoprotein alone, are at risk to develop AIDS. So far, in North America, AIDS is largely confined to persons exposed either to allogeneic cells or alloantibodies which cross the placenta. The corollary of this hypothesis is that HIV infected persons not exposed to allogeneic lymphocytes are at less risk to develop AIDS.

At the heart of this autoimmune theory of AIDS is the prediction of two symmetrically opposed immune responses; one a consequence of HIV gp120 and the other a consequence of allogeneic lymphocytes. Idiotypic complementarity between these two immune responses is consistent with current knowledge of T-cell selection and restriction mechanisms and of the HIV gp120/CD4 interaction. A number of hypotheses arise from this theory which can be evaluated serologically. Implications of this theory extend beyond AIDS to autoimmunity and immune system regulation in general. Evaluation of immune phenomena in AIDS, in the context of this theory and current concepts in autoimmunity, may shed new light on immune system regulatory processes.
Objective

The overall objective of this project was to identify particular idiotypes present in AIDS patients, persons at risk for AIDS and persons progressing to AIDS, which correlate with disease progression. Recent progress in the symmetrical network theory of immune regulation suggests that idiotypic links exist between alloimmune and autoimmune responses. Alloimmunity and autoimmunity are consistent features of persons within AIDS risk groups and it is suggested that these features amplify susceptibility to the effects of HIV. In the context of the symmetrical network theory, this amplification results from complementarity between anti-HIV idiotypes and the idiotypes linking alloimmunity and autoimmunity. We wished to identify autoantibodies specific to individuals within AIDS risk groups and probe for an idiotypic relationship between these autoantibodies, anti-HIV gp120 antibodies, and antibodies associated with alloimmunity.

Cross sectional analysis of serum samples, was employed to identify antibodies associated with the development of AIDS. If antibodies associated with AIDS are linked to alloimmunity this supports the contention that idiotypically mediated immune system deregulation occurring in experimental models of autoimmunity occurs in persons progressing to AIDS, and functions as an important cofactor in AIDS pathogenesis. If particular anti-HIV gp120 antibodies are associated with AIDS, the immune response to HIV will be implicated in immune system destruction. If idiotypic links between alloimmunity, autoimmunity, and anti-gp120 immunity can be demonstrated, this will support the hypothesis that complementary immune responses synergize to provoke AIDS through idiotypic interactions. The experiments done to evaluate the hypotheses regarding network interactions in the pathogenesis of AIDS can be grouped into four sections.
1. Anti-anti-CD4 Antibodies

In the network theory of AIDS, the immune response to gp120 is implicated in the context of opposition to the anti-anti-class II immune response to allogeneic cells. The relevant determinant of gp120 is therefore that part of the molecule that binds to the anti-class II protein, CD4. Previous work has shown that the gp120/CD4 interaction is prevented by certain antibodies against CD4 and that these antibodies bear an internal image of gp 120 (83, 84). If the immune response to this region is critical to the development of AIDS, then at some point in infection, all persons progressing to AIDS should make anti-gp120 antibodies which bind to the internal image of gp120 present on the monoclonal antibodies which prevent the gp120/CD4 interaction. By ELISA, sera were screened for antibodies against an appropriate anti-CD4 monoclonal and the specificity of the detected antibodies confirmed by inhibition with viral gp120. This was done to establish whether such antibodies are made and if they are associated with AIDS. Sera were examined for reactivity with the anti-CD4 monoclonal Leu 3a and the anti-CD8 monoclonal Leu 2a. Leu 2a is related to class I MHC by virtue of complementary to the natural ligands of class I and can thus be used to probe for antibodies against internal images of class I MHC antigens.

2. Anti-anti-class II Antibodies

The important complementary immune response to anti-gp120 is predicted to be anti-anti-class II and should be present in the serum of HIV infected persons progressing to AIDS and in non-HIV infected persons exposed to allogeneic lymphocytes. Sera were examined for antibodies reacting with a monoclonal antibody against the non-polymorphic region of the class II HLA DR antigen and
for antibodies against an anti-gpl20 monoclonal which prevents the CD4/gp120 interaction (85, 86). This monoclonal bears an internal image of the anti-class II region of CD4 so it can also be considered anti-class II.

3. Complementary Immune Responses

In HIV infected persons progressing to AIDS, it is postulated that two immune response symmetrically directed against one another are involved. During progression to AIDS, these immune responses act to destabilize the immune system network at a fundamental level. This suggests that there will be a strong convergence within the anti-gp120 immune response to antibodies bearing the relevant idiotype. Therefore, late in disease most of the anti-gp120 antibodies are expected to be anti-anti-anti-class II antibodies. Serum from AIDS patients was mixed with serum from non-HIV infected persons, who had been exposed to allogeneic lymphocytes and were expressing autoantibodies, in an attempt to inhibit the anti-gp120 ELISA activity of the AIDS serum and thus demonstrate the hypothesized complementary immune responses.

4. Anti-collagen Antibodies

From early experiments in this study, it was observed that antibodies prevalent in AIDS sera reacted with gelatin in commercial monoclonal antibody diluent. Since gelatin is denatured collagen, this probably represented antibodies against denatured collagen, which occur in other immune disorders similar to AIDS. Common antibodies in these disorders may reflect a common underlying idiootypic etiology. The prevalence and levels of this activity were determined in serum samples from distinct clinical subgroups to evaluate the relationship to
AIDS risk and AIDS development. Specificity of the antibodies for collagen was evaluated by anti-gelatin ELISA inhibition with purified human collagens and by immunoblotting. Cross-reactivity with the collagen like regions of the complement component C1q was similarly evaluated. Anti-collagen positive sera and affinity purified anti-collagen antibodies were examined for idiotypic relationship to anti-anti-class II and anti-gp120 antibodies implicated in the pathogenesis of autoimmunity and immunodeficiency.
Materials and Methods

Serum. Serum samples from 16 homosexual AIDS patients and 71 homosexuals not meeting clinical criteria for AIDS were obtained from the Vancouver Lymphadenopathy AIDS Study. Serum samples from 36 asymptomatic hemophiliacs were obtained from the B. C. Center for Disease Control. The sera from B. C. hemophiliacs had previously been tested by ELISA and western blot for antibodies against HIV. Serum samples from 19 HIV- and 12 HIV+ hemophiliacs at various CDC stages of disease were obtained from Dr. C. Tsoukas of Montreal General Hospital. Serum samples from 12 patients with clinically defined rheumatoid arthritis were obtained from the British Columbia Arthritis Center and control sera were obtained from 11 healthy laboratory personnel. All sera were heat inactivated at 60°C for 30 min. Sera not previously tested for anti-HIV antibodies was tested by commercial ELISA (Dupont HTLV-III ELISA) and by recombinant gp120 (Genentech) ELISA for antibodies against HIV.

ELISA Assays. Replicate wells of plastic flat bottomed 96 well microtitre ELISA plates (Dynatech Immulon) were coated overnight at 4°C with 50 μL/well of a 1 ng/μL solution of the desired antigen in carbonate buffer. Following overnight incubation, wells were individually rinsed 4 times with PBS plus 0.5% Tween 20. Residual binding sites were then blocked by a 90 min incubation with 200 μL of 5% fat free casein (BDH) in PBS. Wells were again rinsed 4 times with PBS Tween 20 and 100 μL of each serum sample diluted 1/50 in 1% casein was added to duplicate wells for 90 min. Samples tested against C1q were diluted in both 1% casein and 1% casein with 20 mM EDTA. Wells were then rinsed 6 times and 100 μL of alkaline phosphatase conjugated goat anti-human IgG (Dupont) diluted 1/400 in 1% casein was added to all wells for 60 min. Wells were rinsed a final 6 times and 100 μL of 1
mg/mL p-dinitrophenyl phosphate (Sigma) in 10% diethanolamine buffer was added to each well. After 45 min incubation OD 405 was read on a Dupont microplate reader. All steps other than overnight coating were carried out at room temperature. Specific ODs were calculated by subtracting the OD of each sample obtained in BSA (fraction IV, Sigma) coated wells from the OD obtained in the well of the test antigen. Samples were considered positive if their specific OD reading was 3 or more standard deviations above the mean specific OD of the control samples.

Antigens used in this assay include gp120 (Genentech), leu 3a and leu 2a (Becton-Dickinson), L234 an IgG1 class anti-human class II mouse monoclonal antibody, obtained from the ATCC, anti-gp120 and HTLV-III (Dupont), BSA, gelatin (J.T. Baker Chemical Company), human IgG, human placental collagens type I, III, IV, and V, fibronectin and complement component C1q (Sigma) and anti-human IgG (Technical Research Associates). Modifications to the basic ELISA procedure employed to measure serum IgG levels and binding to antibody bound C1q are described in later sections.

*Inhibition Assays.* Inhibition assays were carried out to assess the ability of human collagens, fibronectin, C1q, IgG, and DNA to inhibit the anti-gelatin ELISA and the ability of gp120 to inhibit the anti-leu 3a ELISA. Usually, various amounts of the potential inhibitors were added to different serum dilutions or dilutions of purified antibodies and incubated overnight at 4°C. ELISAs were then carried out as previously described except that the serum samples were incubated on the ELISA plates for only 30 min. Inhibition of the anti-gelatin ELISA by fibronectin was assessed by adding fibronectin to diluted serum immediately before standard ELISA testing. Collagens were obtained as lyophilized protein and were solubilized by
incubation overnight in 0.1 M acetic acid. Solubilized collagens were heat denatured at 60°C for 30 min. DNA was denatured by heating to 100°C for 10 min.

Antibody Purification and Analysis. Serum antibodies were precipitated with 50% (NH₄)₂SO₄, redissolved in PBS, precipitated with 40% (NH₄)₂SO₄, redissolved in PBS and exhaustively dialyzed against PBS. Dialyzed material was applied three times to gelatin-agarose (Sigma) affinity columns (equilibrated with PBS) and columns were washed with 3 volumes of PBS. Bound material was eluted with 3 volumes of 4M urea pH 7.3 in PBS and immediately dialyzed into PBS. Columns were washed with 3 volumes 8M urea PBS and 5 volumes PBS before reuse. Antibodies were purified from individual and pooled serum samples positive for anti-gelatin antibodies and from equivalent volumes of control serum.

Purified material from the equivalent of 1 µL serum was dissolved in non-reducing 2% SDS, 50% sucrose sample buffer, heated to 90°C for 3 min and electrophoresed on 6% SDS polyacrylamide gels. A continuous buffer system with 192 mM glycine and 25 mM Tris pH 8.3 was used for all electrophoresis and electroblotting. Replicate lanes from 6% gels were then either silver stained as in (87) or electroblotted onto nitrocellulose (BioRad) as in (88). Nitrocellulose strips were rocked for 60 min in 5% skim milk in Tris buffered saline pH 7.3 and then for 120 min with alkaline phosphatase conjugated goat anti-human IgG antibodies diluted 1/1000 in 5% skim milk. Strips were washed 3 times for 5 min with PBS 0.5% Tween and then incubated for 10 min with alkaline phosphatase immunoblotting substrate (89).

Human collagens were dissolved in sample buffer with 5% 2-mercaptoethanol, heated to 100°C for 5 min, electrophoresed on 6% SDS polyacrylamide gels and electroblotted onto nitrocellulose. Nitrocellulose strips were incubated for 60 min in 5% skim milk and overnight with serum or purified
antibodies diluted 1/500 in 5% skim milk. Development of strips was carried out as described above. Collagens were loaded onto gels at approximately 300 ng/lane.

**Measurement of Serum IgG.** Serum IgG was measured by ELISA. Plates were coated with polyclonal goat anti-human IgG antibodies and ELISA was carried out as stated except that serum samples were diluted 1/10^6 in 1% casein. Known concentrations of human IgG (Cappel) were run in parallel with serum samples to construct a standard curve from which serum IgG concentration was estimated.

**Reactivity Against Collagen-Like Sequences of Clq.** Clq was electrophoresed under reducing and non-reducing conditions on 6% and 12% polyacrylamide gels in the presence and absence of 5% 2-mercaptoethanol. The collagen-like fragments of Clq were obtained by pepsin digest (90), and electrophoresed on 12% SDS polyacrylamide gels under the same conditions. Transfer to nitrocellulose and immunoblotting with purified anti-gelatin antibodies was performed as described for collagens. Reduced collagen type I or III was electrophoresed and used as a positive control.

**Reactivity Against Antibody Bound Clq.** In order to determine if collagen-like antigenic epitopes of Clq were exposed upon binding to antibody, a modified ELISA was developed in which IgG antibodies were coated directly onto microtitre plates. Clq was then allowed to react with the bound IgG in the presence of purified anti-gelatin antibodies and control antibodies. Plates were coated as previously described with 500 ng human IgG (Sigma). Clq was added for 90 min at 500 ng/well in 1% casein in the presence of 0.5 μg/mL purified biotinylated anti-gelatin antibodies, or 5 μg/mL biotinylated normal human IgG. Biotinylation was carried out as in (91). Biotinylated material purified by gelatin-affinity chromatography
from normal sera was also used as a control in this ELISA. The ELISA was
developed as previously described except that a 1/400 dilution of avidin conjugated
alkaline phosphatase (Sigma) was used to quantitate the binding of biotinylated
antibodies to ELISA plates. Samples were also added to replicate wells coated with
500 ng of C1q or IgG alone.

**Complement Measurements.** Hemolytic complement activity was measured
in fresh sera from healthy volunteers by the rise in OD 415 following 60 min
incubation with antibody sensitized sheep red blood cells (SRBC) (Sigma) at 20°C.
From 5 to 40 μg purified anti-gelatin antibodies were added to 10 μL of test sera
diluted 1/15 in PBS and 150 μL sensitized SRBC to assess any effect on complement
activity. Manufacturer's instructions were followed for testing except that smaller
aliquots of SRBC were incubated with sera in eppendorf tubes. After incubation,
tubes were centrifuged for 10 min in an eppendorf centrifuge and 100 μL of each
supernatant transferred to individual wells of an ELISA plate. OD 415 was read
immediately after transfer on a microplate reader.

**Statistical Analysis.** Significant differences were assessed by the Student's t
test and correlation between different measures assessed by linear regression
analysis using the Statworks software program.
Results

Section I. Antibodies, HIV Infection, and Disease Progression

**Anti-anti-CD4 Antibodies.** One hundred and seventy-three serum samples were tested by ELISA for antibodies reactive with the anti-CD4 monoclonal antibody leu 3a. The samples included 10 controls, 38 HIV- homosexuals, 33 HIV+ homosexuals, 16 homosexual AIDS patients, 21 HIV+ hemophiliacs, 42 HIV- hemophiliacs, and 12 RA patients. Results of ELISA testing are shown in figures 1 and 4. Few homosexuals in any category express anti-anti-CD4 antibodies and there appears to be no correlation with disease progression. This suggests that anti-anti-CD4 antibodies are not associated with the development of AIDS. The finding that some HIV- homosexuals and hemophiliacs express anti-anti-CD4 antibodies suggests that some of these antibodies do not arise against HIV gp120, but more likely are related to exposure to foreign MHC class II antigens. Soluble purified HIV gp120 prevents only 30 to 40 per cent of the binding to leu 3a (figure 2) in either HIV+ or HIV- homosexual serum samples. Inhibition of anti-anti-CD4 activity of HIV- serum with gp120 suggests that antibodies against MHC class II antigens cross-react with HIV gp120. Thus, there is the potential for interaction between the immune response to MHC antigens and the immune response to gp120.

**Anti-anti-CD8 Antibodies.** The same serum samples as above were tested for reactivity with the anti-CD8 monoclonal antibody leu 2a. Results are shown in figures 3 and 4. In this case, there does appear to be a relationship between infection with HIV, disease progression, and expression of anti-anti-CD8 antibodies. The prevalence of these antibodies varies from 24% (HIV-) to 40% (HIV+) to 50% (AIDS) in serum from homosexuals (figure 3A). Anti-anti-CD8 antibodies probably result
Figure 1. Anti-anti-CD4 ELISA values obtained with individual serum samples within homosexual (A) and hemophiliac (B) subgroups, together with normal control sera. A number of hemophiliac sample values are not visible due to a net OD of zero.
Figure 2. Inhibition of anti-anti-CD4 ELISA reactivity with recombinant gp120. Gp120 inhibits some of the anti-anti-CD4 activity of serum from two HIV infected individuals diluted 1/1000 (A) and 1/250 (B) and of serum from a non-infected homosexual diluted 1/250 (C).
from exposure to foreign MHC class I antigens and our data suggests that such exposure increases the risk of infection with HIV and the risk of HIV related disease progression in homosexuals. A similar, but stronger correlation between HIV infection and anti-anti-CD8 antibodies was seen in hemophiliacs; prevalence in HIV- hemophiliacs (38%), prevalence in HIV+ hemophiliacs (90%) (figure 3B). The sera of hemophiliacs also expressed much higher levels of anti-anti-CD8 activity, presumably as a result of repeated exposure to concentrated preparations containing allo-MHC class I antigens.

Anti-anti-CD8 antibodies were found also in 25% of serum samples from rheumatoid arthritis patients (figure 4). The patient with the highest level of anti-anti-CD8 antibodies had serum drawn during a clinically active disease flare. The presence of anti-anti-CD8 antibodies in persons with autoimmune disease suggests a link between production of these antibodies and aberrant immune system regulation. One of three samples from persons with SLE also contained anti-anti-CD8 antibodies (not shown). These antibodies may be involved in, or signify, disease progression in HIV infected persons. This hypothesis is complicated by the significant correlation of anti-anti-CD8 antibody levels with serum IgG in homosexual AIDS patients and HIV+ hemophiliacs, although these factors are independent in other groups (table 1).

Anti-anti-class II Antibodies. Several monoclonal antibodies were used to test by ELISA for anti-anti-class II MHC antibodies in controls, homosexuals, hemophiliacs, and RA patients. Results using an anti-gp120 monoclonal which bears an internal image of CD4 are shown in figure 5. Low antibody levels, not significantly different from control levels, were expressed in homosexual samples of any category (tables II and III) and no correlation with disease progression was apparent (figure 5A). Hemophiliacs expressed higher levels of anti-anti-class II
Figure 3. Anti-anti-CD8 ELISA values obtained with individual serum samples within homosexual (A) and hemophiliac (B) subgroups, together with normal control sera. A number of hemophiliac sample values are not visible due to a net OD of zero.
Figure 4. Anti-anti-CD4 and anti-anti-CD8 ELISA values obtained with individual serum samples from rheumatoid arthritis patients. Cutoff lines indicate 3 standard deviations above the mean ELISA OD obtained with normal control sera analyzed at the same time, but not shown.
Figure 5. Anti-anti-gp120 (anti-anti-class II MHC) ELISA values obtained with individual serum samples within homosexual (A) and hemophiliac (B) subgroups, together with normal control sera. A number of hemophiliac sample values are not visible due to a net OD of zero. Serum samples from 12 rheumatoid arthritis patients were negative for anti-anti-gp120 activity (not shown).
antibodies (figure 5B), but the levels of these antibodies was significantly correlated with serum IgG (table 1). ELISA results using a monoclonal antibody (L234) against a non-polymorphic region of human MHC class II (HLA DR) were consistently negative (not shown). These results demonstrate difficulty in detecting anti-anti-class II activity in homosexual serum samples using monoclonal antibodies as antigens.

**Anti-gelatin Antibodies.** Anti-gelatin antibody levels were measured by ELISA in 178 serum samples. Samples included 8 controls, 12 rheumatoid arthritis patients, 44 HIV- hemophiliacs, 24 HIV+ hemophiliacs, 38 HIV- homosexuals, 33 HIV+ homosexuals, and 16 homosexual AIDS patients. Results are shown in figures 6A and 6B. The prevalence of autoantibodies against gelatin increased in homosexuals with infection with HIV (32% HIV-, 66% HIV+) and with progression to AIDS (100%). Prevalence also increased with HIV infection in hemophiliacs, but to a much lesser degree than in homosexuals (0% HIV-, 13% HIV+). Although prevalence increased with HIV infection and disease progression in homosexuals, anti-gelatin antibody levels reached titres as high in non-infected homosexuals as in AIDS patients (figure 6A). The mean anti-gelatin activity of the HIV- homosexuals did not differ significantly from controls (table III), even though individuals within the HIV- homosexual population had high levels of anti-gelatin antibodies.

**Serum IgG Levels.** Serum IgG levels were measured to assess correlations between total serum IgG and particular anti-idiotype or autoantibody levels. The mean serum IgG levels of the different serum categories and correlation coefficients with different antibodies are shown in table I. Mean serum IgG was elevated in the
Figure 6. Anti-gelatin ELISA values obtained with individual serum samples within homosexual (A) and hemophiliac (B) subgroups. A number of sample values are not visible due to a net OD of zero. Serum samples from 12 rheumatoid arthritis patients were negative for anti-gelatin activity (not shown).
Table I. **Serum IgG Levels and Correlation Coefficients**

<table>
<thead>
<tr>
<th></th>
<th>IgG/ααCD8</th>
<th>IgG/ααCD4</th>
<th>IgG/ααgp 120</th>
<th>IgG/αgel</th>
<th>mg IgG/mL (mean±sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>.681</td>
<td>.345</td>
<td>.166</td>
<td>.085</td>
<td>16.50±2.11</td>
</tr>
<tr>
<td>HIV+a</td>
<td>.136</td>
<td>.380</td>
<td>.261</td>
<td>.353</td>
<td>17.29±0.81</td>
</tr>
<tr>
<td>HIV-a</td>
<td>.105</td>
<td>.322</td>
<td>.179</td>
<td>.359</td>
<td>11.63±0.36</td>
</tr>
<tr>
<td>HIV+b</td>
<td>.730</td>
<td>.708</td>
<td>.775</td>
<td>.490</td>
<td>30.73±3.52</td>
</tr>
<tr>
<td>HIV-b</td>
<td>.159</td>
<td>.000</td>
<td>.206</td>
<td>.320</td>
<td>19.61±1.48</td>
</tr>
<tr>
<td>RA</td>
<td>.086</td>
<td>.120</td>
<td>.178</td>
<td>.036</td>
<td>13.71±1.20</td>
</tr>
<tr>
<td>Controls</td>
<td>.170</td>
<td>.153</td>
<td>.302</td>
<td>.714</td>
<td>9.94±0.65</td>
</tr>
</tbody>
</table>

Footnotes.

- *a* = homosexuals
- *b* = hemophiliacs
<table>
<thead>
<tr>
<th>Group</th>
<th>( \alpha CD8 )</th>
<th>( \alpha CD4 )</th>
<th>( \alpha gp 120 )</th>
<th>( \alpha Gelatin )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>.284±.030</td>
<td>.265±.023</td>
<td>.092±.010</td>
<td>1.091±.104</td>
</tr>
<tr>
<td>(n=15)</td>
<td>(n=15)</td>
<td>(n=14)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>HIV+a</td>
<td>.223±.013</td>
<td>.252±.038</td>
<td>.096±.004</td>
<td>.643±.078</td>
</tr>
<tr>
<td>(n=33)</td>
<td>(n=33)</td>
<td>(n=33)</td>
<td>(n=33)</td>
<td></td>
</tr>
<tr>
<td>HIV-a</td>
<td>.208±.012</td>
<td>.251±.020</td>
<td>.086±.004</td>
<td>.420±.080</td>
</tr>
<tr>
<td>(n=38)</td>
<td>(n=38)</td>
<td>(n=22)</td>
<td>(n=38)</td>
<td></td>
</tr>
<tr>
<td>HIV+b</td>
<td>.983±.095</td>
<td>.355±.064</td>
<td>.528±.086</td>
<td>.171±.033</td>
</tr>
<tr>
<td>(n=21)</td>
<td>(n=21)</td>
<td>(n=21)</td>
<td>(n=24)</td>
<td></td>
</tr>
<tr>
<td>HIV-b</td>
<td>.353±.054</td>
<td>.199±.041</td>
<td>.302±.060</td>
<td>.095±.011</td>
</tr>
<tr>
<td>(n=43)</td>
<td>(n=43)</td>
<td>(n=43)</td>
<td>(n=44)</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>.301±.031</td>
<td>.218±.031</td>
<td>.032±.01</td>
<td>.077±.010</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(n=12)</td>
<td>(n=12)</td>
<td>(n=12)</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>.179±.009</td>
<td>.186±.009</td>
<td>.080±.004</td>
<td>.126±.024</td>
</tr>
<tr>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=8)</td>
<td></td>
</tr>
</tbody>
</table>

Footnotes.

\( a=\) homosexuals

\( b=\) hemophiliacs
group of HIV infected homosexuals, all hemophiliacs, and persons with rheumatoid arthritis (tables I and III).

Significance Levels. Significance levels were determined by Student's t test to see which parameters distinguished relevant populations (table III) and which parameters differed independently of differences in serum IgG. Mean ELISA OD levels of different antibodies in each different group are shown in table II. Results of Student's t testing for significant differences in population means are shown in table III. Anti-anti-CD4 levels do not distinguish AIDS patients from HIV+ homosexuals, but do distinguish AIDS patients from normal controls. Anti-anti-CD8 levels distinguish AIDS patients from HIV infected homosexuals and other groups but don't distinguish HIV infected homosexuals from non-infected homosexuals. Anti-anti-CD8 levels also distinguish HIV infected from non-infected hemophiliacs. These parameters may relate more to risk factors for infection ie. exposure to alloantigens than to the progression of HIV related disease. Anti-gelatin antibody levels show the best correlation with disease progression, as the population means discriminate between HIV+ homosexuals and homosexual AIDS patients and between HIV+ and HIV- homosexuals and hemophiliacs, but do not distinguish HIV- homosexuals or hemophiliacs from controls.

Section II. Complementary Immune Responses

Serum from HIV- homosexuals expressing autoantibodies and serum from normal controls partially inhibited the binding of antibodies from a particular AIDS patients to gp 120 (figure 7A). Repetition of this inhibition experiment with 4 control sera, 4 autoimmune sera, and 4 different AIDS patients produced highly variable results from individual to individual (figure 7B). There was no consistent
Table III.

### Significance Values

#### A. Serum IgG levels

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. AIDS</td>
<td>≠</td>
<td>ns</td>
<td>.001</td>
<td>.001</td>
<td>ns</td>
<td>ns</td>
<td>.038</td>
</tr>
<tr>
<td>B. HIV+homosexuals</td>
<td>≠</td>
<td>.001</td>
<td>.001</td>
<td>ns</td>
<td>.023</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>C. HIV- homosexuals</td>
<td>≠</td>
<td>.001</td>
<td>.001</td>
<td>.029</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. HIV+hemophiliacs</td>
<td>≠</td>
<td>.002</td>
<td>.001</td>
<td>.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. HIV- hemophiliacs</td>
<td>≠</td>
<td>.011</td>
<td>.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. RA patients</td>
<td>≠</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.027</td>
</tr>
<tr>
<td>G. Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### B. Anti-anti-CD4 levels

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. AIDS</td>
<td>≠</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>.050</td>
</tr>
<tr>
<td>B. HIV+homosexuals</td>
<td>≠</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>C. HIV- homosexuals</td>
<td>≠</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. HIV+hemophiliacs</td>
<td>≠</td>
<td>.050</td>
<td>ns</td>
<td>.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. HIV- hemophiliacs</td>
<td>≠</td>
<td></td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. RA patients</td>
<td>≠</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>G. Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table III. continued

C. Anti-anti-CD8 levels

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AIDS</td>
<td>≠</td>
<td>.050</td>
<td>.005</td>
<td>.001</td>
<td>ns</td>
<td>.040</td>
</tr>
<tr>
<td>B</td>
<td>HIV+homosexuals</td>
<td>≠</td>
<td>ns</td>
<td>.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>C</td>
<td>HIV- homosexuals</td>
<td>≠</td>
<td>.001</td>
<td>.025</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>HIV+hemophiliacs</td>
<td>≠</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HIV- hemophiliacs</td>
<td>≠</td>
<td></td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>RA patients</td>
<td>≠</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Anti-anti-gp 120 levels

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AIDS</td>
<td>≠</td>
<td>ns</td>
<td>ns</td>
<td>.001</td>
<td>ns</td>
<td>.001</td>
</tr>
<tr>
<td>B</td>
<td>HIV+homosexuals</td>
<td>≠</td>
<td>ns</td>
<td>.001</td>
<td>.004</td>
<td>.001</td>
<td>ns</td>
</tr>
<tr>
<td>C</td>
<td>HIV- homosexuals</td>
<td>≠</td>
<td>.001</td>
<td>.012</td>
<td>.001</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>HIV+hemophiliacs</td>
<td>≠</td>
<td>.034</td>
<td>.001</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HIV- hemophiliacs</td>
<td>≠</td>
<td>.021</td>
<td>ns</td>
<td></td>
<td></td>
<td>.011</td>
</tr>
<tr>
<td>F</td>
<td>RA patients</td>
<td>≠</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table III. continued

E. Anti-gelatin levels

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>AIDS</td>
<td>≠</td>
<td>.004</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
</tr>
<tr>
<td>B.</td>
<td>HIV+homosexuals</td>
<td>≠</td>
<td>.050</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>.002</td>
</tr>
<tr>
<td>C.</td>
<td>HIV- homosexuals</td>
<td>≠</td>
<td>.020</td>
<td>.001</td>
<td>.001</td>
<td>.020</td>
<td>ns</td>
</tr>
<tr>
<td>D.</td>
<td>HIV+hemophiliacs</td>
<td>≠</td>
<td>.009</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.</td>
<td>HIV- hemophiliacs</td>
<td>≠</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td>RA patients</td>
<td>≠</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.011</td>
</tr>
<tr>
<td>G.</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Footnotes.

Numbers in the tables represent the probability that the means of the various populations compared by Student's t test are the same. If the probability that the population means were the same was greater than .05 in any test, the difference between those populations was considered non-significant and ns entered into the table. Populations in the table columns are denoted by letters which correspond to the denotations of the populations named in the table rows.
Figure 7. Serum from a normal control (R) and non-HIV infected anti-gelatin positive homosexual (12) slightly inhibited the anti-gp120 ELISA activity of an individual AIDS patients' serum diluted 1/500 (A). There was no consistently greater inhibition by homosexual (26, 7, 35) or other alloimmune (P) serum samples (1/20 dilution) expressing anti-gelatin activity, than by normal control sera (AB, J, H, R) of the anti-gp120 activity of any of four AIDS sera (18, 19, 46, 53) diluted 1/500 (B). Samples shown in legend that do not appear on graph gave zero inhibition.
relationship between supposed exposure to allogeneic cells and subsequent anti-anti-class II antibody response and the ability to inhibit anti-gp120 antibodies from AIDS patients' serum. The relevant immune responses, anti-anti-class II in homosexuals and anti-anti-anti-class II in AIDS patients either exhibit too much individual variation for specific complementarity to be demonstrated by this method or have already formed immune complexes in the AIDS patients' sera. The ability of serum from normal controls to bind anti-gp120 antibodies of AIDS serum was unexpected and is of unknown significance.

Section III. Characterization of Anti-gelatin Antibodies

Titration of Anti-Gelatin Activity. Titration of anti-gelatin antibody activity in 3 homosexual serum samples showed titres 100 times those of control sera. Anti-gelatin antibodies were routinely detectable at a 1/10,000 dilution of sera (figure 8).

Antibody Nature of Anti-gelatin Activity. Material purified by gelatin agarose affinity chromatography from control, HIV+, and AIDS serum was shown by SDS PAGE sizing to have a molecular weight of 150,000 kd and was shown by immunoblot analysis to be human IgG (figure 9). Much higher levels of antibody were purified from AIDS and HIV+ serum relative to control serum, but some IgG was purified from control serum by gelatin-agarose affinity chromatography.

Anti-collagen Specificity of Anti-gelatin Antibodies. Four types of native and heat denatured human collagens were used to inhibit the anti-gelatin ELISA. Figure 10 shows that all collagens inhibited the anti-gelatin ELISA more effectively after heat denaturation. Almost complete inhibition occurred with 50 ng of heat
Figure 8. Titration of anti-gelatin activity in representative homosexual and control serum samples shows that the level of anti-gelatin activity is roughly 100 times higher in the homosexual samples. Difference between these samples and control samples was easily detectable at dilutions up to 1/10,000.
Figure 9. Gelatin-agarose affinity purified material from control (A), HIV+ homosexual (B), and AIDS (C) sera was subjected to 6% non-reducing SDS PAGE and transferred to nitrocellulose for immunoblot visualization with alkaline-phosphatase conjugated goat anti-human IgG antibodies (1) or silver stained along with molecular weight standards (2).
Figure 10.

A

% inhibition

\[ \mu g/mL \text{ denatured collagen} \]

B

% Inhibition

\[ \mu g/mL \text{ collagen (non denatured)} \]
Figure 10. All human collagens tested (types I, III, IV, and V) inhibited the anti-gelatin ELISA activity of a representative anti-gelatin positive homosexual serum sample diluted 1/100 (A&B) or 1/200 (C). Denatured collagens (A) inhibit the ELISA more effectively than non-denatured collagens (B&C). Native and denatured collagens at 20 μg/mL did not inhibit the anti-gp120 ELISA activity of HIV+ serum (not shown).
Figure 11. Purified fibronectin inhibits the anti-gelatin ELISA activity of representative anti-gelatin positive sera diluted 1/100. Fibronectin was added to diluted serum immediately prior to the ELISA and the incubation times were as for a standard ELISA. Anti-gelatin positive sera does not react with fibronectin directly in standard ELISA testing (not shown). Inhibition of the anti-gelatin ELISA by fibronectin is through direct reaction of fibronectin with gelatin and masking of the antibody reactive determinants. Fibronectin at up to 20 \( \mu g/mL \) did not inhibit the anti-gp120 ELISA activity of HIV+ serum (not shown).
denatured types I and II collagen added to 100 μL of a 1/100 dilution of serum (figure 10A). Roughly 80 times as much native collagen type I or III was required to elicit the same degree of inhibition (figure 10A, B). Figure 11 shows the ability of fibronectin to inhibit antibody binding to gelatin. This suggests that the anti-gelatin antibodies bind to the same determinants of collagen as does fibronectin.

Figure 12 demonstrates the strong immunoblot reactivity of an anti-collagen positive serum and of purified anti-collagen antibodies with the alpha chains of reduced collagens type I and III, confirming the preferential reactivity demonstrated by inhibition studies.

**Correlation With Serum IgG Levels.** Figure 13 and table I summarize the relationship by group of anti-gelatin activity with serum IgG levels. Only control sera shows significant correlation of these measures. This suggests anti-gelatin antibodies are independent of polyclonal B cell activation.

**Anti-Collagen Profiles.** In order to see whether overall differences in anti-collagen activity existed between homosexual HIV-, HIV+, and AIDS patients, ELISA activity was measured in six samples from each group against all four collagens in both native and heat denatured conformations. Relative reactivity was very consistent within and between these groups as shown by the similar profiles and small standard error bars, but also very different from the control group (figure 14). Reactivity in the "anti-gelatin positive" sera from homosexuals rose selectively against denatured collagens, especially against denatured types I and III, compared to control sera. This also argues against polyclonal activation as a source of autoantibodies against gelatin.
Figure 12. Immunoblot analysis of reduced electrophoresed collagens (6% SDS PAGE) with anti-gelatin positive serum (A) and affinity purified anti-gelatin antibodies (B) shows preferential reactivity with the alpha chains of collagens type I and III. Only slight reactivity is seen with collagen type IV and reactivity with type V collagen is barely detectable.
Figure 13. Serum IgG levels and anti-gelatin ELISA activity plotted against each other for individual serum samples from homosexuals (HIV\(^+\), HIV\(^-\), and AIDS), hemophiliacs (HIV\(^+\), HIV\(^-\)), rheumatoid arthritis patients, and normal controls. No correlation between these variables is evident, which suggests that anti-gelatin activity is not due to polyclonal activation.
Figure 14. The average relative reactivity (± sem) of six sera from each of four groups against native and denatured forms of collagen types I, III, IV, and V. Relative reactivity is defined as the ELISA OD against a given collagen divided by the highest ELISA OD obtained under identical conditions against a native or denatured collagen with the same serum sample. The different specificity profile for control sera compared to the "anti-gelatin positive" sera from the homosexual AIDS, HIV+, and HIV- groups argues against anti-gelatin activity arising through polyclonal activation.
Section IV. Reactivity of Anti-gelatin Antibodies With C1q

Direct ELISA Reactivity. Complement component C1q is known to express collagen-like sequences and triple helical conformation. Since the anti-gelatin antibodies reacted to some degree with all collagens tested, it seemed reasonable to expect some degree of reactivity with C1q as well. Reactivity with native C1q bound to ELISA plates, however, did not correlate with anti-gelatin activity and was unaffected by the addition of 20 mM EDTA to the serum diluent (not shown). This concentration of EDTA abrogates binding of C1r and C1s to C1q and prevents any inhibition of antibody binding to the collagen like region of C1q. Antibody preparations used in this study reacted non-specifically with immobilized C1q, presumably through the Fc binding regions of C1q. Since C1q is used to measure serum immune complexes in a similar ELISA format, this form of ELISA is inappropriate for the measurement of specific anti-C1q antibodies. Although some anti-gelatin positive sera had greater ELISA reactivity against C1q than control sera, it is not clear what the C1q ELISA measures; total serum IgG, serum immune complexes, or anti-C1q antibodies. Soluble C1q inhibited the anti-gelatin ELISA to a measurable, but minor extent, suggesting some weak cross-reactivity of the anti-gelatin antibodies with soluble C1q (figure 15). It is possible that this small amount of reactivity is obscured in a solid phase binding assay such as an ELISA, by the non-specific binding of IgG via Fc portions to C1q.

Immunoblot Reactivity. Purified anti-gelatin antibodies did not react with the primary chains of C1q produced upon reduction of intact C1q (figure 16). Similar to the C1q ELISA, non-specific antibody binding to intact C1q and to C1q constituent chain oligomers occurred in immunoblot testing (figure 17). After digestion of C1q with pepsin to prepare collagen-like fragments, there was no
Figure 15. Purified complement component Clq inhibits the anti-gelatin activity of a representative anti-gelatin positive sera (diluted 1/100) to a minor extent. Clq was added to diluted serum in the presence of 20 mM EDTA and incubated overnight at 4°C before anti-gelatin ELISA testing. Clq at up to 20 μg/mL did not inhibit the anti-gp120 ELISA activity of HIV+ serum (not shown).
Figure 16. Molecular weight standards (1), fibronectin (2), gp120 (3), C1q in 8M urea (4), C1q in sample buffer (5), type III collagen (6), and reduced C1q (7) were subjected to 6% SDS PAGE and silver stained (A). Reduced C1q (1), type III collagen (2), C1q in sample buffer (3), C1q in 8M urea (4), gp120 (5) and fibronectin (6) were transferred from the same gel to nitrocellulose for immunoblotting with purified anti-gelatin antibodies (B). Proteins were loaded at approximately 250 ng/lane. Anti-gelatin antibodies reacted with type III collagen (B2) and non-reduced C1q (B3,4), but not with recombinant gp120 (B5), fibronectin (B6), or reduced C1q (B1).
Figure 17. Binding of purified anti-gelatin antibodies to non-reduced C1q (A1) and type I collagen (A4), but not to reduced C1q (A3) was seen by immunoblotting. Samples were subjected to 12% SDS PAGE and transferred to nitrocellulose. Gelatin at 0.5% inhibited binding to reduced type I collagen (B3), but not to C1q (B5). Immunoblot strips (C) show that purified anti-gelatin antibodies (1), purified normal human IgG (2), and goat anti-human antibodies (3) all bind to non-reduced C1q transferred to nitrocellulose following 6% SDS PAGE. This suggests that non-specific binding of antibodies to immobilized C1q occurs through the Fc binding regions of C1q.
Figure 18. Demonstration that anti-gelatin antibodies do not react on immunoblots with the collagen-like regions of C1q or acetylcholinesterase. Non-reduced pepsin alone (lane 1), non-reduced C1q digest (lane 2), non-reduced acetylcholinesterase digest (lane 3), (standards lanes 4 and 5), reduced pepsin alone (lane 6), reduced C1q (lane 7), reduced C1q digest (lane 8) reduced acetylcholinesterase digest (lane 9), and reduced type I collagen (lane 10) were subjected to 12% SDS PAGE and silver stained (A) or transferred to nitrocellulose for immunoblot analysis with purified anti-collagen antibodies (B). Immunoblot shows reactivity of purified antibodies only with type I collagen (B10) and residual intact C1q left after pepsin digest (B2).
immunoblot reactivity of the anti-gelatin antibodies or anti-gelatin positive sera with reduced or non-reduced fragments (figure 18).

IgG Enhancement of Anti-C1q Reactivity. When C1q and purified anti-gelatin antibodies were allowed to react in the presence of solid phase bound human IgG, there was a significant enhancement of anti-C1q activity. This enhancement was not seen with normal IgG or with material purified by gelatin-agarose affinity chromatography from normal sera (figure 19). This suggests that a conformationally altered form of C1q present upon binding to the Fc region of IgG reacts specifically with anti-gelatin antibodies.

Hemolytic Complement Testing. Anti-gelatin antibodies added to diluted fresh control serum at up to an equivalent amount by weight of the C1q present in the test serum, did not inhibit complement hemolytic activity (not shown). Although this represents a large excess of anti-gelatin compared to the relative amounts of C1q and anti-gelatin antibodies found in AIDS sera, the in vitro complement assay may create artificial conditions under which hemolysis is heavily favoured over normal conditions in vivo. This possibility need be considered, although these results suggest that anti-gelatin antibodies binding to activated C1q do not inhibit complement function and are not responsible for the depressed hemolytic complement activity seen in HIV+ homosexuals.
Figure 19. The binding of purified anti-gelatin antibodies to C1q is enhanced when C1q interacts with immobilized IgG antibodies (IgG + C1q), compared to the binding to C1q or IgG alone (end groupings). Enhanced binding was not seen with material affinity purified from normal serum with gelatin-agarose or with 10 times the quantity of purified IgG. Columns represent the mean OD obtained from three separate experiments ± sem.
Discussion

Infection with HIV can be asymptomatic for long periods or rapidly devastating (92). Severe immunodeficiency follows disappearance of the T4 cells necessary for specific immune responses (93). Before clinical signs of immune deficiency develop, immunological signs of hyperactivity are pronounced. B cell hyperplasia, elevated serum immunoglobulins and circulating immune complexes arise soon after infection (47). These symptoms can reflect direct activation of B cells by HIV or other agents, but they also occur in the absence of infection (94). Immune activation in autoimmune diseases, for example, produces similar serological and pathological signs. Increased growth factor production, direct B cell activation or effects on T cell regulation could explain the early signs of HIV infection. HIV induces more immunoglobulin synthesis in lymphocyte cultures of B and T cells than in cultures of B cells alone, which suggests, but doesn't prove, that HIV exerts some effects on B cells through an effect on T cells (95). The maintenance of immune activation, even in periods of limited viral replication, suggests that an immunoregulatory disorder develops early in the course of HIV infection. This immunoregulatory disorder may pertain to the later development of overt AIDS.

Network theories of immune system regulation maintain that idiotypes recognizing foreign antigens such as HIV are themselves recognized by idiotypes present on the cell surface of T and B lymphocytes (96). The character of the network topology in the immune system is postulated to be largely determined by MHC proteins. Interaction between T cell idiotypes depends upon complementarity of antigen responsive idiotypes to self MHC and mimicry of MHC by internal idiotypes. Selection criteria for internal idiotypes impels recognition of MHC restriction elements, while antigen responsive idiotypes are selected for
recognition of short foreign peptides (77). The regulatory idiotypes must resemble MHC but differ in that there is no constraint to bind short peptides directly. Diversity among antigen responsive idiotypes is favoured, while convergence to shapes most closely resembling the MHC sites of T cell interaction is favoured among regulatory T cells. T cell regulation through this type of internal recognition is postulated to involve a network focussing topology (77). Focussing to highly conserved internal regulatory idiotypes could occur if similarities between MHC molecules are more recognizable than differences. In this case the regulatory structures of immune systems would be highly conserved. Alternatively, there may be many regulatory idiotypes, each corresponding to a different MHC protein involved in presenting conserved sets of peptides. Regulation of multiple antigen reactive cells restricted to particular MHC proteins could occur through one regulatory T cell in either case.

Although the role of suppression in the ontogeny and maintenance of self tolerance is sometimes questioned, self reactive T and B lymphocytes do exist (97). In some circumstances, polyclonal activation, autoantibody production, and immune complex production seem to occur through defective suppression of self reactive cells. In human lupus, autoantibodies with different antigenic specificity express a common idiotype defined by a monoclonal antibody anti-16.6 (98). These autoantibodies may share an idiotype which is related to the idiotype of regulatory cells which normally suppress autoantibody production. Constraints of the symmetrical network theory dictate that suppressor cells have high network connectivity and interact idiotypically with a large number of lymphocytes (99). Thus point idiotypic defects in suppression can have widespread effects on the immune system network. Polyclonal activation may actually result from idiotypically specific effects on regulatory cells and thus produce antibodies with common idiotypes.
HIV confronts the central axis of the immune system network with its external envelope glycoprotein, gp120. Gp120 is complementary to the cell surface protein CD4, which is found most commonly on T lymphocytes and monocytes (80). CD4 normally functions in signal transduction by interacting with MHC class II proteins and this interaction is inhibited by gp120 (100). HIV thus relates to class II MHC proteins in manner analogous to the internal regulatory idiotypes. HIV gp120 recognizes MHC restriction elements on the CD4 protein and the regulatory idiotypes recognize MHC restriction elements on T cell receptors. This relationship of gp120 to CD4 and class II MHC and potential mimicry of regulatory idiotypes is the basis of the network theory of AIDS. In the context of this theory, gp120 expresses an internal image of class II. Theoretically, prohibition of immune responses against internal images of class II MHC is not as strictly enforced as against class II proteins themselves. Subtle differences between class II proteins and internal image of class II proteins, elicited through different selection criteria, may render internal images of class II MHC on regulatory idiotypes targets for the immune response against HIV gp120.

Since it is the anti-CD4 portion of gp120 which intersects with the immune system network, an hypothesis of the network theory of AIDS is that the immune response against this portion of gp120 is involved in AIDS pathogenesis. Detailed mapping of the gp120/CD4 interaction with monoclonal antibodies has identified anti-CD4 antibodies which bind to the same region of CD4 as does gp120 (83). These antibodies mimick gp120 and can be used to detect anti-gp120 antibodies of the relevant specificity produced in HIV infection (84).

The network theory of AIDS also maintains that the pathogenesis of AIDS depends on coincident immune responses against HIV gp120 and against allogeneic cells (77). Most people who develop AIDS are exposed to allogeneic cells in blood products or semen. Lymphocytes present in these fluids will transiently proliferate
in response to host MHC proteins and expose the host immune system to anti-host MHC receptors. These anti-host MHC receptors induce an anti-anti-class II antibody response in experimental alloimmunizations (79). Anti-anti-class II MHC antibodies are idiotypically related to class II proteins in an analogous manner to both gp120 and the internal regulatory idiotypes of the immune system. Potential complementarity between the immune response to gp120 and the anti-anti-class II immune response exists such that the anti-anti-class II response will stimulate the anti-gp120 response and vice versa. Two symmetrically opposed immune responses along the central axis of the regulatory network may synergize to effect the gradual erosion of network stability. This potential synergy implies that antibodies complementary to the anti-anti-CD4 antibodies may be involved in AIDS pathogenesis and that the specificity of these antibodies would be anti-anti-class II MHC.

Disruption of the internal regulation of the immune system network by HIV is a possible cause of polyclonal activation and eventually of T4 cell depletion and immunodeficiency. Autoreactive antibodies and T cells could result if the regulatory functions of T cells were undermined by inappropriate immune responses. If AIDS results from specific, idiotypically related immune responses to conserved antigens, as suggested by this theory, this should be reflected by the emergence of common idiotypes in the sera of AIDS patients. This study focussed on the identification of antibodies which, according to the network theory of AIDS, reflect common targets of a pathogenic immune response associated with HIV infection and are involved in the induction of AIDS. A primary objective was to assess the prevalence of anti-anti-class II antibodies and antibodies against that portion of gp120 which binds to CD4. Association between the appearance or level of these antibodies and disease progression should occur if they are involved in destabilizing the immune system network. A secondary objective was to identify
and characterize antibodies associated with disease progression, as these antibodies should be idiotypically related to network destabilizing immune responses if such immune responses are occurring and leading to AIDS.

Direct ELISA testing in this study revealed very few HIV infected homosexuals expressing antibodies against the CD4 binding region of gp120. A similar low prevalence was observed by Lundin et al. using an alternative method of detection (101). Their data also corroborates our observation of high levels of anti-anti-CD4 antibodies in sera from a small minority healthy HIV infected persons. The sensitivity of our ELISA to the anti-anti-CD4/anti-gp120 antibodies of interest was confirmed using soluble recombinant gp120 to inhibit the anti-leu 3a ELISA. Surprisingly, inhibition studies indicated that only about half of the anti-anti-CD4 activity was related to HIV gp120. Although this could be due to variability in HIV gp120, the CD4 binding region is expected to be highly conserved. It is more likely that the remaining 50% of the anti-anti-CD4 antibodies, and 100% of the anti-anti-CD4 antibodies seen in HIV negative persons are actually anti-foreign class II antibodies, which potentially include anti-anti-CD4 activity. If exposure to alloantigens induces the anti-anti-CD4 antibodies, anti-anti-CD8 antibodies should be more prevalent. The CD8 protein has a relationship to class I proteins analogous to that of class II proteins and CD4. In alloresponses, class I antigens induce the predominant alloantibody response and in our homosexual and hemophiliac serum samples, as expected, there are higher levels and a greater prevalence and of anti-anti-CD8 antibodies than anti-anti-CD4 antibodies. There also appears to be some correlation between this anti-foreign response and HIV infection and progression to AIDS. This is expected in that exposure to HIV often occurs coincident with exposure to alloantigens and allogeneic cells have been implicated in the network model as a cofactor in AIDS pathogenesis. There were also much higher levels and a greater prevalence of anti-anti-CD8 antibodies in
hemophiliac sera than homosexual sera, and the presence of these antibodies correlated well with HIV infection. Presumably this reflects that the more blood products a hemophiliac is exposed to the greater the allo-MHC response and the greater the chance of HIV infection. Since nearly all HIV infected hemophiliacs are also alloimmune it is not possible to associate the anti-anti-CD8 response with AIDS in this group.

Our results and those of Lundin et al. (101) suggest that anti-anti-CD4 antibodies are not involved in the pathogenesis of AIDS. Anti-anti-CD4 antibodies were present in the sera of some healthy HIV infected homosexuals and did not increase in level or prevalence in AIDS patients. It is possible that the regulatory T-cell idiotypes are not conserved to the degree that an immune response against them is detectable in a varied population with a single idioype. A relationship between anti-HIV immunity and anti-MHC immunity is apparent, however, from the ability of HIV gp120 to inhibit the anti-anti-CD4 antibodies in sera from HIV-persons. Cellular immunity of the corresponding specificity may be more important in AIDS pathogenesis. Recent work showed that individuals not infected with HIV mount an \textit{in vitro} cytotoxic T cell response against gp120 characteristic of previous priming to gp120 determinants (102). This may reflect, as proposed in the network focussing topology, prior T cell selection for recognition of MHC mimicking idiotypes present on regulatory T cells, which are themselves mimicked by gp120. Possibly, the T cell cytotoxic response stimulated by HIV cross-reacts with regulatory T cell idiotypes. We would then expect, as has been observed, that despite the unusually high anti-gp120 CTL response induced in \textit{vitro}, that there will be little anti-gp120 CTL activity detectable in unstimulated peripheral blood lymphocytes from HIV infected persons (102). T cells in HIV infected persons which are cytotoxic towards normal T cells have been reported, but specificity for T cell receptor idioypic determinants has not been determined (22).
The interaction between MHC class II and CD4 has not been mapped in the same detail as the gp120/CD4 interaction, so the selection of appropriate anti-class II monoclonals with complementarity to the anti-anti-class II antibodies implicated in the network theory of AIDS is problematic. Direct ELISA testing with a monoclonal antibody (L234), against a non-polymorphic region of HLA DR, revealed no anti-anti-class II antibodies in AIDS patients or HIV infected persons. ELISA testing using an anti-gp120 monoclonal which bears an internal image of CD4, and is thus anti-class II, revealed very low levels of anti-anti-class II antibodies in sera from some AIDS patients and HIV infected persons. The low levels and relative prevalence of these antibodies do not support a role for the antibodies in AIDS pathogenesis.

Complementarity between anti-gp120 antibodies in AIDS sera and putative anti-anti-class II antibodies in serum from non HIV infected homosexuals was also not apparent by inhibition of anti-gp120 ELISA. This could reflect absence of the antibodies of the predicted idiotypic specificity or prior formation of antibody antibody immune complexes in the AIDS patients' sera. It is also possible that the complementary idiotypes on the anti-gp120 antibodies are expressed independently of the gp120 binding site so that the formation of complexes does not preclude binding of anti-gp120 antibodies to gp120. This phenomenon was observed with anti-gp120 and anti-anti-gp120 antibodies in immune complexes eluted from the platelets of HIV infected persons (58).

Overall, these results don't support the hypothesis that complementary anti-anti-CD4 and anti-anti-class II antibodies cause AIDS. If such conserved immune responses cause AIDS, they may occur at the cellular level. Alternatively, the idiotypic mechanism involved is considerably more complicated and less conserved than the model predicts. Evaluation of cellular immunity pertinent to the network theory of AIDS will require development of new techniques for the
generation and analysis of cytotoxic T cells. Whether an idiotypic mechanism
operates at the cellular or humoral level and whether or not it involves exactly the
idiotypes predicted, a common idiotypic destructive mechanism should be reflected
in the emergence of common idiotypes in affected persons.

Some of the focus on aberrant immune regulation in AIDS stems from the
the association between autoimmunity and HIV infection. Autoantibodies in AIDS
patients are often attributed to polyclonal B cell activation, induced by HIV directly,
other pathogens, or by HIV induced depletion of T4+ suppressor inducer cells.
Autoantibodies generated by polyclonal activation are generally of low affinity and
reflect the composition of the B cell repertoire (103). Levels of these autoantibodies
correlate with serum immunoglobulin levels and usually don't reach the levels of
antibodies produced in specific immune responses. If AIDS is an
immunoregulatory problem induced by specific idiotypes, common specific
autoantibodies should appear in AIDS patients and these antibodies should not
exhibit the characteristics of autoantibodies produced by polyclonal activation.
Although many different autoantibodies have been described in AIDS patients,
most occur with low frequency and probably reflect individual differences in
immunological or genetic background, rather than an idiotypic mechanism of
AIDS pathogenesis.

We have detected common specific autoantibodies in AIDS patients which
are independent of polyclonal activation and which occur in several immune
disorders with features similar to AIDS. The distribution of these autoantibodies
suggests they are related to an idiotypic mechanism involved in the induction of
severe immune disorder and immunodeficiency. Characterization of these
antibodies and identification of factors associated with their presence may therefore
reveal important factors in the development of AIDS.
These autoantibodies were detected by reaction with the diluent of the anti-CD4 commercial monoclonal leu 3a, which contains mostly gelatin. Sera from seven different groups were therefore systematically assayed for ELISA reactivity with gelatin. A clear association between anti-gelatin antibodies and HIV infection and progression to AIDS was observed with homosexual serum samples. Association of anti-gelatin antibodies with HIV infection was observed in hemophiliac sera. No anti-gelatin antibodies were seen in control sera, sera from non-HIV infected hemophiliacs, or sera from rheumatoid arthritis patients. The prevalence of these antibodies in different groups suggests they are useful indicators of the risk of developing AIDS. If a specific idiootypic mechanism is involved in AIDS pathogenesis, it is possible that these anti-gelatin antibodies are related to this mechanism.

Several experiments demonstrated that anti-gelatin antibodies are not due to polyclonal activation. The level of anti-gelatin antibodies in many serum samples is over 100 times the level in normal sera and anti-gelatin reactivity is easily detectable at 1/10,000 dilutions of sera. Linear regression analysis showed that serum IgG levels do not correlate significantly with anti-gelatin levels except for in control sera. The correlation of anti-gelatin activity with total serum IgG in controls probably reflects a minor increase in background dependent upon serum IgG. The collagen type specificity profile of antibodies in control sera differs from the anti-gelatin positive sera in that in the positive sera there is specific expansion of antibody reactivity with denatured collagens type I and III. Inhibition and immunoblot studies showed that determinants most commonly expressed on denatured type III collagen are targets for anti-gelatin antibodies present in AIDS sera. These studies suggest that anti-gelatin antibodies result from a specific immune response occurring in association with progression to AIDS.
Serum fibronectin shows a reactivity profile with collagen similar to that of the autoantibodies seen in this study (104), and in inhibition experiments, prevented most of the interaction of anti-gelatin antibodies with gelatin (104). Anti-gelatin reactivity was shown by ELISA, SDS PAGE sizing, and immunoblot to be IgG in composition and unrelated to fibronectin. Fibronectin levels have been measured in AIDS sera and are not different from levels of normal sera (105). It is difficult to attribute the anti-gelatin antibodies to the presentation of new determinants to the immune system since the determinants involved are likely to be masked by fibronectin. Although immune responses against collagen occur in disorders characterized by polyclonal activation, the antibodies we describe are unrelated to polyclonal activation. The conditions under which tolerance to collagen is broken during, or before, progression to AIDS suggest an immunoregulatory problem exists. The key questions are how this immunoregulatory problem develops and affects the production of specific anti-gelatin antibodies.

The broad reactivity of the anti-gelatin antibodies with all collagens tested implies that they react with other proteins containing collagen-like sequences. Two such proteins are the enzyme acetylcholinesterase (Ach) and the complement component C1q, both of which also interact with serum fibronectin (106, 107). Autoantibodies against Ach have been described in association with neuromuscular disorders and autoantibodies against C1q occur in human lupus in association with elevated levels of immune complexes and decreased hemolytic complement activity (108, 109). This is relevant in the context of AIDS in light of the elevated levels of immune complexes and decreased complement activity observed in this syndrome (47, 48).

Experimentally produced anti-collagen antibodies cross-react with C1q and bind to the same region of C1q as do C1r and C1s (110). C1q is composed of three
different chains, A, B, and C, which are disulphide linked as CC homodimers and AB heterodimers. Six AB dimers and three CC dimers associate into the intact molecule bearing six globular heads and a stalk region which exhibits collagen-like triple helical structure. The primary A, B, and C chains of Clq are each composed of approximately one third collagen like sequence (106). When intact Clq interacts via its globular heads with the Fc region of bound immunoglobulin molecules, a conformational change in the collagen like region of Clq results which enables activation of C1r and C1s (111). One theory proposed to explain the production of anti-C1q antibodies in human lupus is the presence of high levels of conformationally altered Clq in circulating immune complexes (109). This might result in the presentation of neoantigens to the immune system and the induction of antibodies reactive with collagen like sequences. Previously described monoclonal antibodies which cross-react with Clq and collagen are specific for denatured collagen (112). Since the proposed mechanism of autoantibody production against Clq is consistent with the symptoms of HIV infection, reactivity of the anti-gelatin antibodies with Clq was investigated.

Immunoblot analysis showed no reactivity between purified anti-gelatin antibodies and the reduced primary chains of Clq. This is somewhat surprising in that the anti-gelatin antibodies reacted with primary determinants of each of the four collagens tested. Apparently, the repeating sequence XYgly expressed in the A, B, and C chains of Clq does not itself confer cross-reactivity with the primary determinants against which the anti-gelatin antibodies are directed. Solid phase bound Clq used in this study reacted with all antibody preparations when intact or when individual dimers were associated into oligomers larger than 100 kd. Reduction of Clq abrogated all antibody binding to Clq. ELISA measures of serum reactivity showed no difference in the presence or absence of EDTA suggesting there was no competition between antibody binding to Clq and the Ca++
dependent binding of C1r and C1s to C1q. Soluble C1q inhibits the anti-gelatin ELISA only to a slight extent compared to inhibition seen with denatured collagens. Purified anti-gelatin antibodies did not inhibit complement function even when added in equivalent amounts (by weight) to the C1q concentration used in the complement assay. However, the same preparation of anti-gelatin antibodies reacted strongly with purified C1q bound to immobilized IgG. When C1q is digested with pepsin so that collagen like sequences alone are left intact, reactivity with anti-gelatin antibodies is still absent. These results suggest that only under select circumstances is C1q a specific target of the anti-gelatin antibodies described in this study. These circumstances could involve depletion of other complement components or the entrapment of C1q in circulating immune complexes. Such circumstances exist in HIV infection, but it is still speculative to attribute the production of anti-gelatin antibodies to immune complex bound C1q. Conceivably, anti-gelatin antibodies are caused by conformationally altered C1q and this would provide a simple explanation for the loss of self tolerance to collagen. Cross reactivity of the anti-gelatin antibodies with C1q may, however, simply be a biochemical phenomenon without physiological relevance. Reactivity with select conformations of C1q present in immune complexes is an attractive explanation for production of anti-gelatin antibodies, but more biological evidence is needed to evaluate this possibility.

Antibodies with the idiotypes predicted to cause AIDS were found, in this study not to be linked to the development of AIDS. Exposure to alloantigens and a subsequent immune response against MHC class I antigens was linked to HIV infection and AIDS in this study, but the data does not distinguish whether exposure to allogeneic proteins or cells increases just the risk of infection or also the risk for developing AIDS. It is possible that immune disorder caused by HIV itself induces an anti-class I like antibody response. Similar antibodies, anti-anti-CD8
were detected in a number of lupus and rheumatoid arthritis patients not exposed
to alloantigens. Whether these antibodies are a cause or consequence of immune
disorder is not clear, but it is interesting that they are associated with immune
disorder outside of HIV infection and exposure to allogeneic cells. In the
homosexual AIDS patients and HIV infected hemophiliacs, anti-anti-CD8 levels
correlate significantly with serum IgG levels. The reason for this relationship is
also obscure, but perhaps some form of selection occurs during progression to AIDS
which expands the relative numbers of cells involved in this immune response.
Alternatively, alloantigens inducing the anti-anti-CD8 antibodies or the anti-anti-
CD8 antibodies themselves cause polyclonal activation in certain circumstances. In
the context of idiotypic network regulation, polyclonal activation does not
necessarily imply non-specific activation.

Antibodies against gelatin were clearly linked in this study to progression to
AIDS. Yet high levels of these antibodies also occur without HIV infection in
homosexuals and persons with lupus, leprosy, or graft versus host disease (113).
This suggests there is a common element in the immunopathogenesis of these
disorders. The anti-gelatin antibodies are not related to polyclonal activation, but
do seem to correlate with exposure to alloantigens or their equivalent.
Homosexuals are exposed to allogeneic lymphocytes and often show
immunological priming against alloantigens (114). Graft versus host disease
involves B and T cell mediated immunity against host MHC antigens. An
immunodominant cell surface protein of mycobacterium leprae, the cause of
leprosy, shows immunological cross-reactivity with HLA DR proteins, and with
HIV proteins (115, 116). A mouse monoclonal antibody against HIV gp120 reacts
with a monocyte cell surface antigen involved in antigen presentation and
antibodies against HIV gp41 react with human MHC class II antigens (73, 74). Lupus
is not associated with alloimmunity, but in a murine model of lupus, the MRL
lpr/lpr strain, anti-anti-self MHC antibodies precede the usual autoantibodies characteristic of lupus (78). Interestingly, this strain of mouse also expresses high levels of anti-gelatin activity.

Though alloimmunity correlates with anti-gelatin antibodies, it is not clear if, and how, these immune responses are biochemically or idiotypically linked. Purified anti-gelatin antibodies don't bind lymphocyte cell surface antigens or inhibit or compete with anti-anti-CD4 antibodies, anti-anti-CD8 antibodies, or anti-anti-class II antibodies. Anti-gelatin antibodies don't appear to be anti-MHC themselves, complementary to anti-MHC antibodies, or to share idiotypes with anti-MHC or anti-anti-MHC antibodies. It is possible though, as is seen with other antibodies, that the idiotype anti-gelatin antibodies have in common with the MHC related antibodies is expressed independently of the antigen binding region and will require anti-idiotypes for its demonstration.

A possible clue to the origin of the anti-gelatin antibodies is their relative prevalence in HIV infected homosexuals and hemophiliacs. Homosexuals, in common with persons with graft versus host disease and leprosy, are exposed to MHC or MHC-like antigens on viable cells or organisms, whereas hemophiliacs are exposed to alloantigens in lyophilized protein preparations. Viable cells or organisms capable of proliferation or active infection are more likely to induce cytotoxic T cell mediated immunity. Perhaps it is this form of alloimmunity that is specifically linked to the production of anti-gelatin antibodies. The capacity of HIV to mimic class II antigens in the induction of cytotoxic T cell responses is consistent with a role for HIV in anti-gelatin antibody production and is also consistent with a synergistic effect of HIV infection and exposure to allogeneic cells. In lupus, perhaps T cell subset abnormalities favour the induction of T cell immunity against MHC or MHC mimicking proteins. T-cells isolated from the synovium of severely
affected rheumatoid arthritis patients often proliferate in response to autologous lymphocytes.

The correlation between T cell alloimmunity and anti-gelatin antibodies does not explain how the anti-gelatin antibodies arise or if and how AIDS is related. The T cell alloimmune response may include a response against the MHC mimicking, regulatory idiotypes of the network focussing topology and may help to provoke the network instability envisioned in the network theory of AIDS. If this hypothesis is correct, then what we see in autoimmune diseases such as lupus may be simply a less virulent form of AIDS. HIV alone, or in concert with exposure to allogeneic lymphocytes, would amplify T cell alloimmunity and, as a result of retroviral persistence, eventually result in AIDS. This may explain the chronically elevated levels of cytotoxic T8 cells seen in HIV infection and the failure of these cells to protect against AIDS.

In conclusion, the etiology of AIDS remains a mystery. The susceptibility of the human immune system to a poorly replicating, but persistent retrovirus, in the minds of a number of immunologists is best explained by the unique relationship of HIV antigens to the regulatory structures of the immune system. The similarities to other immunoregulatory disorders and the negative effects of anti-HIV immunity speak for an immune mechanism of AIDS pathogenesis that will not be counteracted by anti-virals, nor without a better understanding of the internal regulation of the immune system.
Bibliography


56. Grant, M. D., Weaver, M. S., Tsoukas, C., and Hoffmann, G. W. Distribution of antibodies against denatured collagen in AIDS risk groups and homosexual AIDS patients suggests a link between autoimmunity and the immunopathogenesis of AIDS. 1989 submitted for publication.


78. Kion, T. A. unpublished.


