# THE ROLE OF CYTOTOXIC T LYMPHOCYTES IN HIV-INFECTION

By

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# THE ROLE OF CYTOTOXIC T LYMPHOCYTES IN HIV-INFECTION

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Abstract

The acquired immune deficiency syndrome (AIDS) is an incurable disease caused by infection with the human immunodeficiency virus (HIV). In the eight years following identification of HIV as the cause of AIDS, little progress has been made in understanding the complex pathogenesis of AIDS or in developing effective therapies to forestall disease progression. All current strategies to treat HIV infection assume that disease progression is directly related to infection of and replication in $CD4^+$ lymphocytes by HIV.

Recent theoretical and experimental studies have raised the possibility that the pathogenesis of AIDS depends upon indirect effects of HIV infection; namely the induction of deleterious immune responses. In this study, we examined the effects of HIV infection on cytotoxic T lymphocyte (CTL) activity. Three aspects of CTL activity in HIV infection were examined: the role of anti-HIV CTL in suppressing viral replication and disease progression; the role of autoimmune CTL in $CD4^+$ lymphocyte depletion in HIV infection; and changes in the CTL repertoire in HIV infection and the relationship of these changes to progressive $CD4^+$ lymphocyte depletion.

Although a possible role for circulating anti-HIV CTL in suppressing HIV replication *in vivo* was seen, there was no evidence that these CTL protect against $CD4^+$ lymphocyte depletion. This suggests a dissociation between viral replication and $CD4^+$ lymphocyte depletion. Autoimmune CTL that lysed uninfected activated $CD4^+$ lymphocytes were found in HIV-infected individuals. The natural history of $CD4^+$ lymphocyte depletion suggested a relationship between this CTL activity and *in vivo* depletion of $CD4^+$ lymphocytes. Autoimmune CTL had a conventional phenotype, but were not HLA-restricted. The characteristics of these CTL suggested a mechanism of $CD4^+$ lymphocyte depletion involving recognition of $CD4^+$ T cell receptor (TCR) idiotypes by the autoimmune CTL.

Skewing of the CTL repertoire was also demonstrated in HIV infection. The increased incidence of skewing of TCR variable domain (V) gene usage observed in parallel to $CD4^+$ lymphocyte depletion is consistent with an interdependence of the selective expansion of CTL and depletion of $CD4^+$ lymphocytes.

Results of this thesis suggest that autoimmune CTL play a role in the pathogenesis of AIDS and are rational targets for novel therapeutic approaches to the treatment of HIV infection.

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## Preface

This thesis follows new guidelines established by the School of Graduate Studies at McMaster University allowing the inclusion verbatim of manuscripts prepared for publication as chapters in the written thesis. Three manuscripts have been included as chapters in this thesis. Chapter one, " The influence of lymphocyte counts and disease progression on circulating and inducible anti-HIV CTL activity in HIV-1$^+$ Humans" is a study of the possible role of anti-HIV CTL in protection from disease progression. This manuscript was published in 1992 in *AIDS* 6;1085. Chapter two, " Evidence of a role for autoimmune cytotoxic T lymphocytes in CD4$^+$ lymphocyte depletion in HIV-1-infection" is a study of the possible autodestructive effects of CTL in HIV infection. This manuscript has been submitted to the *Journal of Immunology*. Chapter three, "Changes in the cytotoxic T cell repertoire of HIV-1-infected individuals: relationship to disease progression" is a study of the relationship between skewing of the CTL repertoire and CD4$^+$ lymphocyte depletion. This manuscript was published in 1993 in *Viral Immunology* 6;85.

These chapters build on each other in that the first demonstrates the failure of anti-HIV CTL to protect against CD4$^+$ depletion despite inhibiting HIV replication, the second demonstrates the presence of autoimmune CTL in HIV infection and provides evidence for their role in CD4$^+$ lymphocyte depletion and the third establishes a relationship between the progressively more stringent selection of CD4$^+$ and CD8$^+$ lymphocytes that occurs in HIV infection. The theory which threads these studies together is based on an hypothesis developed by the candidate and all experimental work and design was the direct responsibility of the candidate. In this way, these three manuscripts become the candidates thesis.

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# Introduction

Understanding the pathogenesis of acquired immune deficiency syndrome (AIDS) requires analysis of the interactions between the human immunodeficiency virus (HIV) and the human immune system. Many effects of HIV on the immune system have been recognized, but few are well understood. The common assumption that HIV replication alone is the critical element of disease progression is not solidly supported by data, has led to little progress in the treatment of AIDS and is now seriously questioned by both the AIDS research community and organized, well informed advocacy groups representing people infected with HIV. The objective of this thesis was to investigate one potentially critical aspect of the interaction between HIV and the immune system; the effect of HIV infection on cytotoxic T lymphocyte (CTL) activity.

## A. Cytotoxic T Lymphocytes and Viral Infections

CTL play a key role in protection from, control of, and elimination of intracellular parasites such as viruses. CTL combat viral infection primarily by recognizing peptide fragments of viral proteins presented by major histocompatibility complex (MHC) molecules on the surface of infected host cells. This recognition causes death of the infected host cells in *in vitro* assays and there is good evidence that host cell death due to the action of anti-viral CTL occurs *in vivo* as well (1). CTL are highly efficient killers which usually peak in number and activity within several weeks of infection and disappear quickly as infection subsides. Induction of CTL and subsequent failure to clear infection has been associated with immunopathology and even death. The best studied example of this negative effect of CTL is murine infection with lymphocytic

choriomeningitis virus (LCMV) (2). Recognition of their autodestructive capacity has branded CTL the "double edged sword" of immune effector function. Appropriate regulation of these effector cells is therefore crucial to health and survival.

## B. Anti-HIV Cytotoxic T Lymphocytes in HIV Infection

The "double edged sword" caveat is especially relevant for evaluating the role of CTL in HIV infection. Since HIV is an intracellular parasite, it is a potential target for anti-HIV specific CTL. The induction of anti-HIV CTL might help resolve acute infection with HIV and later limit reactivation and spread of HIV. However, HIV escapes and eventually defeats the immune system and the persistence of ineffective CTL could actually favor their immunopathologic potential. A brief review of the literature on CTL in HIV infection illustrates some realization of their bipartite potential.

One of the most prominent early features of HIV infection is a marked increase in the absolute number of $CD8^+$ lymphocytes in peripheral blood (3) and lymph nodes (4). The $CD8^+$ lymphocyte subset includes the majority of CTL, therefore, this increase may reflect the induction and recruitment of specific anti-HIV CTL. A similar elevation in $CD8^+$ lymphocyte number occurs in Epstein-Barr virus (EBV) infection, but numbers return to normal coincident with remission of acute symptoms (5). In HIV infection, $CD8^+$ lymphocyte numbers remain elevated from the time of seroconversion through the late stages of infection (6). This may reflect continuous stimulation of anti-HIV CTL due to chronic replication of HIV. Specific anti-HIV CTL were first described in the lungs and peripheral blood of infected persons (7, 8). This detection was in no way associated with, or limited to cases of acute infection. An extremely unusual feature of anti-HIV CTL is their presence at high enough frequency for direct detection in freshly isolated

lymphocytes from peripheral blood and bronchoalveolar lavage fluid without *in vitro* stimulation (7, 8). These findings demonstrate that infection with HIV induces a specific CTL response that persists for years after infection and is probably several orders of magnitude greater than CTL responses elicited by other viruses. Furthermore, the anti-HIV CTL response in a particular individual does not focus on particular immunodominant peptides, but includes CTL against multiple peptide determinants of viral structural and regulatory proteins and viral enzymes (9). These findings have generated optimism among vaccine researchers, since if such a response could be induced by a preventive vaccine it might block infection, or if such a response could be maintained by post-infection vaccination, it might prevent disease progression. Conversely, these findings have also fueled pessimism, on the basis that since the enormous response to natural infection doesn't prevent disease progression, protective responses may be impossible to generate. Arguments that anti-HIV CTL do prevent disease progression, at least in the short term, and that only through their eventual reduction is HIV able to cause AIDS, continue to spark some optimism and direct clinical immunotherapeutic research.

### C. Evidence of a Protective Role for Cytotoxic T Lymphocytes in HIV-infection

Given the central role attributed to anti-HIV CTL in combating disease progression, it is unfortunate that experimental and clinical evidence for this role remains scarce, circumstantial and anecdotal. Several groups have shown that *in vitro* replication of HIV occurs to a greater extent in peripheral blood lymphocyte cultures depleted of $CD8^+$ lymphocytes (10, 11). This indicates that $CD8^+$ T cells inhibit viral replication *in vitro* and suggests they do so *in vivo* as well. Furthermore, the degree of inhibition

mediated by $CD8^+$ lymphocytes tends to decrease or disappear as clinical deterioration occurs (12). This $CD8^+$ lymphocyte mediated inhibition appears to involve both cytolytic and non-cytolytic mechanisms (13, 14).

Mutation of antigenic determinants of HIV *in vivo* away from CTL recognition has been documented and interpreted as evidence for a protective role for anti-HIV CTL (15). However, persistence of HIV variants *in vivo* expressing CTL epitopes in the presence of circulating anti-HIV CTL specific for those epitopes has also been reported (16). Thus, on the basis of currently available data, it is debatable whether immune selection of HIV variants insensitive to anti-HIV CTL is an integral component of pathogenesis.

In one HIV-infected individual studied over 3 years, an initially high level of circulating anti-HIV specific CTL activity fell gradually and eventually disappeared as this individual developed AIDS (17). Investigators also reported selective depletion of anti-HIV specific CTL in this subject and mentioned similar findings in 3 additional AIDS patients (17). In contrast to these findings, a different study of a larger number of subjects reported non-selective disappearance of CTL activity with the occurence of opportunistic infections (18).

Several groups have estimated the precursor frequency of anti-HIV CTL in peripheral blood of small numbers of HIV-infected individuals. Results of the first study suggested that anti-HIV CTL precursor frequency declined coincident with disease progression (19), but this finding was based on the study of only two individuals and measured only HLA-A2 restricted anti-*env* CTL. A later study suggested the measured decrease in anti-HIV CTL precursors actually reflected accumulation of suppressor cells and that anti-*env* CTL precursor frequency increased with disease progression (20). Another study, measuring levels of circulating anti-HIV CTL activity, found no

correlation with disease stage, since 2 patients with AIDS-related complex (ARC) retained circulating anti-HIV CTL activity (21). However, no investigators have detected circulating anti-HIV CTL in AIDS patients.

Even if the contentious issue of whether anti-HIV CTL or anti-HIV CTL precursor levels specifically fall in parallel with disease progression was resolved, this would not establish a cause and effect relationship. CTL levels might fall and precipitate increased viral replication and disease progression or CTL levels might simply fall as a consequence of disease progression. Ongoing and future experiments may help clarify this issue. Post-infection vaccination with killed virus and recombinant gp160 has had some positve effects on clinical status and $CD4^+$ lymphocyte numbers (22-24). Although these treatments may stimulate anti-HIV CTL, there is as yet no experimental evidence of such stimulation, nor of a role for anti-HIV CTL in mediating the positive effects of these treatments. Adoptive immunotherapy with autologous polyclonal phytohemagglutinin (PHA)-activated $CD8^+$ lymphocytes and autologous anti-HIV CTL clones may directly demonstrate or refute the importance of anti-HIV CTL in protecting HIV-infected individuals from AIDS.

Several studies using scid mice reconstituted with human lymphocytes have demonstrated some efficacy of anti-HIV CTL in protecting the mice from infection with HIV (25). Protection sometimes occurred even when HLA-mismatched anti-HIV CTL clones were transferred into reconstituted scid mice challenged with HIV (25). This parallels the inhibition of HIV replication by $CD8^+$ cells in peripheral blood of HIV-infected individuals in that HLA-restricted T cell receptor (TCR) mediated cytotoxic immunity as well as non-HLA-restricted non-cytotoxic immunity is associated with inhibition of viral replication (13, 14).

In summary, current evidence for protection against disease progression by anti-HIV CTL is at best controversial, yet several observations offer compelling circumstantial support for this possibility. HIV levels fall in acute HIV infection before neutralizing antibodies can be detected, suggesting a role for cell-mediated immunity in limiting the initial infection (26). Most, if not all, asymptomatic HIV-infected individuals possess circulating anti-HIV CTL, while circulating anti-HIV CTL are not detectable in AIDS patients. Furthermore, there is some evidence that decreases in anti-HIV CTL precursor frequency (19) and mutation by HIV away from recognition by anti-HIV CTL are associated with disease progression (15). There is no direct evidence that anti-HIV CTL protect against $CD4^+$ lymphocyte depletion or limit plasma or cellular HIV load *in vivo*. Since the hypothesis that anti-HIV CTL protect against disease progression rests on limiting viral replication in, and and thus lysis and loss of $CD4^+$ lymphocytes, this appears to be a key area requiring further research and evaluation.

## D. Evidence for An Immunopathologic Role for Cytotoxic T Lymphocytes in HIV-infection

The natural history of HIV infection, with estimates of 100% of HIV infected individuals eventually suffering AIDS, is sufficient evidence alone to question a protective role for anti-HIV CTL against disease progression. The unusually high intensity of the anti-HIV CTL response also raises questions about the propriety of this response. Two unique aspects of the anti-HIV CTL response have been reported. The first is the well documented presence of detectable circulating anti-viral CTL. Secondly, it has been reported that detectable anti-HIV CTL can be easily generated *in vitro* by primary stimulation of lymphocytes from non-HIV-infected individuals (19). Generation

of primary CTL *in vitro* was previously described only against allogeneic MHC antigens. In the study demonstrating induction of anti-HIV CTL in uninfected individuals, the frequency of anti-HIV CTL precursors was estimated to be comparable to that of CTL precursors for allogeneic MHC antigens (19). Cross-reactivity between both HIV *env* (27, 28) and *nef* (29) and MHC antigens has been reported and could explain the high precursor frequencies of these anti-HIV CTL, but there are also high frequencies of CTL recognizing HIV *gag* (30) and *pol* (31) antigens. No satisfactory explanation for the inordinately high circulating anti-HIV CTL activity in HIV-infected subjects nor for the primary induction of anti-HIV CTL in lymphocytes from non-HIV-infected subjects has been established.

In light of the eventual failure of anti-HIV CTL to protect against disease progression, it has been suggested that the intense anti-HIV CTL response actually contributes to disease progression (32). This idea was first proposed based on immunohistological analysis of lymph nodes from HIV-infected persons. Histological evidence was consistent with the possibility that infiltrating $CD8^+$ lymphocytes accumulated in, and later destroyed the lymph nodes and killed $CD4^+$ lymphocytes (33). Recently, a similar role for $CD8^+$ lymphocytes in SIV infected macaques was also suggested from immunohistological analyses of lymph nodes (34). $CD8^+$ lymphocytes are associated with extralymphatic immunopathology in HIV-infection as well, including vasculitis (35), alveolitis (36), sicca-syndrome (37) and neuritis (38). It is not known whether the $CD8^+$ lymphocytes involved are predominantly anti-HIV CTL, but anti-HIV CTL have been isolated from the lungs of subjects with alveolitis (36).

Although $CD4^+$ HLA class II-restricted anti-*env* CTL are not apparent in natural HIV infection, some $CD4^+$ HLA class II-restricted anti-*env* CTL clones from seronegative individuals lyse uninfected $CD4^+$ lymphocytes exposed to soluble HIV

gp120 (39). This suggested a possible *in vivo* pathogenic mechanism whereby uninfected CD4+ lymphocytes binding secreted HIV gp120 become sensitive to lysis by class II-restricted anti-*env* CTL (40). The possibilty of a more direct mechanism arose from the demonstration that CD8+ CTL from HIV-infected individuals lyse uninfected CD4+ lymphocytes never exposed to HIV products (41). This finding implied a significantly different pathogenic mechanism in that these CTL were not anti-HIV CTL mediating bystander lysis of uninfected CD4+ lymphocytes, but were genuine autoimmune CTL directed against an autoantigen on uninfected CD4+ lymphocytes. Together with previous histological evidence that CD8+ lymphocytes infiltrate the lymph nodes and kill CD4+ lymphocytes (33, 34), the concept of autoimmune CTL contributing to the pathogenesis of AIDS is also supported by a correlation between rapid progression to AIDS and higher CD8+ lymphocyte counts at the time of first medical examination (42). Therefore, the potential autodestructive activity of CTL in HIV infection is another area of research requiring further investigation.

## E. The Cytotoxic T Cell Repertoire in HIV-infection

Inspired by the finding that self-superantigens encoded by endogenous murine retroviruses mediate deletion of T cells utilizing particular TCR β-chain variable (V) genes (43-45), investigators have begun to examine the T cell repertoire in HIV-infected individuals. The report of specific deletion of T cells utilizing Vβs 15–20 from the T cell repertoire of HIV-infected subjects with severe CD4+ lymphocyte depletion suggested the activity of an HIV-encoded superantigen (46). However, to my knowledge these results have never been reproduced. Another report recently identified Vβ12 as the target of an HIV associated superantigen (47). Some investigators believe that different strains of

HIV or even isolates derived from the same individual at different times might target different Vβ regions (48). Although the primary focus of T cell repertoire analysis has been on identification of Vβ specific deletions or expansions caused by a potential superantigen (or Vβ selective element) encoded by HIV, autoimmune theories of HIV induced pathogenesis also predict effects on the T cell repertoire (49, 50). These theories propose that an autoimmune response directed against TCR variable regions is responsible for the elimination of $CD4^+$ T cells. Both the autoimmunity and superantigen theories suggest negative and positive selection of T cells based on V region specificity. The superantigen hypothesis predicts selection based only on Vβ expression whereas the autoimmune theory predicts selection based on the TCR idiotype. Idiotypic selection is likely to preferentially affect certain Vβ and Vα regions without absolute restriction to those Vβ or Vα regions. The autoimmune theory provides a rationale for selective depletion of $CD4^+$ lymphocytes based on a discernably different three dimensional shape imposed upon the TCR by HLA-class II restriction versus HLA-class I restriction. However, if $CD8^+$ CTL mediate the autoimmune destruction of $CD4^+$ lymphocytes, then positive selection of specific autoimmune $CD8^+$ lymphocytes would be reflected by changes in the $CD8^+$ CTL repertoire as well. Hence, characteristic changes in both the $CD8^+$ and $CD4^+$ T cell repertoires are predicted to accompany disease progression related to autoimmunity. An experimentally appealing aspect of changes in the CTL repertoire is that these changes are amenable to direct functional analysis using antibodies against TCR V region gene products in redirected lysis assays. If $CD8^+$ CTL are responsible for the autoimmune destruction of $CD4^+$ lymphocytes, it may be more meaningful to quantitiate Vβ and Vα gene expression on $CD8^+$ lymphocytes functionally, rather than phenotypically.

Determining whether skewing of the T cell repertoire is a component of HIV disease progression and whether the character of this skewing relates to the process of disease progression is critical to establishing a realistic perspective on the pathogenesis of AIDS. The paradigm of non-selective $CD4^+$ lymphocyte depletion based on direct infection and lysis has to date overwhelmingly dominated approaches to treatment of HIV infection. If this paradigm is even partially wrong, a radical shift in the direction of treatment research is necessary. Therefore, the effects of HIV infection on the CTL repertoire is another area which warrants further investigation.

## F. Specific Objectives

### i. Anti-HIV Specific Cytotoxic T Lymphocytes in HIV Infection

Studies on anti-HIV CTL activity were designed to address several specific questions related to controversial issues raised in the introduction, in the hope that the answers might provide insight into the role of anti-HIV CTL in HIV infection. The first questions centered on the characterization of anti-HIV CTL in natural infection. What HIV antigens do they predominantly recognize, *gag*, *pol* or *env*? Are the anti-HIV CTL predominantly HLA-class I or class II restricted? The remaining questions dealt with the relationship between anti-HIV CTL activity and disease progression. Did the presence of circulating or inducible anti-HIV CTL correlate with parameters of disease progression such as $CD4^+$ lymphocyte count, $CD8^+$ lymphocyte count, HIV plasma antigenemia and clinical stage of disease? Did the requirements for induction of anti-HIV CTL activity *in vitro* relate to parameters of disease progression? Were anti-HIV CTL precursors activated in subjects without detectable circulating anti-HIV CTL activity? The answers

to these questions might suggest whether or not anti-HIV CTL protect against HIV replication, CD4+ lymphocyte depletion, or clinical progression. Also, detection of specific impairments arising in anti-HIV CTL activity might suggest how HIV escapes the CTL immune response. Finally, a better understanding of the role of anti-HIV specific CTL in HIV infection might enable evaluation of competing theories of pathogenesis. The results of these studies are presented in form of a manuscript published in 1992 in *AIDS* 6;1085, entitled "The influence of lymphocyte counts and disease progression on circulating and inducible anti-HIV-1 CytotoxicT cell activity in HIV-1+ Humans" by M. D. Grant, F. M. Smaill, D. P. Singal and K. L. Rosenthal. Dr. Smaill was attending physician to the HIV-infected individuals volunteering for this study and made available on a confidential basis results of CD4+ and CD8+ lymphocyte counts and clinical evaluations. Dr. Singal conducted HLA-class I A, B and C typing of study volunteers.

### ii. Autoimmune Cytotoxic T Lymphocytes in HIV Infection

Studies on autoimmune CTL in HIV infection were designed to address a possible role for autoimmune CTL in the pathogenesis of CD4+ lymphocyte depletion. The first objective was to confirm their presence and levels in HIV-infected individuals relative to non-infected individuals. The next questions focussed on characterization of the autoimmune CTL. What was the phenotype of the CTL? What receptors do the CTL use to recognize and kill uninfected CD4+ lymphocytes? Are the autoimmune CTL HLA-restricted? Are the CTL specific for CD4+ lymphocytes? Do they recognize all CD4+ lymphocytes equally? What are the characteristics of the antigen on CD4+ lymphocytes that is recognized by the autoimmune CTL? What stimuli can be used to activate

autoimmune CTL *in vitro*? The last questions addressed the possible role of autoimmune CTL in the *in vivo* depletion of CD4+ lymphocytes. Do HIV-infected individuals with detectable autoimmune CTL lose CD4+ lymphocytes more rapidly than HIV-infected individuals without autoimmune CTL activity? Does the loss of CD4+ lymphocytes *in vivo* correlate with the elimination of specific targets of the autoimmune CTL? The answers to these questions might help in understanding the mechanisms leading to the induction of autoimmune CTL and to evaluate the possible role for autoimmune CTL in disease progression. The results of studies addressing these questions are presented in the form of a manuscript submitted for publication in *The Journal of Immunology* entitled "Evidence of a Role for Autoimmune Cytotoxic T lymphocytes in CD4+ Lymphocyte Depletion in HIV-1-infection" by M. D. Grant, F. M. Smail and K. L. Rosenthal. Dr. Smaill was attending physician to the HIV-infected individuals volunteering for this study and made available on a confidential basis results of CD4+ and CD8+ lymphocyte counts.

### iii. The Cytotoxic T cell Repertoire in HIV Infection

Studies were designed to determine whether skewing of the cytotoxic T cell repertoire is a component of disease progression in HIV infection. The first objective was to establish a normal range for functional V gene utilization by CTL from non-HIV infected individuals. After this was established, functional V gene utilization by CTL from HIV-infected individuals at different stages of CD4+ lymphocyte depletion was analysed. The main questions asked were whether or not skewing of the CTL repertoire occurred in HIV infection and if so, was it related to the degree of CD4+ lymphocyte depletion. Comparing functional to phenotypic levels of V gene utilization by CD8+ CTL in HIV infection might suggest whether selected subsets of CTL with certain V$\alpha$ or V$\beta$

regions are activated by specific antigens, or whether superantigen mediated activation of all T cells with a certain Vα or Vβ region is responsible for skewing of the repertoire. The results of these studies are presented in the form of a manuscript published in *Viral Immunology* entitled "Changes in the Cytotoxic T cell Repertoire of HIV-1 Infected Individuals: Relationship to Disease Progression" By M. D. Grant, F. M. Smaill, K. Laurie and K. L. Rosenthal. Karen Laurie prepared the cells for flow cytometry. Dr. Smaill was attending physician to the HIV-infected individuals volunteering for this study and made available on a confidential basis results of $CD4^+$ and $CD8^+$ lymphocyte counts.

## G. An Hypothesis

At the extreme, there are only two alternative views on the pathogenesis of AIDS: HIV directly kills the $CD4^+$ lymphocytes that disappear following HIV infection; or the immune system kills the $CD4^+$ lymphocytes that disappear following HIV infection. My hypothesis is that the immune system kills $CD4^+$ lymphocytes and that this is caused by an autoimmune response against TCR idiotypes specific to HLA-class II restricted T lymphocytes. One of the fundamental tenets of this hypothesis is the existence of an idiotypic network of interactions between T lymphocytes. This conflicts with the established view that T cells recognize only small linear peptide sequences in the context of MHC antigens. There are well documented exceptions to this current belief, but the significance of these exceptions lies mainly in the fact that there is no absolute restriction of TCRs to recognition of peptides in association with MHC molecules, nor any functional restriction to activation through such recognition. For example, alloantigens (51), superantigens (52) and anti-TCR antibodies (53) activate T cells in a non-MHC-restricted manner. In addition, non-MHC-restricted T cells with specificity for

conformational determinants of myoglobin (54), a molecule expressed on activated lymphocytes (55), fluorescein (56), B cell idiotypes (57) and tumor-associated mucins (58), have been well characterized. While T cell responses to foreign antigens undoubtedly involve MHC restriction, T-T interactions may be restricted to loci distinct from the MHC such as the restriction of suppressor T cells to the enigmatic I-J loci (59) and to Igh loci (60). From the point of view of T lymphocytes, there may be two distinct antigenic universes: an external one composed of self-peptides and peptides from foreign proteins complexed to self-MHC; and an internal one composed of complementary shapes within the V regions of other lymphocytes. As a consequence of connectivity within the internal antigenic universe, T cells may recognize, react to and regulate each other via idiotypic complementarity between their respective TCRs. Further, an appropriate level of complementarity between TCRs may be essential in selection and maintenance of the T cell repertoire. Because extensive diversity is favored to ensure responsiveness to an almost limitless array of foreign antigens, any one lymphocyte is less likely to contact its complementary external foreign peptide, than an internal complementary receptor. Whereas these contacts via complementary V regions require and also maintain connectivity between TCRs, MHC restriction is imposed by early selection criteria in the thymus, where survival depends an appropriate affinity for self-MHC.

What relates the idiotypic T cell network to the pathogenesis of AIDS is the possible role of T-T interactions in the selection of MHC-restricted anti-foreign T cells. If T cells possess V region connectivity, two forces might drive the emergence of dominant T cells in response to foreign peptides; competition for foreign peptide in the context of MHC and the ability to escape internal interactions to an extent allowing dominance over other clones. In this scheme, autoreactivity is a natural consequence of

any reaction to foreign antigens and in fact helps to determine that the dominant T cells are minimally self-reactive (fall within network defined limits of connectivity) and optimally MHC-restricted to foreign peptides. This self-regulatory scheme can be circumvented by antigens that resist restriction to self-MHC antigens. These antigens activate T cells independently of MHC presentation and consequently impel no competitive selection of MHC-restricted peptide specific T cells. Without this competitive selection, autoreactivity against the V regions of the activated T cells may cross a threshold beyond which immunity against the V regions of T cells activated in a non-MHC restricted manner occurs. This would eventually result in the elimination of all T cells with V region complementarity to the persistent antigen. There are a number of ways that HIV may activate T cells in a non-MHC restricted manner. Our results will be interpreted in light of this hypothesis.

# The Influence of Lymphocyte Counts and Disease Progression on Circulating and Inducible Anti-HIV-1 Cytotoxic T cell Activity in HIV-1$^+$ Humans

Running head Anti-HIV-1 CTL Activity in HIV-1$^+$ Humans

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## Abstract

**Objective:** The objective of this study was to evaluate specific anti-HIV CTL activity in relation to basic clinical and laboratory parameters used to follow HIV infection.

**Methods:** Lymphocytes from HIV$^+$ subjects with different clinical and immunological features of HIV infection were tested for circulating and inducible anti-HIV CTL activity using autologous B lymphoblastoid cells infected with recombinant vaccinia viruses expressing the HIV gag, pol and env genes as targets. Anti-HIV CTL were induced *in vitro* by stimulation with HIV-infected autologous lymphoblasts.

**Results:** We detected circulating anti-HIV CTL exclusively in asymptomatic subjects and found a significant association ($p<.01$) between CD8$^+$ lymphocyte counts ≥900/μl blood and detectable levels of circulating anti-HIV CTL. The subjects with circulating anti-HIV CTL also had a higher mean CD8$^+$ lymphocyte count than those without detectable circulating activity ($p<.001$), but no significant difference in mean CD4$^+$ lymphocyte count. CD8$^+$ HLA class I-restricted anti-HIV CTL were induced in all HIV$^+$ subjects tested following *in vitro* stimulation with HIV-infected autologous lymphoblasts. In subjects without detectable circulating anti-HIV CTL, circulating HLA-DR$^+$ cells contributed to anti-HIV CTL activity induced *in vitro* by stimulation with HIV or Concanavalin A (Con A).

**Conclusions:** Circulating anti-HIV CTL activity is associated with CD8$^+$ lymphocyte counts ≥900/μl. Anti-HIV CTL retain proliferative and functional capacity following *in vitro* stimulation with HIV and IL-2 through all stages of HIV infection. Persistent inducible anti-HIV CTL activity in subjects with advanced HIV disease and without circulating CTL suggests impaired activation and/or proliferation of the CTL *in vivo*.


**Keywords: HIV, cytotoxic T lymphocytes, AIDS, chromium release assay**

## Introduction

Infection with HIV induces characteristic immunodeficiency over a variable time period. Cellular immunologic signs of infection include a decreased CD4/CD8 lymphocyte ratio in peripheral blood and progressive reduction of $CD4^+$ lymphocyte number and function. $CD8^+$ lymphocytes usually persist at normal or elevated levels long after $CD4^+$ T-cell depletion and impairment have occurred. In later stages of HIV infection, $CD8^+$ lymphocytes also decrease in number, without apparent functional impairment or infection with HIV [1, 2]. Determining the role of $CD8^+$ suppressor and cytotoxic lymphocytes in HIV infection and AIDS, with regard to control of infection, immunopathology, and immune system regulation, is critical for evaluation of immunotherapeutic protocols and vaccines, and for understanding the pathogenesis of AIDS.

$CD8^+$ cytotoxic T lymphocytes capable of killing cells expressing HIV antigens and non-cytotoxic $CD8^+$ lymphocytes which suppress HIV replication *in vitro* circulate in $HIV^+$ subjects [3, 4]. These $CD8^+$ lymphocytes may help maintain low levels of $CD4^+$ lymphocyte infection during the long asymptomatic period of HIV infection. In many subjects, HIV replication increases before the onset of AIDS, suggesting that immune system surveillance is somehow overwhelmed, suppressed or breached [5-7]. A major research focus in HIV infection concerns defects in $CD4^+$ lymphocyte function. Reduced antiviral CTL activation *in vitro*, reversible by addition of IL-2 or costimulation with allogeneic cells probably reflects defective $CD4^+$ T-cell function [8-10], however, specific $CD8^+$ lymphocyte defects have also been described. Anti-HIV CTL precursor cells were found to decrease prior to AIDS defining illness and an increase in $CD8^+CD57^+$ suppressor cells which inhibit CTL activity was reported to parallel disease progression [11, 12]. Selective disappearance of anti-HIV CTL from an otherwise functional cytotoxic $CD8^+$ lymphocyte pool and the accumulation of $HLA\text{-}DR^+$ $CD8^+$ lymphocytes with defective

proliferative potential have also been reported [1, 13, 14]. Any or all of these defects might contribute to reduced anti-HIV immune surveillance and progression of HIV disease.

Alternatively, anti-HIV CTL may not protect against disease progression. The anti-HIV CTL response is unusually strong in that CTL measurable without *in vitro* stimulation persist for years in peripheral blood [1, 15, 16]. In spite of this initial $CD8^+$ CTL-mediated response, $CD4^+$ T cell depletion and disease progression eventually occur. Thus $CD8^+$ anti-HIV CTL may not protect aginst disease progression. In addition, immunopathology associated with HIV infection appears to be caused by $CD8^+$ T-cells [17-19]. Several researchers have even suggested a role for anti-HIV CTL in $CD4^+$ lymphocyte depletion [20-22]. To study the role of anti-HIV CTL and CTL regulation in progressive HIV infection, we measured and characterized circulating and inducible anti-HIV gag, pol, and env CTL in a cohort of $HIV^+$ subjects at different stages of disease and with different levels of peripheral blood $CD4^+$ and $CD8^+$ lymphopenia. Our data demonstrate the presence of circulating anti-HIV CTL in CD4 lymphopenic, but otherwise asymptomatic persons with $\geq$900 $CD8^+$ lymphocytes/$\mu$l blood. HLA-$DR^+$ and $DR^-$ anti-HIV CTL precursors and responses could be stimulated *in vitro* through later stages of HIV infection in all subjects tested. Immunocompetence in the $CD8^+$ lymphocyte population even after AIDS onset suggests that CTL activity might be exploited in immunotherapy, but also that the natural anti-HIV CTL immune response is either ineffective or overcome.

## Methods

### Subjects

Subjects were recruited from the McMaster University Special Immunology Services Clinic. HIV-1 infection was determined by anti-HIV antibody ELISA and confirmed by Western blot. Subjects were clinically assessed in tandem with CD4/CD8 lymphocyte analysis, anti-HIV CTL analysis, and plasma p24 quantitation. Although most subjects were asymptomatic, $CD8^+$ lymphocyte counts were increased and $CD4^+$ lymphocyte counts and CD4/CD8 lymphocyte ratios decreased relative to values for the general population. Informed consent was obtained from all subjects for drawing of blood samples and our studies received local ethics approval.

### Lymphocyte separation

Blood was collected in heparinized vacutainers, diluted with an equal volume of PBS (phosphate buffered saline), centrifuged for 15 min at 500Xg, and the cell free supernatant collected and stored at -70°C for subsequent plasma HIV antigen measurement. Blood cells were rediluted to 2X original volume with PBS, layered over Ficoll-Paque gradient separation medium (Pharmacia Chemicals, Dorval, Que.) and centrifuged at 400Xg for 30 min. Interface cells were collected, washed three times in PBS with 1% FCS (fetal calf serum) and used as fresh effector cells or cultured appropriately. Medium used for all studies was RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% FCS (Bockneck, Burlington, Ont.), 10mM hepes, 2mM L-glutamine, 1% penicillin/streptomycin, (all from GIBCO) and $2X10^{-5}$ M 2-mercaptoethanol (Sigma Chemical Company, St. Louis, MO). HLA class I A, B and C antigen typing was done on peripheral blood mononuclear cells (PBMC) by the microdroplet lymphocyte cytotoxicity test [23]

**Generation of B lymphoblastoid cell lines (BLCL)**

$5X10^6$ freshly isolated PBMC were incubated in 2.5 ml cell free supernatant from EBV producing B95-8 cells (American Type Culture Collection, ATCC CRL1612). After 24 hours, the cells were washed and incubated in medium containing 20% FCS and 1μg/ml cyclosporin A (Sandoz Canada, Montreal, Que.). After 10 days, BLCL were maintained in standard media or frozen in 20% FCS, 10% DMSO supplemented medium and stored in liquid $N_2$.

***In vitro* induction of anti-HIV CTL**

A modification of the method established by Plata and coworkers was used to stimulate anti-HIV CTL *in vitro* [24]. Briefly, freshly separated PBMC were cultured ($1X10^6$/ml) in standard medium (90% of cells) or resuspended in 1-2 ml E-line supernatant with 5μg/ml purified phytohemagluttinin (PHA-P) (Wellcome Diagnostics, Dartford, England) for 3 days (10% of cells). E-line cells, constitutively producing HIV-$1_{SF2}$ were kindly supplied by J. Levy (UCSF, CA). After three days, the infected cells were washed in medium, irradiated at 5000 rad, resuspended in fresh medium and mixed with with the original uninfected cultured PBMC at a 1 stimulator/10 responder ratio. After 3 days of coculture, cells were resuspended in fresh medium, usually supplemented with 5U/ml recombinant IL-2 (Genzyme, Cambridge MA) to expand activated cells, and tested for anti-HIV cytotoxic activity 4 days later. Cells were retested after 3 additional days if specific lysis was not apparent and cell lines could be maintained in IL-2 for up to 8 weeks by restimulation with HIV infected irradiated autologous PHA activated blasts every 10 days. For Concanavalin A (Con A) stimulation, PBMC cultures were supplemented with 10 μg/ml Con A (Difco, BDH, Toronto, Ontario, Canada) for 3 days, followed by 7 days with 5U/ml IL-2.

**Cytotoxicity assays**

Targets for anti-HIV CTL assays were generated by infecting autologous BLCL overnight at a multiplicity of infection of 15 with recombinant vaccinia virus vectors expressing the HIV gag gene (Vsc40) [25], the HIV pol reverse transcriptase gene (Vcf21) [15], the HIV env gene (Vsc25) [26], and as control, the nucleoprotein gene of pichinde virus (VVNP) [27]. Vsc40, Vcf21, and Vsc25 were the generous gifts of Dr. B. Moss, (NIH) and VVNP was constructed in our laboratory [27]. All recombinants were constructed by transfection of vaccinia virus (wild type strain WR) infected CV-1 cells with plasmids containing the E. coli β-galactosidase gene and the desired HIV-1 gene. Recombinants were identified as blue plaques by development with β-galactosidase substrate, plaque purified, expanded, and expression of the relevant HIV insert in infected cells confirmed by radioimmunoprecipitation. Infected BLCL were incubated for 90 min with 200 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA), washed 4 times in PBS with 1% FCS, resuspended at $1X10^5$/ml and 50μl added to round bottom wells of microtitre test plates. Effector cells were washed and resuspended in fresh medium at $1X10^7$/ml (circulating CTL assays) or $5X10^6$/ml (*in vitro* induced CTL assays) and 50, 25, and 12.5 μl added to replicate wells. The final volume in each well was adjusted to 300μl with medium or with OKT3 hybridoma supernatant (ATCC CRL 8001). Each target was added in duplicate to wells containing medium alone or 1 N HCL to generate background and maximum $^{51}Cr$ release values. Microtitre test plates were incubated for 5 hours at 37° after which 100μl of supernatant was removed from each well for γ counting. Per cent specific lysis was calculated as previously described [28]. Spontaneous $^{51}Cr$ release was less than 25% in all cases. Variation between the lysis of replicate VVNP infected control targets averaged less than 3%, therefore lysis greater than 10% above that of control targets was taken as significant HIV-specific lysis.

### $CD4^+/CD8^+/DR^+$ lymphocyte depletions

To investigate the contributions of $CD4^+$, $CD8^+$ and HLA-$DR^+$ lymphocytes to anti-HIV CTL activity, subsets were depleted or selected from starting populations either immediately before cytotoxicity assays or before initiation of *in vitro* stimulation. Lymphocytes were incubated at $3X10^6$/ml in PBS with 1% bovine serum albumin (BSA) for 30 min at 4°C with either OKT8 (ATCC CRL8014), OKT4 (ATCC CRL8002), or anti-HLA-DR L243 (ATCC HB55) antibodies. Hybridomas were obtained from ATCC and grown *in vivo* in $CAF_1$ mice (OKT4 and OKT8) or Balb C mice (L243). Monoclonal antibodies were concentrated from ascitic fluid by $(NH_4)_2SO_4$ precipitation, redissolved in 2x original volume PBS, exhaustively dialyzed against PBS and used at 1/200 dilutions. After incubation with antibodies, the lymphocytes were washed twice in PBS with 1% BSA and incubated with goat anti-mouse IgG conjugated magnetic dynabeads (DYNAL Inc., Great Neck, NY) for 1 hour at 4°C. Unbound cells were removed after magnetic displacement of beads. This resulted in depletion of >95% of the intended cell population as measured by flow cytometry. Positive selection using the magnetic beads resulted in 99% purity of the selected population.

### Plasma p24 quantitation

Plasma collected from blood coincidentally with preparation of PBMC for circulating CTL assays was used for p24 quantitiation by HIV antigen capture ELISA (Organon Teknika, Scarborough, Ontario, Canada). Recombinant p24 obtained from the NIAID AIDS reference reagent program [29] was serially diluted with control plasma and used to generate a standard curve. The detection limit of this assay was ~30 pg p24/ml of plasma.

## Statistical Analysis

Associations between CTL activity and lymphocyte counts were assessed by the Chi-square test with Yates correction. Correlations between different measures were assessed by linear regression analysis and differences between means assessed with Student's *t* test using the Statworks statistical analysis program.

## Results

### Circulating anti-HIV CTL activity

This activity was assessed in 20 HIV$^+$ subjects using freshly isolated lymphocytes and autologous EBV transformed BLCL infected with recombinant vaccinia viruses expressing HIV gag, pol, and env genes. Autologous BLCL infected with VVNP, a recombinant vaccinia virus expressing Pichinde virus nucleoprotein, served as control targets for these and all other studies. Of 20 HIV$^+$ subjects tested, 6 had detectable circulating anti-HIV CTL activity against gag, pol, or env antigens. Circulating cellular cytotoxicity against HIV was shown to be mediated by T cells by inhibition of lysis by OKT3 for subjects AO87 and AO75. All subjects with circulating anti-HIV CTL activity were CDC class II, asymptomatic (Table 1). Since all persons with circulating anti-HIV CTL activity had CD8$^+$ lymphocyte counts of 900/μl or greater, we tested for association between CD8 lymphocyte counts ≥900/μl and circulating CTL activity. Circulating anti-HIV CTL activity was significantly ($p<.01$ $\chi^2=7.54$) associated with CD8$^+$ lymphocyte counts ≥900/μl blood. Mean CD8$^+$ lymphocyte count was also higher in the group of subjects with circulating anti-HIV CTL activity ($p<.001$); mean±SEM 1075±93 versus 713±75. The CD4$^+$ lymphocyte counts of subjects with circulating anti-HIV CTL activity ranged from approximately normal (830/μl AO89) to extremely low (90/μl AO75). There was no significant difference between the mean CD4$^+$ lymphocyte count of the group of subjects with and without circulating anti-HIV CTL ($p>.25$); mean±SEM 348±102 versus 239±46.

Although no statistically significant association ($p>0.40$) between the presence or levels of circulating anti-HIV CTL and plasma HIV antigenemia was observed, only 1 of the 6 subjects with circulating anti-HIV CTL activity had detectable HIV antigenemia, compared to 7 of the 14 subjects without circulating anti-HIV CTL activity. This is unlikely to be due to anti-viral treatment, since 5 of the 8 subjects positive for plasma HIV antigen were receiving AZT at the time of testing (Table 1). These results demonstrate that detection of

circulating anti-HIV CTL is associated with CD8 counts ≥ 900/μl and are consistent with a role for circulating anti-HIV CTL in limiting HIV replication during asymptomatic infection. However our data does not prove a causal role for circulating anti-HIV CTL in limiting replication of HIV *in vivo*.

**Induction of anti-HIV CTL activity**

Anti-HIV CTL activity was measured after *in vitro* stimulation with HIV and IL-2 for 7 and 10 days. The results following 7 days *in vitro* stimulation are shown in Table 1. Anti-HIV CTL directed against pol and env antigens were commonly detected following restimulation. Anti-HIV CTL against HIV gag antigens were seen less frequently than reported in other studies [14, 16], which might relate to our cohort of subjects or more likely to the particular recombinant vaccinia virus vector used to express HIV gag antigens. In eight subjects, anti-HIV activity was shown to be inhibitible with OKT3, confirming the T cell nature of this activity. HLA restriction of the anti-HIV CTL was shown using autologous and partially HLA-matched target cells (Table 2). Thus, classical anti-HIV CTL can be induced by this method in most HIV$^+$ subjects. Three subjects, AO86, A0123 and AO102 showed no CTL activity after 7 days stimulation (Table 1). This was somewhat surprising in the case of subject AO86 because of the presence of circulating anti-HIV CTL. The lack of activity after 7 days of stimulation may be related to terminal differentiation of the circulating anti-HIV CTL *in vivo*, as previously suggested by Gotch *et al.* (14). Anti-HIV CTL activity developed for two of these subjects (AO123 and AO102) who were retested after 10 days of stimulation (Fig. 1). In addition, higher CTL activity was present after 10 days of *in vitro* stimulation compared to 7 days of stimulation (AO105). Thus, competent CTL precursors were always present, although different incubation times and conditions were necessary for their activation. Since our original cohort included only two subjects classified under then current guidelines as having AIDS (CDC group IV), we wished to confirm the presence of inducible anti-HIV CTL in additional group IV subjects. Results shown in Table 3 confirm

the persistence of anti-HIV CTL precursors following the occurrence of AIDS defining opportunistic infections or neoplasms. Two of the subjects tested in this group were also part of the original cohort, one (AO103) having progressed to AIDS over the course of study and the other (AO78), one of the original 2 group IV subjects. Subject AO78 has retained anti-HIV CTL precursor activity for >24 months post AIDS. For a number of subjects exogenous IL-2 was not necessary for induction of anti-HIV CTL in 7 day cocultures, while for others, additional IL-2 was necessary or significantly augmented the response (Figs. 2a and 2b). After stimulation without exogenous IL-2, not enough cells were obtained with two subjects with AIDS (AO78 and AO103) to even perform assays, while AIDS subject AO136 showed equivalent anti-HIV CTL responses with and without exogenous IL-2. Thus, neither clinical staging nor immunological staging (CD4 lymphocyte counts) can be generally applied to predict requirements for *in vitro* induction of anti-HIV CTL in $HIV^+$ subjects.

**Contribution of different lymphocyte subsets to anti-HIV CTL**

In order to determine which subset of lymphocytes mediated the anti-HIV CTL activity and from which subsets the CTL originated, selection and depletion experiments were carried out using anti-CD4, anti-CD8 and anti-HLA-DR monoclonal antibodies and anti-mouse IgG coated magnetic beads. After restimulation with HIV and IL-2, effector cells from 6 subjects were depleted of either $CD8^+$ or $CD4^+$ lymphocytes immediately before CTL assays. In all cases, depletion of $CD4^+$ cells had little effect, while removal of $CD8^+$ cells reduced anti-HIV CTL activity practically to background (Fig. 3). Thus $CD8^+$ lymphocytes mediated the HIV env and pol specific lysis of autologous BLCL infected with recombinant vaccinia viruses.

In order to determine the initial phenotype of the anti-HIV CTL present following HIV/IL-2 stimulation or Con A/IL-2 stimulation, freshly isolated lymphocytes were separated into

control and HLA-DR$^-$ populations by depletion of HLA-DR$^+$ cells. Stimulation with HIV and IL-2 induced anti-HIV CTL activity in both populations, but the activity was lower in the population depleted of HLA-DR$^+$ cells (Fig. 4). This suggests that both the HLA-DR$^+$ and the HLA-DR$^-$ populations contain anti-HIV CTL precursor cells. However, removal of HLA-DR$^+$ cells might decrease the induced anti-HIV CTL activity by removal of accesory cells, rather than removal of CTL precursors. To address this possibility, freshly isolated PBMC were separated into HLA-DR$^+$ and HLA-DR$^-$ populations by positive selection of HLA-DR$^+$ cells, stimulated non-specifically with Con A and IL-2 and tested for anti-HIV CTL activity. This method of stimulation also resulted in higher anti-HIV CTL activities with the HLA-DR$^+$ starting populations (Fig. 5). Therefore, some HLA-DR$^+$ cells contribute to the anti-HIV CTL activity generated *in vitro* by stimulation with HIV and IL-2 or Con A and IL-2. The HLA-DR antigen is an activation marker on T lymphocytes, therefore it is notable that HLA-DR$^+$ cells present *in vivo* contribute to *in vitro* inducible anti-HIV CTL activity in subjects without detectable circulating anti-HIV CTL activity and low numbers of CD8$^+$ lymphocytes.

## Discussion

Understanding the role of anti-HIV CTL in the natural history of HIV infection is important for understanding the pathogenesis of AIDS. Anti-HIV CTL activity is potentially a critical determinant of the course of infection and also a window on the immune status of HIV infected subjects. In this cross-sectional study, we examined anti-HIV CTL activity in a cohort of HIV infected subjects at different stages of disease. Interestingly, a number of subjects with $CD4^+$ lymphocyte counts indicative of progressive disease had high levels of circulating anti-HIV CTL activity. We observed a strong association between circulating anti-HIV CTL activity and the numbers of $CD8^+$ lymphocytes. Furthermore, the mean $CD8^+$ lymphocyte count was higher in subjects with circulating anti-HIV CTL activity than in subjects without circulating anti-HIV CTL. HIV pol antigens were most commonly recognized by circulating CTL from subjects studied with our assay system. In contrast, anti-HIV CTL inducible by *in vitro* stimulation with HIV infected autologous lymphoblasts and IL-2 were present in all subjects tested. These CTL were HLA class I-restricted $CD8^+$ T lymphocytes. Requirements for induction of anti-HIV CTL activity *in vitro* varied between individual subjects; many required only coculture with HIV infected autologous lymphoblasts for 7 days, while others also required exogenous IL-2 and 10 days of coculture. The levels of inducible activity were independent of $CD4^+$ or $CD8^+$ lymphocyte counts and disease status. Anti-HIV CTL activated *in vitro* were derived from both $CD8^+$ $HLA\text{-}DR^+$ and $CD8^+$ $HLA\text{-}DR^-$ circulating lymphocytes in subjects with and without detectable circulating anti-HIV CTL. Thus, anti-HIV CTL or their precursors were often activated *in vivo*, as demonstrated by HLA-DR expression, in subjects with <900 $CD8^+$ lymphocytes/μl blood and no detectable circulating anti-HIV CTL.

The absence of circulating anti-HIV CTL in subjects showing strong anti-HIV CTL responses following *in vitro* stimulation suggests there is *in vivo* impairment of some aspect

of anti-HIV CTL generation. The lack of correlation between circulating anti-HIV CTL activity and $CD4^+$ lymphocyte number and the ability of subjects without circulating anti-HIV CTL to generate anti-HIV CTL *in vitro* without exogenous IL-2 indicates that this impairment is not wholly due to defective helper T cell function. A soluble factor released by $CD8^+CD57^+$ T cells, which increase in number during HIV infection [12], was shown to inhibit the effector phase of CTL function [31]. Further, transforming growth factor β, which inhibits the differentiation and expansion of CTL, is produced in greater amounts by PBMC cultures of $HIV^+$ subjects than controls [32]. These soluble factors might play a role in anti-HIV CTL impairment. Recently, our laboratory demonstrated that exposure of CTL to human fibroblasts infected with herpes simplex virus renders the CTL inactive [C.M. Posavad and K.L. Rosenthal: submitted for publication]. Therefore, a number of features associated with HIV infection, other than defective $CD4^+$ helper cell function, may conspire to compromise the effectiveness of the anti-HIV CTL response.

Anti-HIV CTL responses were detectable in all HIV infected subjects following *in vitro* restimulation, but the activation requirements of these CTL varied between subjects, suggesting a hierarchy of defects in CTL responsiveness. It is likely that anti-HIV CTL can be induced by coculture with HIV infected cells without exogenous IL-2 before HIV associated deficiencies occur. Either functional helper lymphocytes responsive to HIV are still present or the $CD8^+$ CTL produce IL-2 and proliferate in an autocrine fashion. A fraction of $HIV^+$ subjects required exogenous IL-2 for the generation of anti-HIV CTL activity. In these subjects, HIV specific helper function was insufficient or possibly suppressor cells which are diluted out in IL-2 were present. Still other $HIV^+$ subjects required longer periods of stimulation with HIV infected cells and exogenous IL-2, indicating lower CTL precursor frequency, increased suppressor cell frequency, or other factors reducing the efficiency of CTL generation.

Our subjects were all asymptomatic at time of testing, including those with previous AIDS defining opportunistic infections. Previous reports of failure to detect anti-HIV CTL precursors in subjects with AIDS defining opportunistic infections and of decreasing anti-HIV CTL precursor frequency following opportunistic infections [11], could be due to the effects of concurrent infection, rather than specific effects of HIV disease. Two previous studies of circulating non-specific CTL activity showed that AIDS patients had elevated circulating CTL activity which fell upon occurrence of an opportunistic infection [33, 34].

The presence of circulating anti-HIV CTL activity in subjects with low $CD4^+$ lymphocyte counts is interesting in light of current models of the pathobiology of HIV infection. Elevation of $CD8^+$ lymphocyte numbers occurs before significant $CD4^+$ lymphocyte depletion [35] and the association between $CD8^+$ lymphocyte counts and circulating anti-HIV CTL activity suggests that at least some of the $CD8^+$ lymphocytosis during infection is due to activation of anti-HIV CTL. $CD4^+$ lymphocyte depletion may therefore occur despite anti-HIV CTL activity. $CD8^+$ cells are known to at least suppress viral production by HIV infected lymphocytes [4, 36], but it is clear that infection is not eliminated and that complete viral suppression never occurs *in vivo*. There is, therefore, speculation that clinical deterioration occurs only when the level of viral production crosses a certain threshold. In this model of pathogenesis, a role for anti-HIV CTL in delaying disease progression seems probable. However, if the pathogenic effects of HIV are mediated by direct destruction of $CD4^+$ lymphocytes, this progressive destruction in the face of few productively infected lymphocytes and an unusually strong anti-viral CTL response is paradoxical. In light of this, indirect mechanisms of HIV induced $CD4^+$ lymphocyte depletion which would not be hindered, and in some cases would be hastened by anti-HIV CTL activity have recently been proposed [20, 37-39].

In summary, it is clear that *in vivo* anti-HIV CTL activity and *in vitro* anti-HIV CTL responses persist long after significant HIV induced $CD4^+$ lymphocyte depletion has occurred. These CTL suppress HIV replication *in vitro*, although they do not eliminate HIV infected cells [40]. If there are as few productively infected lymphocytes as reported [2, 41, 42], it is unclear why $CD4^+$ lymphocyte numbers decrease so dramatically. Infection of T cell progenitors is one possible explanation [43] but this would have an effect on $CD8^+$ lymphocytes as well, given the passage of $CD8^+$ precursors through a $CD4^+CD8^+$ stage [44]. As yet, there is no evidence that class I-restricted anti-HIV CTL contribute to $CD4^+$ lymphocyte depletion and class II-restricted anti-HIV CTL are not apparent in natural infection. Human HIV infection is enigmatic in terms of the profound $CD4^+$ lymphocyte depletion occurring despite low levels of infection. Less research has focussed on elevated $CD8^+$ numbers than on reduced $CD4^+$ numbers, but this is an early and persistent characteristic of HIV infection [35]. An epidemiological study reported more rapid progression to AIDS in subjects with the highest $CD8^+$ lymphocyte counts at the time of first examination [45] and $CD8^+$ T-cells from $HIV^+$ subjects which specifically lyse uninfected $CD4^+$ T cells have also been described [46]. Although some $CD8^+$ lymphocytosis probably relates to anti-HIV activity, the pathological changes in $CD8^+$ lymphocyte activity observed in HIV infection imply additional processes in maintaining elevated $CD8^+$ lymphocyte numbers and inducing the accumulation of $CD8^+$ lymphocytes with unusual characteristics. This indirect effect of HIV infection could relate more directly to the pathogenesis of AIDS than the infection of small numbers of $CD4^+$ lymphocytes.

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## References


1. Pantaleo G, DeMaria A, Koenig S, *et al*: **CD8+ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of human immunodeficiency-virus-specific cytotoxicity.** *Proc Natl Acad Sci USA* 1990, 87:4818-4822.

2. Schnittman SM, Psallidopoulos MC, Lane HC, *et al*: **The reservoir for HIV in human peripheral blood is a T-cell that maintains expression of CD4.** *Science* 1989, 245:305-308.

3. Walker BD, Chakrabarti S, Moss B, *et al*: **HIV-specific cytotoxic T lymphocytes in seropositive individuals.** *Nature* 1987, 328:345-348.

4. Walker CM, Moody DJ, Stites DP, Levy JA: **CD8+ lymphocytes can control HIV infection *in vitro* by suppressing virus replication.** *Science* 1986, 234:1563-1566.

5. Venet A, Lu W,Beldjord K, Andrieu J: **Correlation between CD4 cell counts and cellular and plasma viral load in HIV-1-seropositive individuals.** *AIDS* 1991, 5:283-288.

6. Ho DD, Moudgil T, Alam M, *et al*: **Quantitation of human immunodeficiency virus type 1 in the blood of infected persons.** *N Engl J Med* 1989, 321:1621-1625.

7. Coombs RW, Collier AC, Allain JP: **Plasma viremia in human immunodeficiency virus infections.** *N Engl J Med* 1989, 321:1626-1631.

8. Shearer G M, Salahuddin SZ, Markham PD, *et al*: **Prospective study of cytotoxic T lymphocyte responses to influenza and antibodies to human T lymphotropic virus-III in homosexual men.** *J Clin Invest* 1985, 76:1699-1704.

9. Shearer GM, Bernstein DC, Tung KSK, *et al*: **A model for the selective loss of major histocompatibility complex self restricted T-cell immune responses during the development of acquired immunodeficiency syndrome [AIDS].** *J Immunol* 1986, **137**:2514-2521.

10. Clerici MN, Stocks J, Zajac RA, Boswell RN, Via CS, Shearer GM: **Circumvention of defective CD4 T helper cell function in HIV-infected individuals by stimulation with HLA alloantigens.** *J Immunol* 1990, **144**:3266-3271.

11. Hoffenbach A, Langlade-Demoyen P, Dadaglio G, *et al*: **Unusually high frequencies of HIV-specific cytotoxic T lymphocytes in humans.** *J Immunol* 1989, **142**:452-462.

12. Joly P, Guillon J, Mayaud C, *et al*: **Cell-mediated suppression of HIV-specific cytotoxic T lymphocytes.** *J Immunol* 1989, **143**:2193-2201.

13. Pantaleo G, Koenig S, Baseler M, Lane HC, Fauci AS: **Defective clonogenic potential of $CD8^+$ T lymphocytes in patients with AIDS.** *J Immunol* 1990, **144**:1696-1704.

14. Gotch FM, Nixon DF, Alp N, McMichael AJ, Borysiewicz LK: **High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals.** *Int Immunol* 1990, **2**:707-712.

15. Walker BD, Flexner C, Paradis D, *et al*: **HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals.** *Science* 1988, **240**:64-66.

16. Koup RA, Sullivan JL, Levine PH, *et al*: **Detection of Major histocompatibility complex class I-restricted HIV-specific cytotoxic T lymphocytes in the blood of infected hemophiliacs.** *Blood* 1989, **73**:1909-1914.

17. Calabrese LH, Estes M, Yen-Lieberman B, *et al*: **Systemic vasculitis in association with human immunodeficiency virus infection.** *Arthritis Rheum* 1989, 32:569-576.

18. Itescu S, Brancato LJ, Buxbaum J, *et al*: **A diffuse infiltrative CD8 lymphocytosis syndrome in human immunodeficiency virus (HIV) infection; a host immune response associated with HLA-DR5.** *Ann Intern Med* 1990, 112:3-10.

19. Autran B, Mayaud CM, Raphael M, *et al*: **Evidence for a cytotoxic T-lymphocyte alveolitis in human immunodeficiency virus-infected patients.** *AIDS* 1988, 2:179-185.

20. Zinkernagel, RM: **Virus-trigerred AIDS: a T-cell mediated immunopathology?** *Immunol Today* 1988, 9:370-372.

21. Germain RN: **Antigen processing and CD4$^+$ T cell depletion in AIDS.** *Cell* 1988, 54:441-443.

22. Siliciano RF, Lawton T, Knall C, *et al*: **Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4$^+$ cell depletion.** *Cell* 1988, 54:561-575.

23. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y: **Microdroplet testing for HLA-A, -B, -C and -D antigens.** *Am J Clin Pathol* 1978, 69:103-120.

24. Langlade-Demoyen P, Michel F, Hoffenbach A, *et al*: **Immune recognition of AIDS virus antigens by human and murine cytotoxic T lymphocytes.** *J Immunol* 1988, 141:1949-1958.

25. Flexner C, Broyles SS, Earl P, Chakrabarti S, Moss B: **Characterization of human immunodeficiency virus gag/pol gene products expressed by recombinant vaccinia viruses.** *Virol* 1988, 166:339-349.

26. Chakrabarti S, Robert-Guroff M, Wong-Staal F, Gallo RC, Moss B: **Expression of the HTLV-III envelope gene by a recombinant vaccinia virus.** *Nature* 1986, **320**:535-537.

27. Ozols DY, Harnish DG, Rawls WE, Rosenthal KL: **Assessment of the specificity of cytotoxic T lymphocytes for the nucleoprotein of pichinde virus using recombinant vaccinia viruses.** *Arch Virol* 1990, **115**:209-225.

28. Walker CM, Rawls WE, Rosenthal KL: **Generation of memory cell-mediated immune responses after secondary infection of mice with pichinde virus.** *J Immunol* 1984, **132**:469-474.

29. Steimer KS, Puma JP, Power MD, *et al*: **Differential antibody responses of individuals infected with AIDS-associated retroviruses surveyed using the viral core antigen p25 gag expressed in bacteria.** *Virol* 1986, **150**:283-290.

30. Centers for Disease Control: **Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections.** *Morbid Mortal Weekly Rep* 1986, **35**:334-339.

31. Autran B, Leblond V, Sadat-Sowti B, *et al*: **A soluble factor released from bone marrow transplanted patients inhibits cell-mediated cytolysis.** *Blood* 1991, **77**:2237-41.

32. Kekow J, Wachsman W, McCutchan, JA, Cronin M, Carson DA, Lotz M: **Transforming growth factor β and noncytopathic mechanisms of immunodeficiency in HIV infection.** *Proc Natl Acad Sci USA* 1990, **87**:8321-8325.

33. Vanham G, Kestens L, Gigase P, *et al*: **Evidence for circulating activated cytotoxic T cells in HIV-infected subjects before the onset of opportunistic infections.** *Clin exp Immunol* 1990, 82:3-9.

34. Vanham G, Kestens L, Penne G, *et al*: **Subset markers of CD8(+) cells and their relation to enhanced cytotoxic T-cell activity during human immunodeficiency virus infection.** *J Clin Immunol* 1991, 11:345-356.

35. Gaines H, von Sydow MAE, von Stedingk LV, *et al*: **Immunological changes in primary HIV-1 infection.** *AIDS* 1990, 4:995-999.

36. Kannagi M, Masuda T, Hattori T, *et al*: **Interference with human immunodeficiency virus (HIV) replication by CD8+ T cells in peripheral blood leukocytes of asymptomatic HIV carriers *in vitro*.** *J Virol* 1990, 64:3399-3406.

37. Hoffmann GW, Kion TA, Grant MD: **An idiotypic network model of AIDS immunopathogenesis.** *Proc Natl Acad Sci USA* 1991, 88:3060-3064.

38. Ameisen JC, Capron A: **Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis.** *Immunol Today* 1991, 12:102-105.

39. Habeshaw JA, Dalgleish A: **The relevance of HIV/envCD4 interactions to the pathogenesis of acquired immune deficiency syndrome.** *J Acq Immun Def Synd* 1989, 2:457-468.

40. Tsubota H, Lord CI, Watkins DI, Morimoto C, Letvin NL: **A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes.** *J Exp Med* 1989, 169:1421-1434.

41. Harper ME, Marsalle LM, Gallo RC, Wong-Staal F: **Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization.** *Proc Natl Acad Sci USA* 1986, 83:772-776.

42. Brinchmann JE, Albert J, Vartdal F: **Few infected CD4+ T cells but a high proportion of replication-competent provirus copies in asymptomatic human immunodeficiency virus type 1 infection.** *J Virol* 1991, 65:2019-2023.

43. Schnittman SM, Denning SM, Greenhouse JJ, *et al*: **Evidence for susceptibility of intrathymic T-cell precursors and their progeny carrying T-cell antigen receptor phenotypes TCRαβ+ and TCRγδ+ to human immunodeficiency virus infection: a mechanism for CD4+ (T4) lymphocyte depletion.** *Proc Natl Acad Sci USA* 1990, 87:7727-7731.

44. Fowlkes BJ, Schwartz RH, Pardoll DM. **Deletion of self-reactive thymocytes occurs at a CD4+8+ precursor stage.** *Nature* 1988, 334:620-623.

45. Anderson RE, Shiboski SC, Royce R, Jewell NP, Lang W, Winkelstein WJr: **CD8+ T lymphocytes and progression to AIDS in HIV-infected men: some observations.** *AIDS* 1991, 5:213-215.

46. Zarling JL, Ledbetter JA, Sias J, *et al*: **HIV-infected humans, but not chimpanzees, have circulating cytotoxic T lymphocytes that lyse uninfected CD4+ cells.** *J Immunol* 1990, 144:2992-2998.

Fig. 1

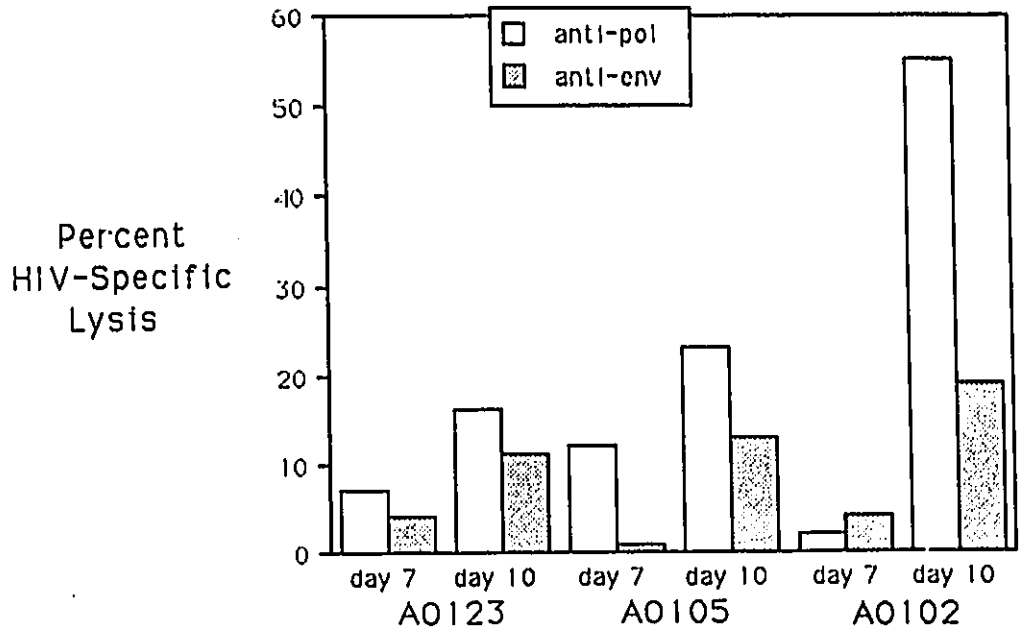
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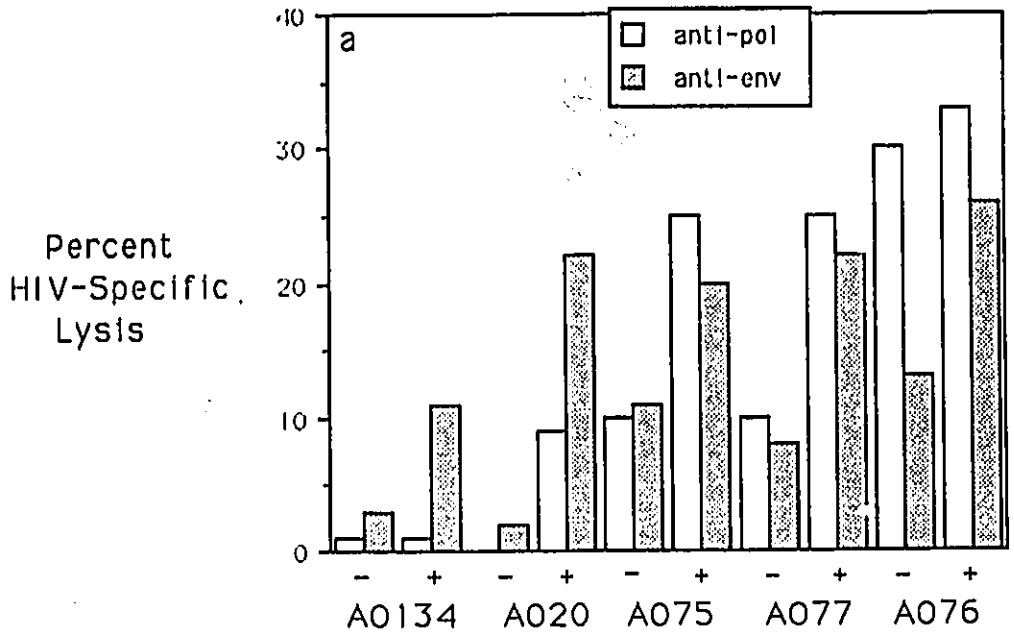
Fig. 2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HIV-Specific
Lysis
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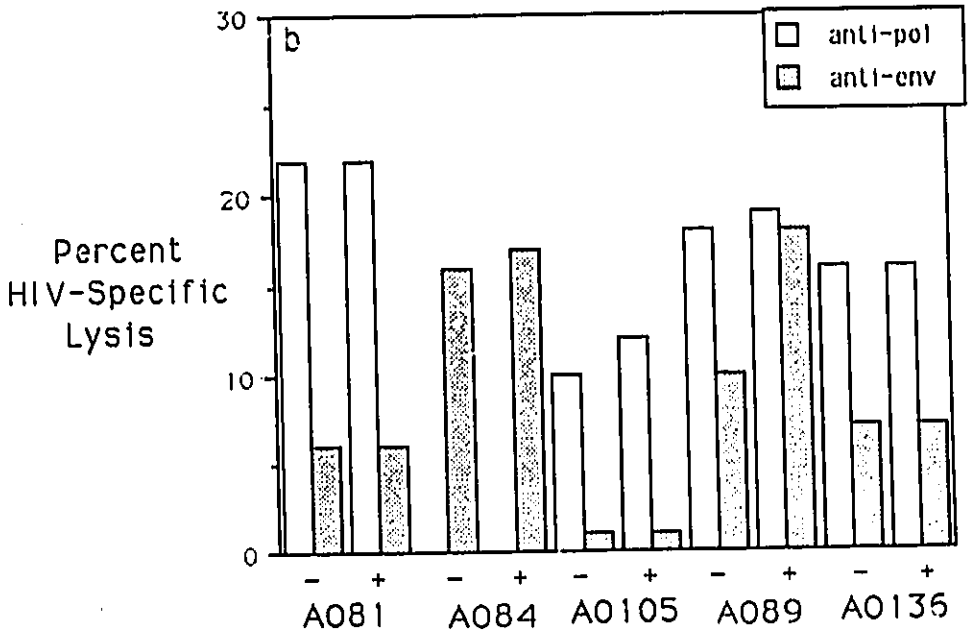
30
20
10
0
a
anti-pol
anti-env
- + - + - + - + - +
A0134
A020
A075
A077
A076

Fig. 2


b
anti-pol
anti-env
Percent HIV-Specific Lysis
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20
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0
- + - + - + - + - +
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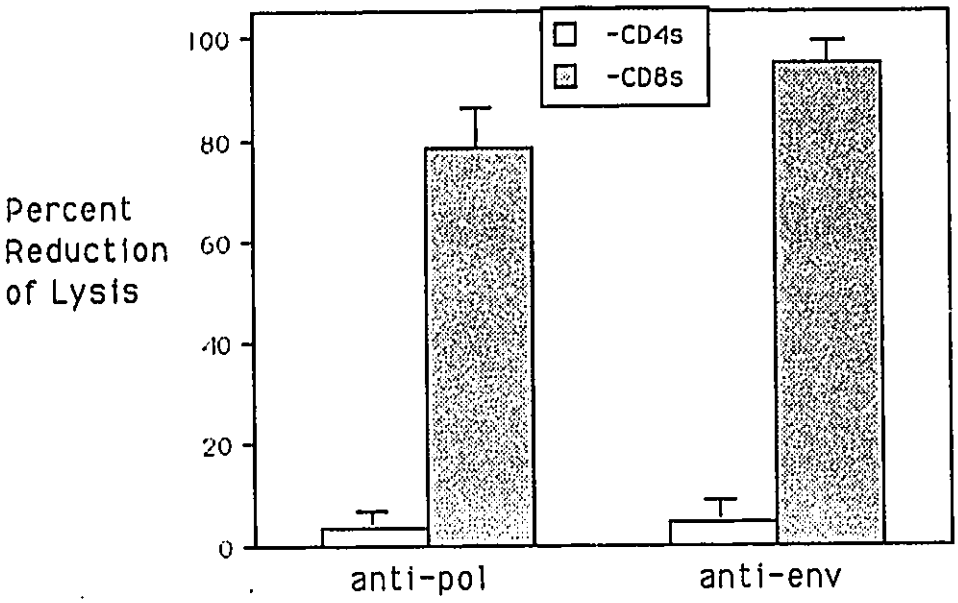
Fig. 3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Reduction
of Lysis
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60
40
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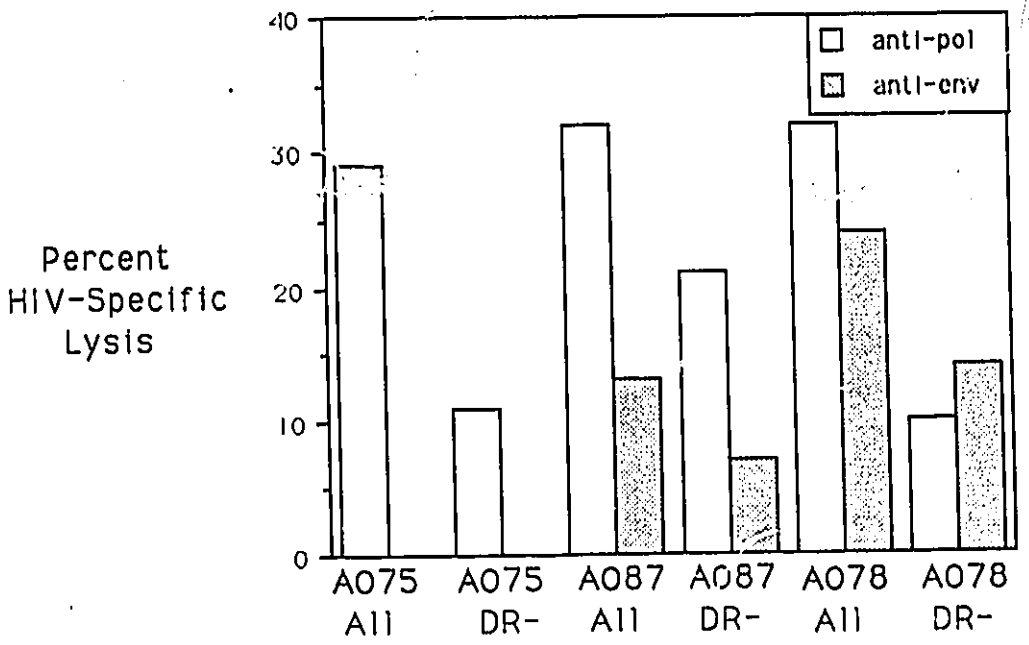
-CD4s
-CD8s
anti-pol
anti-env

Fig. 4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HIV-Specific
Lysis
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20
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All
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A087
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A078
All
A078
DR-

Fig. 5

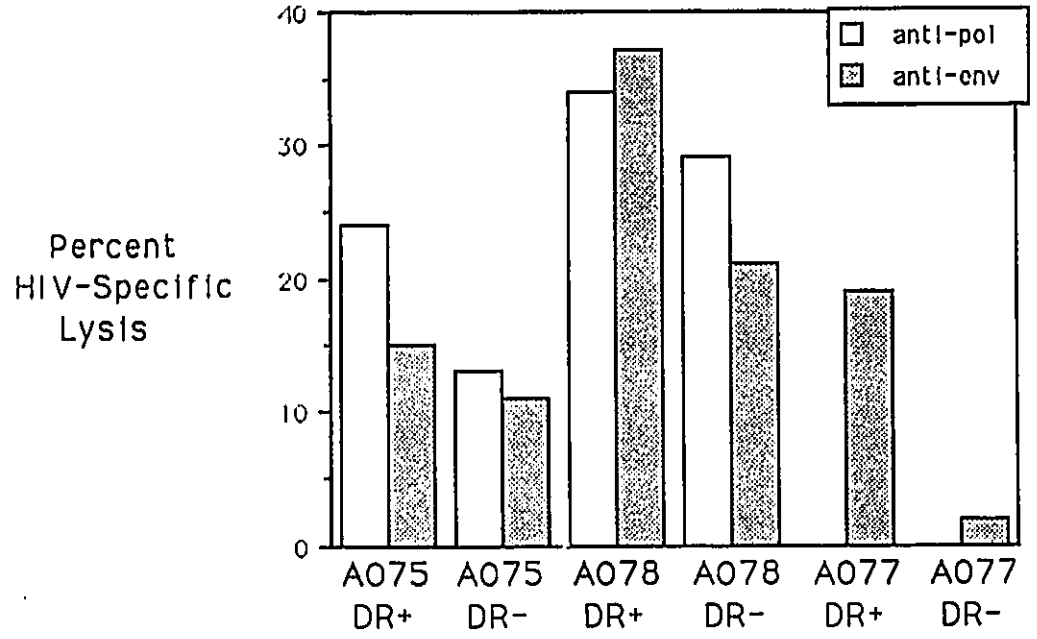
Percent
HIV-Specific
Lysis
40
30
20
10
0
anti-pol
anti-env
A075 DR+
A075 DR-
A078 DR+
A078 DR-
A077 DR+
A077 DR-

Figure Captions

**Fig. 1.** Comparison of 7 and 10 day *in vitro* stimulation of anti-HIV CTL. All subjects were tested after 7 days for consistency (Table 1), but subjects whose activity after 7 days was insignificant were retested on day 10. Results shown are with an effector:target ratio of 50:1 with lysis of control VVNP infected targets subtracted for calculation of percent specific lysis. Lysis of VVNP-infected targets in these experiment ranged from 1% to 7%.

**Fig. 2.** Effect of exogenous IL-2 on *in vitro* induction of anti-HIV CTL activity. [a] In 5 subjects, addition of 5U/ml IL-2 (+) after 3 days to cells stimulated with autologous infected lymphoblasts was necessary for, or at least enhanced, induction of anti-HIV CTL. [b] In another 5 subjects, addition of IL-2 (+) after 3 days had little effect on anti-HIV CTL responses. Results shown are with an effector:target ratio of 50:1 with lysis of VVNP infected control targets subtracted for calculation of percent specific lysis. Lysis of VVNP-infected targets in these experiment ranged from 6% to 41%.

**Fig. 3.** Effect of T cell subset depletions on anti-pol and anti-env CTL activity. Depletion of $CD4^+$ or $CD8^+$ cells was carried out immediately before CTL assays on starting populations set at an effector:target ratio of 50:1. Results shown represent the mean reduction in percent specific lysis (±SEM) in depletion experiments with 6 separate subjects. Initial specific lysis of vacc-pol infected targets ranged from 16%-66% and initial specific lysis of vacc-env expressing targets from 12-60% in these experiments.

**Fig. 4.** Contribution of HLA-DR$^+$ cells to the inducible anti-HIV CTL response. Fresh PBMC were split into two populations, one of which was depleted of HLA-DR$^+$ cells (DR$^-$) prior to stimulation with HIV infected autologous lymphoblasts and IL-2 for 7 days. Anti-HIV CTL activity was then measured in both populations. Effector:target ratio was 20:1 for subject AO75 and 50:1 for subjects AO78 and AO87. Lysis of control VVNP infected targets was subtracted for calculation of percent specific lysis. Lysis of VVNP-infected targets in these experiment ranged from 2% to 13%.

**Fig. 5.** Anti-HIV CTL induced *in vitro* derive from the circulating HLA-DR$^+$ lymphocyte population. Positively selected HLA-DR$^+$ cells (DR$^+$) and the reciprocal cell population depleted of HLA-DR$^+$ cells (DR$^-$) were stimulated with Con A for 3 days, followed by 5U/ml IL-2 for 7 days before assessment of anti-HIV CTL activity using autologous BLCL infected with recombinant vaccinia viruses. Results shown are with an effector:target ratio of 50:1 with lysis of VVNP infected control targets subtracted for calculation of percent specific lysis. Lysis of VVNP-infected targets in these experiment ranged from 2% to 13%.

Table 1. HIV+ Subjects' Clinical Status and CTL Activity Against HIV Antigens

| Subject | CDC Stage[a] | Lymphocytes/µl Blood CD4+ | CD8+ | AZT | HIV Plasma Ag[b] | % Specific Lysis[c] Circulating gag | pol | env | pich | Restimulated gag | pol | env | pich |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO89[d] | II | 830 | 1160 | - | - | 14 | 0 | 9 | 0 | 14 | 28 | 3 | 9 |
| AO101 | II | 660 | 825 | - | - | 0 | 1 | 0 | 0 | 43 16[e] | 47 17 | 44 25 | 31 |
| AO123 | II | 420 | 630 | - | - | 0 | 4 | 0 | 0 | 4 | 13 | 10 | 6 |
| AO76 | II | 390 | 1300 | - | - | 3 | 2 | 3 | 3 | 21 4 | 62 8 | 44 8 | 25 |
| AO80 | II | 310 | 1030 | - | - | 0 | 3 | 10 | 0 | 26 | 26 | 39 | 17 |
| AO86 | II | 300 | 1500 | - | + | 8 | 12 | 16 | 2 | 41 | 33 | 44 | 35 |
| AO84 | II | 300 | 1000 | - | + | 0 | 0 | 0 | 0 | 14 | 21 | 26 9 | 9 |
| AO81 | II | 280 | 930 | - | - | 4 | 25 | 6 | 0 | 29 | 51 | 35 | 28 |
| AO87 | II | 280 | 930 | + | - | 7 5 | 27 10 | 8 7 | 5 | 12 | 34 | 43 | 13 |
| AO134 | II | 250 | 590 | + | - | 0 | 8 | 2 | 0 | 9 | 15 | 25 | 14 |
| AO105 | II | 240 | 1200 | + | + | 0 | 0 | 5 | 0 | 11 | 18 | 7 | 6 |
| AO77 | Ii | 230 | 380 | + | - | 0 | 3 | 6 | 0 | 38 24 | 51 29 | 50 28 | 23 |
| AO78 | IVc-1 | 220 | 730 | + | + | 0 | 0 | 1 | 0 | 30 3 | 30 5 | 35 7 | 17 |
| AO103 | II | 220 | 550 | + | + | 0 | 2 | 3 | 0 | 14 | 30 | 50 | 5 |
| AO88 | II | 180 | 600 | + | + | 0 | 0 | 4 | 0 | 42 17 | 50 18 | 53 17 | 40 |
| AO85 | III | 150 | 750 | - | + | 10 | 5 | 8 | 5 | 14 | 17 | 14 | 5 |
| AO75 | II | 90 | 900 | + | - | 8 0 | 26 0 | 11 0 | 3 | 12 | 45 9 | 31 6 | 14 |
| AO72 | II | 40 | 400 | + | - | 8 | 7 | 9 | 10 | 5 | 9 | 25 7 | 9 |
| AO118 | IVc-1 | 30 | 560 | + | + | 0 | 0 | 5 | 0 | 2 | 9 | 33 | 3 |
| AO102 | II | 10 | 470 | + | - | 0 | 0 | 5 | 0 | 7 | 9 | 11 | 7 |

Table 1. Legend

[a]CDC status assessed according to published classification criteria [30].

[b]Subjects positive for antigenemia if >30 pg p24/ml plasma present.

[c]Percent Specific Lysis values shown at an effector:target ratio of 100:1 for circulating CTL and 50:1 for restimulated CTL.

[d]Subjects underlined had detectable circulating anti-HIV CTL activity.

[e]Underlined values are percent specific lysis in the presence of OKT3, which inhibits T cell-mediated lysis.

Table 2. Class I HLA Restriction Specificity of anti-HIV CTL

| Subject | HLA Class I Type | | Target cell | % Specific Lysis[a] | | | |
|---|---|---|---|---|---|---|---|
| | A | B | HLA Match | gag | pol | env | pich |
| AO75 | 2, 24 | 44 | autologous | 12 | 45 | 31 | 14 |
| | | | A2 | ND | 18 | 30 | 10 |
| | | | A24 | ND | 12 | 16 | 17 |
| | | | B44 | ND | 24 | 3 | 2 |
| AO76 | 2, 28 | 18, 44 | autologous | 23 | 62 | 44 | 25 |
| | | | A2 | ND | 20 | 25 | 8 |
| | | | A28 | ND | 22 | 25 | 12 |
| | | | B18 | ND | 15 | 18 | 22 |
| | | | B44 | ND | 11 | 11 | 13 |
| AO80 | 1, 3 | 7, 35 | autologous | 27 | 27 | 39 | 17 |
| | | | A1 B35 | 15 | 22 | 23 | 12 |
| | | | A1 | 19 | 28 | 28 | 18 |
| | | | B7 | 44 | 41 | 49 | 25 |
| AO81 | 2, 28 | 44, 51 | autologous | 29 | 51 | 35 | 28 |
| | | | A2 B51 | 8 | 12 | 9 | 5 |
| | | | A28 B51 | 11 | 10 | 20 | 10 |
| | | | A2 | 8 | 10 | 12 | 4 |

[a]Percent specific lysis is shown at an effector:target ratio of 50:1.

Table 3. Inducible anti-HIV CTL activity in CDC group IV Subjects

| Subject | Lymphocytes /μl Blood | | % Specific Lysis[a] Effector cells alone | | | | Effectors + OKT3[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4+ | CD8+ | gag | pol | env | pich | gag | pol | env | pich |
| AO103 | 10 | 170 | 3 | 17 | 19 | 2 | 2 | 5 | 5 | 2 |
| AO78 | 30 | 150 | 11 | 39 | 37 | 6 | 0 | 3 | 0 | 4 |
| AO188 | 40 | 130 | 16 | 29 | 19 | 11 | 7 | 2 | 14 | 7 |
| AO207 | 20 | 600 | 13 | 30 | 20 | 9 | 13 | 15 | 16 | 13 |
| AO130 | 10 | 690 | 6 | 6 | 10 | 0 | 2 | 2 | 4 | 0 |
| AO136 | 290 | 580 | 47 | 57 | 48 | 41 | 24 | 28 | 27 | 17 |
| AO185 | 30 | 280 | 26 | 40 | 25 | 23 | 11 | 14 | 8 | 7 |
| AO20 | 0 | 280 | 7 | 9 | 22 | 3 | 14 | 12 | 17 | 8 |

[a]Percent specific lysis is shown at an effector:target ratio of 50:1.

[b]Percent specific lysis in the presence of monoclonal antibody OKT3 was measured to confirm that the lysis was mediated by T cells.

# Autoimmune Cytotoxic T Lymphocytes in HIV-1-infection

# Evidence of a Role for Autoimmune Cytotoxic T lymphocytes in CD4+ Lymphocyte Depletion in HIV-1-infection

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Infection of humans with HIV results in depletion of CD4+ lymphocytes by poorly understood mechanisms. Here, we demonstrate that HIV-infected individuals possess CTL that kill autologous activated CD4+ lymphocytes and activated CD4+ lymphocytes from heterologous HIV-infected and uninfected individuals. HIV or Con A induced this activity in PBL cultures from HIV-infected, but not uninfected individuals. CTL present in HIV-infected individuals lysed activated CD4+ but not CD8+ lymphocytes from the same HIV-infected or uninfected individuals. Effector cells mediating this activity were shown to be CD3+ CD8+ and TCR αβ+, yet lysis mediated by these cells was neither HLA-restricted, nor HIV-specific. CTL-mediated lysis of heterologous CD4+ lymphocytes was not inhibited by a mAb against HLA-A, B and C antigens and occurred when targets and effectors were mismatched at HLA-A and B class I antigens. Cytotoxicity was much higher against activated than non-activated CD4+ lymphocytes. These CTL distinguish between activated CD4+ cells from different HIV-seronegative individuals, suggesting recognition of a polymorphic antigen specific to CD4+ lymphocytes and unrelated to HIV. CTL activity against autologous CD4+ lymphocytes was detected at any one time in only a minority of HIV-infected subjects tested, but varied markedly over time in individual HIV-infected persons, suggesting that there are periodic peaks of detectable activity. A group of HIV-infected individuals with this CTL activity upon initial testing demonstrated a 36% drop in mean CD4 lymphocyte count over a six month follow-up period, whereas a corresponding group without this CTL activity showed no significant change in CD4 lymphocyte count over an equivalent period ($p<.01$). Further, several individuals with CTL activity against their own CD4+ lymphocytes at one time point did not lyse their own CD4+ lymphocytes at a later time point when their CD4 count had fallen, but continued to lyse CD4+ lymphocytes from an HIV-seronegative donor. The association of CTL activity against CD4+ lymphocytes with HIV infection, prediction of CD4+ T cell loss and elimination of susceptible

autologous CD4+ T cell targets over time are features consistent with a role for this activity in the *in vivo* depletion of CD4+ T cells and in the pathogenesis of AIDS.

The mechanisms by which HIV infection leads to CD4+ T cell depletion and the development of AIDS are not clear. It has been argued that HIV infection of and replication in CD4+ lymphocytes is responsible for this depletion, but enumeration of HIV-infected CD4+ cells *in vivo* has raised doubt that this is the only mechanism of CD4+ T cell depletion (1-3). Alternative mechanisms have been proposed and are now receiving considerable attention (4-10). Evidence was recently presented for HIV-associated superantigen-mediated deletion of T cells (11) and there is also evidence that CD4+ T cell deletion may involve induction of T cell anergy (12) and *in vivo* priming of T cells for apoptosis (13). These alternate mechanisms are based on abnormal T cell activation which might mimic ontogenetic selection events leading to anergy or deletion. In the inappropriate setting of the mature immune system, the result could be tolerance to foreign antigens, immunodeficiency and T cell depletion, the hallmarks of AIDS. It is unclear, however, how superantigens mediate depletion of peripheral T cells, whether HIV genes encode a superantigen and exactly how peripheral T cells become primed for apoptosis. Evidence has also accumulated supporting a role for autoimmunity in the pathogenesis of AIDS, including reports of cell-mediated cytotoxicity against uninfected lymphocytes (14-18). The demonstration by Zarling *et al.* (19) that HIV-infected humans, but not HIV-infected chimpanzees have circulating CTL that lyse uninfected CD4+ lymphocytes is especially provocative.

Elevated CD8+ lymphocyte numbers and enhanced cytotoxicity of peripheral blood lymphocytes are common features of HIV infection (20-23). In fact, immunopathology associated with HIV infection includes CD8+ T cell-mediated alveolitis, sicca-syndrome, vasculitis and neuritis (24-27). Increased levels of activated HLA-DR+ CD8+ T lymphocytes were found to accompany disease progression in several studies (28-30) and a positive correlation between the number of CD8+ lymphocytes/µl blood at first examination and rate of progression to AIDS has also been noted (31). The abnormally high level of activation of CD8+ lymphocytes in HIV infection is also

reflected in the unusual ease of detection and high frequency of circulating anti-HIV CTL in asymptomatic HIV-infected individuals (32-34). Although increased $CD8^+$ T cell numbers and activation in HIV infection partially reflects a protective immune response, it is important to recognize that T cell-mediated cytotoxicity may contribute to T cell loss, immunopathology and disease progression. Reports of autoimmune cytotoxic activity suggest more than one set of cytotoxic cells with pathogenic potential may be activated in HIV infection. Zarling *et al.* (19) reported the presence of circulating, putatively HLA-restricted $CD8^+$ T cells which lysed uninfected $CD4^+$ lymphocytes and did not lyse EBV-transformed B cells, whereas Israel-Biet *et al.* (18) reported non-HLA-restricted $CD4^-$ $CD8^-$ T cells which lysed autologous EBV-transformed B cells, as well as activated $CD4^+$ and $CD8^+$ T cells. Since the effect of HIV infection is to specifically deplete $CD4^+$ T cells and the activity reported by Israel-Biet *et al.* (18) showed no clear relationship to markers of disease progression, we focussed on characterizing T cell-mediated cytotoxicity specific for $CD4^+$ lymphocytes. After we were unable to detect circulating CTL which lysed uninfected $CD4^+$ lymphocytes in 10/10 HIV-infected individuals tested, we tried *in vitro* activation with Con A and IL-2. Since activation and expansion of T cells with mitogens does not alter their clonotypic specificity, *in vitro* stimulated cells were used to characterize CTL specific for uninfected $CD4^+$ lymphocytes.

We have confirmed the presence of CTL in HIV-infected individuals which lyse uninfected activated $CD4^+$ T cells and further investigated the nature of this CTL activity and its relationship to *in vivo* $CD4^+$ T cell depletion. The CTL lysing uninfected $CD4^+$ T cells were $CD3^+$ $CD8^+$ TCR $\alpha\beta^+$ and target cell recognition was mediated by the clonotypic $\alpha\beta$ TCR, but recognition by these cells was neither HIV-specific nor HLA-restricted. Activated $CD4^+$, but not $CD8^+$ lymphocytes from both HIV-infected and uninfected individuals were lysed by these CTL. $CD4^+$ cells from different HIV-seronegative subjects were selectively lysed by CTL from different HIV-infected individuals, suggesting that the target of these CTL is a polymorphic antigen other than a

classical HLA antigen. CTL capable of specifically lysing uninfected activated $CD4^+$ lymphocytes were efficiently induced in PBL from HIV-infected individuals by stimulation with Con A or HIV, but not by stimulation with EBV. The natural history of subjects with CTL activity against autologous $CD4^+$ cells supports a role for these CTL in the *in vivo* depletion of $CD4^+$ lymphocytes.

# MATERIALS AND METHODS

*Subjects.* HIV-1-infected individuals were recruited through the Chedoke-McMaster Special Immunology Services Clinic. Infection with HIV-1 was determined by ELISA and confirmed by Western blot. Subjects were clinically assessed and $CD4^+$ and $CD8^+$ lymphocyte counts determined at 3 month intervals whenever possible. Uninfected HIV-seronegative volunteers were recruited from hospital and laboratory personnel. HLA-typing for some subjects was performed by microcytotoxicity assay (35).

*Lymphocyte Separations.* Blood was collected in heparinized vacutainers, diluted with an equal volume of PBS, layered over Ficoll-paque gradient separation medium (Pharmacia Chemicals, Dorval, Que.) and centrifuged at 400x*g* for 30 min. Interface cells were collected, washed 3 times in PBS containing 1% FCS, and counted. Cells were resuspended in PBS containing 0.1% BSA at $3x10^6$/ml with sufficient OKT4 coated magnetic beads (DYNAL Inc., Great Neck, NY) to ensure a 5:1 bead-to-target cell ratio and rotated together for 1 h in a 15 ml conical tube at 4°C. After magnetic displacement of beads, unbound cells were removed by pipetting. The beads bearing the positively selected cells were washed twice with PBS containing 0.1% BSA before culture.

*Lymphocyte Stimulation.* Positively selected $CD4^+$ lymphocytes were resuspended at approximately $10^6$/ml in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% FCS (Bocknek, Burlington, Ont.), 10 mM HEPES, 2mM L-glutamine, 1% penicillin-streptomycin, (all from Gibco) and $2x10^{-5}$ M 2-ME (Sigma Chemical Co. St. Louis MO) and stimulated with 5 µg/ml PHA-P (Wellcome Diagnostics, Dartford, England). After 3 days of PHA stimulation, $CD4^+$ cultures were further supplemented with 5 U/ml recombinant IL-2 (Genzyme, Cambridge, MA). Following an additional 2-3 days in IL-2, $CD4^+$ cells were removed from the beads by vigorous pipetting in a 15 ml conical tube, magnetic displacement of beads, removal of unbound cells, resuspension of

beads in PBS containing 1% BSA and repetition of this procedure a total of 3 times. Cells were not used for at least 48 hours after removal from beads to allow reexpression of surface antigens. Cultures so treated contained a mean of 95% $CD4^+$ lymphocytes as determined by flow cytometry (data not shown).

$CD4^+$ lymphocyte depleted cultures were resuspended at approximately $10^6$/ml in lymphocyte medium supplemented with 10 µg/ml Con A (Difco, BDH, Toronto, Ont., Canada) for 3 days after which 5 U/ml IL-2 was added. After a total of 7 days these cultures contained a mean of 90% $CD8^+$ T cells by flow cytometry (data not shown).

For stimulation with HIV, 10% of the freshly isolated PBMC were cultured with 5 µg/ml PHA-P for 3 days to activate replication of endogenous HIV, washed, irradiated at 5000 Rad, and added to the remaining 90% of the PBMC which had been maintained in unsupplemented medium. After 3 days of coculture, 5 U/ml IL-2 was added. For stimulation with EBV, autologous EBV-transformed BLCL were irradiated at 5000 Rad and added at a ratio of 1:10 to 3 day cultures of PBMC. After 3 days coculture, IL-2 was added at 5 U/ml. Stimulated cells were routinely tested for cytotoxicity or used as targets after 7 days in IL-2 or a total of 10 days after isolation. To generate EBV-transformed B-lymphoblastoid cell lines (BLCL), $5X10^6$ freshly isolated PBMC were incubated in 2.5 ml cell-free supernatant from EBV-producing B95-8 cells (American Type Culture Collection, ATCC CRL1612). After 24 hours, the cells were washed and incubated in medium containing 20% FCS and 1µg/ml cyclosporin A (Sandoz Canada, Montreal, Que.). After 10 days, BLCL were maintained in standard medium or frozen in medium supplemented with 20% FCS and 10% DMSO and stored in liquid $N_2$.

*Cytotoxicity Assays.* Autologous or heterologous $CD4^+$ or $CD8^+$ lymphocytes cultured as described above for between 10-14 days were incubated for 90 min with 200 µCi $Na^{51}CrO_4$ (New England Nuclear, Boston, MA), washed 4 times in PBS containing 1% FCS, and resuspended at $10^5$/ml in medium. 50 µl of target cell suspension were added to duplicate wells of 96 well round bottom microtitre plates (Nunclon, Roskilde,

Denmark) for each E:T ratio and for minimum and maximum controls. Effector cells were resuspended in media at $5x10^6$/ml and 50, 25 and 12.5 µl added to duplicate wells of the microtitre plates to attain E:T ratios of 50:1, 25:1 and 12.5:1. A constant volume of 300 µl in each well was attained by adding either medium or hybridoma supernatants to test wells, medium alone to minimum wells and by adding 1N HCl to maximum wells. Following addition of effector and target cells, microtitre plates were incubated at 37°C in humidified 5% $CO_2$ incubators for 5 hours and 100 µl of supernatant was removed from each well for γ counting. Percent specific lysis was calculated by the following formula: (Test $^{51}Cr$ release-Spontaneous release)÷ (Maximum $^{51}Cr$ release-Spontaneous release) x 100.

In all assays, spontaneous $^{51}Cr$ release was <30% of total $^{51}Cr$ release. Ten percent specific lysis was considered positive since this was always greater than 3 times the standard deviation of the mean spontaneous release.

*Characterization of Effector Cells.* Inhibition studies with various monoclonal antibodies were carried out to determine the phenotype of the cytotoxic cells. Hybridoma supernatants from OKT3 (ATCC CRL8001) and OKT8 (ATCC CRL8014) were added to the microtitre plates in some cases, while in other cases, effector cells were incubated for 30 min with 1 ml OKT3 hybridoma supernatant and washed 2 times with PBS containing 1% FCS before inclusion in cytotoxicity assays. In further tests, effector cells were incubated with 1 µg of anti-αβ TCR mAb BMA-031 (Behring Diagnostics, Ont.) or 1 ml OKT3 hybridoma supernatant for 30 min and washed as above before inclusion in assays. In other assays, target cells were incubated with hybridoma supernatants from anti-HLA class I mAb PA2.6 (ATCC HB118), OKT4 (ATCC CRL8002), or anti-HLA-DR mAb L243 (ATCC HB55) or 2 µg anti-CD54 ICAM (84H10, Amacine, Westbrook, ME) for 30 min and washed as above before use as targets. These studies were performed to evaluate the functional relevance of cell surface molecules to target cell recognition and effector cell function.

*Statistical Analysis.* Wilcoxon Signed Rank Test and Student's *t* test were carried out using the Statworks™statistical analysis software program.

# RESULTS

*Demonstration and Initial Characterization of CTL from HIV-1-Infected Individuals which Lyse CD4+ Lymphocytes.* Using Con A-stimulated CD8+ lymphocytes from HIV-seropositive individuals as effector cells and panels of purified CD4+ lymphocytes as targets, we demonstrated killing of autologous and heterologous CD4+ lymphocytes obtained from HIV-seropositive and uninfected individuals (Table I). Specifically, results in Table I show effective lysis of purified autologous CD4+ lymphocytes by CD4-depleted Con A-stimulated effector cells from seven HIV-1-infected individuals. In addition, CD8+ effector cells from HIV-infected individuals were able to lyse purified CD4+ cells from heterologous HIV-seropositive individuals (Table I). The results in Table I also show that CD8+ effector cells from HIV-infected individuals were cytotoxic for uninfected CD4+ target cells obtained from HIV-seronegative individuals. Since uninfected CD4+ lymphocytes were lysed by CD8+ effector cells from HIV-infected individuals, recognition of the target cells was independent of expression of HIV antigens. In contrast, following the same *in vitro* culture conditions used to stimulate effector cells from HIV-infected individuals, none of eight HIV-seronegative individuals tested displayed similar cytotoxic activity against uninfected CD4+ target cells (Table I). These results confirm that CTL activity against uninfected activated CD4+ lymphocytes occurs in HIV-1 infection. In order to determine the phenotype of the effector cells and the signalling or accessory molecules involved in target cell lysis, inhibition studies with mAb against T cell surface markers were performed. Killing of autologous and heterologous CD4+ cells from HIV+ individuals and killing of heterologous CD4+ lymphocytes from uninfected individuals was inhibited when antibodies against either CD3 or CD8 were present throughout the assay (Table I). In addition, killing of CD4+ cells was inhibited when effector cells alone were reacted with anti-CD3 and washed (Table I). These results

show that HIV-1 infected individuals possess $CD3^+$ $CD8^+$ CTL which lyse uninfected $CD4^+$ lymphocytes and that lysis by these CTL is triggered through the CD3-associated T cell receptor complex.

*Specificity of CTL for Activated $CD4^+$ Lymphocytes.* Since some $HIV^+$ individuals demonstrate elevated CTL responses to alloantigens (36), we wished to determine if the lysis of heterologous $CD4^+$ lymphocytes involved recognition of alloantigens. Therefore, we compared the ability of the CTL from $HIV^+$ subjects to lyse $CD4^+$ and $CD8^+$ lymphocytes obtained from the same HIV-infected or uninfected individual. Results shown in Table II demonstrate that $CD8^+$ effector cells from HIV-infected individuals lysed heterologous $CD4^+$ lymphocytes but not $CD8^+$ lymphocytes obtained from an HIV-infected or uninfected individual. In contrast, alloantigen-specific CTL generated by MLR lysed $CD4^+$ and $CD8^+$ target cells equally (Table II). The susceptibility of $CD4^+$ lymphocytes to CTL-mediated lysis was also shown to be dependent on the activation state of the target cells. Results shown in Table III demonstrate that activated autologous $CD4^+$ cells or activated $CD4^+$ cells from heterologous uninfected individuals were more susceptible to lysis than unstimulated $CD4^+$ target cells. Thus, CTL from HIV-seropositive individuals recognize a target antigen present on activated $CD4^+$ but not $CD8^+$ lymphocytes. These results also suggest that lysis of heterologous $CD4^+$ lymphocytes is not due to allorecognition. Lysis of autologous $CD4^+$ lymphocytes or heterologous $CD4^+$ lymphocytes from HIV-seronegative individuals was also not inhibited by the anti-HLA-A, B and C mAb (PA2.6), although this same antibody inhibited alloantigen-specific CTL and class I-restricted anti-viral CTL (Table IV). This reinforces the evidence that recognition of uninfected $CD4^+$ lymphocytes by $CD8^+$ CTL was not due to recognition of HLA class I antigens and further suggests that lysis of autologous $CD4^+$ lymphocytes was not due to HLA class I-restricted recognition of HIV antigens. Indeed, lysis of heterologous $CD4^+$ T cells from HIV-infected and uninfected individuals occurred in the absence of HLA-A or B identity (Table V).

By examining panels of CD4+ lymphocytes obtained from HIV+ and HIV- individuals as target cells, we found that CD8+ CTL from different HIV-infected individuals preferentially recognized CD4+ lymphocytes from particular subjects (Table VI). This did not reflect an inherent resistance to lysis for a particular subject's CD4+ lymphocytes, since all were sensitive to lysis by CTL from at least one HIV-seropositive individual. We interpret this pattern of recognition as suggesting that CTL from the HIV+ subjects recognize a polymorphic antigen specific to CD4+ T lymphocytes.

*T Cell Receptor Phenotype and Mechanisms of Activation of CTL Recognizing CD4+ Lymphocytes.* Since the recognition of activated CD4+ lymphocytes by CD3+ CD8+ CTL from HIV-infected individuals was not HLA-restricted or HIV-specific, we attempted to determine the T cell receptor phenotype of the effector CTL. Separate effector CTL populations were preincubated with either OKT3 or BMA-031. OKT3 binds to CD3 and inhibits both αβ and γδ T cell-mediated cytolysis, while BMA-031 binds to the αβ TCR and inhibits only αβ T cell-mediated cytolysis. In each case, lysis of CD4+ lymphocyte targets was markedly inhibited when effector cells were incubated with antibodies against either CD3 or the αβ T cell receptor (Table VII). Therefore, CTL from HIV-infected individuals that lyse CD4+ T cells in an unrestricted manner are predominantly TCR αβ+ CTL.

We have shown that Con A stimulation induces CTL activity aginst activated CD4+ lymphocytes in a number of HIV-infected individuals. To determine the relationship of this activity to HIV infection, we examined whether stimulation with HIV would also induce this activity, and if so, whether this was a specific property of HIV. Autologous lymphoblasts stimulated with PHA were used as HIV+ stimulator cells since this has previously been shown to stimulate anti-HIV CTL (40). Results shown in Table VIII demonstrate that stimulation of effector CTL from HIV-infected individuals with either Con A or autologous HIV-infected cells led to the activation of CTL against autologous and heterolgous CD4+ lymphocytes. Stimulation with HIV was as effective as

stimulation with Con A (Table VIII). Further, the cytotoxic activity against CD4+ lymphocytes stimulated by Con A or HIV was inhibited in the presence of anti-CD3 (Table VIII). In contrast, stimulation with autologous EBV transformed B lymphoblastoid cells (BLCL) induced CTL activity against autologous BLCL, but did not stimulate anti-CD3 inhibitable CTL activity against autologous or heterologous CD4+ lymphocytes (Table VIII). Therefore, Con A and HIV, but not EBV, were able to stimulate CTL activity in PBMC from HIV-infected individuals against autologous and heterologous activated uninfected CD4+ lymphocytes.

*Relationship of CTL Activity Against Autologous CD4+ Lymphocytes to In Vivo T Cell Depletion.* Over 50 HIV+ subjects have been tested at least once for cell-mediated cytotoxicity against autologous CD4+ lymphocytes. Of these, 16 showed clear evidence of anti-CD3 inhibitable cell-mediated immunity against autologous CD4+ lymphocytes. We have obtained follow-up CD4 counts for the 6 month period following detection of this activity for seven individuals who at time of testing had >50 CD4+ lymphocytes/μl blood. All of these subjects lost circulating CD4+ lymphocytes over the follow-up period (Fig. 1). The mean fall (±SD) in CD4+ lymphocytes/μl blood for this group was 121± 84 or 36 % compared to a mean rise (±SD) of 40±99 or 10 % for a corresponding group which clearly had no CTL activity against autologous CD4+ lymphocytes (Fig. 1). Thus for this group of HIV+ subjects who demonstrated CTL activity against autologous CD4+ lymphocytes, a significant drop ($p<.01$, Wilcoxon Signed Rank Test) in CD4 counts occurred, whereas a comparable group of HIV-infected individuals who did not demonstrate this CTL activity showed no significant change in their CD4 counts over the six month period (Fig. 1)

Further evidence of a role for CTL activity against autologous CD4+ T cells in T cell depletion comes from repeated testing for CTL activity against autologous CD4+ lymphocytes over time. Results in Table IX show the natural history of CD4 counts and CTL activity against autologous CD4+ lymphocytes for six HIV-infected subjects. In

these subjects, CTL activity against autologous CD4+ lymphocytes fell dramatically or disappeared coincident with an *in vivo* fall in CD4 counts. Since it was possible that the inability to detect lysis of autologous CD4+ cells was due to the loss of susceptible autologous target cells *in vivo* in the HIV-infected individual, we concurrently tested lysis of heterologous CD4+ lymphocytes from an uninfected individual by CTL from several HIV+ subjects at multiple time points. In these cases, even though lysis of autologous CD4+ lymphocytes decreased coincident with a fall in CD4 number, lysis of CD4+ lymphocytes obtained from an uninfected individual remained relatively constant (Table IX). Therefore, autologous CD4+ lymphocytes sometimes became insensitive to lysis following an *in vivo* fall in CD4+ lymphocyte numbers, whereas CD4+ lymphocytes from an uninfected person, in whom numbers and subset representation should remain fairly constant, were still lysed by the CTL. This pattern of lysis could occur due to *in vivo* depletion of susceptible CD4+ targets by the CTL in HIV+ subjects.

# DISCUSSION

In this paper, we have confirmed the presence in HIV-infected individuals of CTL which lyse uninfected activated $CD4^+$ lymphocytes. This lysis was specific in that it was mediated through the TCR of $CD8^+$ $\alpha\beta^+$ CTL which did not lyse $CD8^+$ lymphocytes from the same target cell donor. Recognition was not restricted by classical HLA antigens since lysis occurred with unrelated $CD4^+$ lymphocytes from HLA-mismatched, heterologous donors and was not inhibited by an antibody specific for HLA-A, B and C encoded antigens. However, preferential recognition of certain HIV- subject's $CD4^+$ lymphocytes from within a panel of such targets suggests recognition of a polymorphic ligand specific to $CD4^+$ T cells. A relationship between this CTL activity and HIV infection was suggested by the absence of such CTL from HIV-seronegative controls and the ability of HIV, but not EBV, to induce these CTL following *in vitro* stimulation. Furthermore, a role for these CTL in the *in vivo* depletion of $CD4^+$ lymphocytes in HIV infection is suggested by the selective disappearance of susceptible autologous $CD4^+$ target cells from HIV-infected individuals and the relationship between detection of this CTL activity and subsequent loss of $CD4^+$ lymphocytes *in vivo*. These observations and the unusual characteristics of the CTL themselves, raise questions regarding the pathogenesis of AIDS and the role of non- HLA-restricted T cell recognition.

A role for CTL-mediated lysis of uninfected $CD4^+$ lymphocytes in the pathogenesis of AIDS was first suggested by Zarling *et al.* (19), who described the presence of such CTL in the circulating blood of HIV-infected humans, but not chimpanzees. This difference is a compelling explanation for the failure of HIV to induce disease in chimpanzees. In our study, the consistency with which detection of this activity predicted an iminent fall in $CD4^+$ lymphocyte numbers supports this possibility. They also observed that CTL activity against autologous $CD4^+$ lymphocytes was often less than against heterologous HIV-uninfected lymphocytes (19) and raised the possibility that the CTL

activity had eliminated sensitive targets *in vivo* in the HIV-infected subjects. This suggestion was supported by our demonstration that CD4+ lymphocytes from time points preceding a fall in CD4+ lymphocyte number were more sensitive to lysis than CD4+ lymphocytes isolated from HIV-infected subjects following an *in vivo* fall in CD4+ lymphocyte number.

Our identification of CD8+ TCR αβ+ CTL capable of specifically lysing uninfected CD4+ cells concurs with the phenotype of autoimmune effectors reported by Zarling *et al.* (19). Despite our inability to detect these CTL directly from circulating PBL, the similarities to the activity reported by Zarling *et al.* (19) suggest that these CTL are active *in vivo* in HIV-infection. Under identical culture conditions, 10 HIV-seronegative controls did not generate the CTL activity displayed by HIV-infected individuals. Therefore, this CTL activity is related to HIV infection and not an artefact of *in vitro* stimulation. Israel-Biet *et al.* (18) reported non-HLA-restricted cytotoxic effectors that were mostly CD4- CD8- and lysed activated CD4+ and CD8+ lymphocytes and EBV-transformed B cells. We found that stimulation with EBV-transformed B cells induced effector cells which killed CD4+ lymphocytes, but this activity was not inhibited by anti-CD3 antibodies. Israel-Biet *et al.* (18) found no obvious association between this activity and CD4+ lymphocyte depletion. In contrast, we found an association between the specific anti-CD3 inhibitable CTL activity against autologous CD4+ lymphocytes and CD4+ lymphocyte depletion. Several distinct cell-mediated cytotoxic activities capable of lysing activated uninfected CD4+ T cells may be present in HIV-infected individuals, but only the anti-CD3 inhibitable cytotoxicity specific for CD4+ lymphocytes and mediated by CD8+ TCR αβ+ CTL shows a relationship to CD4+ lymphocyte depletion *in vivo*. The phenotype, specificity and functional utilization of the clonotypic αβ TCR suggests a specific immune response directed against CD4+ lymphocytes in HIV infection.

The lack of HLA-restriction of CTL capable of lysing uninfected activated CD4+ lymphocytes is unusual. For this reason and because TCR γδ+ CTL capable of lysing

autologous activated lymphocytes have been described (41), we considered the possibility that CTL recognizing uninfected CD4+ lymphocytes were γδ lymphocytes. This would also be consistent with the elevated numbers of γδ lymphocytes observed in HIV infection (42, 43). Results from our studies indicated, though, that CTL capable of lysing activated uninfected CD4+ lymphocytes were predominantly, if not exclusively, αβ+ T cells. It also seemed possible that the killing of heterologous CD4+ T cells might be due to allospecificity, but this was ruled out by demonstrating lysis of CD4+ but not CD8+ lymphocytes and by the lack of inhibition with an anti-HLA-A, B and C mAb which inhibited both alloantigen-specific and class I-restricted CTL. Despite the dissociation of T cell recognition and HLA presentation, CTL from HIV-infected individuals were able to distinguish between CD4+ T cells from different donors, suggesting recognition of a polymorphic determinant.

We also found that activated CD4+ lymphocytes were preferentially lysed by the CTL. This could be due to activation-induced expression of neoantigens, upregulation of accessory molecules critical to conjugate formation and lysis or due to activation-induced intracellular programming to receive signals inducing apoptosis. Pre-incubation of CD4+ target cells with mAb against ICAM did not inhibit lysis (data not shown), suggesting that upregulation of this accessory molecule was not involved in the susceptibility of activated CD4+ cells. A role for activation-induced HLA class II expression in recognition is also unlikely, based on the CD8+ phenotype of the CTL (Table I), differential recognition of activated CD4+ versus CD8+ lymphocytes (Table II) and the failure of an anti-HLA-class II mAb to inhibit lysis (data not shown). Amiesen and Capron (5) have proposed that the inappropriate reemergence of a T cell death program in response to activation could account for the CD4+ defects seen in HIV-infected individuals. They recently demonstrated that activation of CD4+ lymphocytes from HIV-infected individuals by HLA-class II-dependent superantigens or PWM resulted in cell death by apoptosis (13). Since one of the mechanisms by which CTL kill their targets is induction of apoptosis

(44), it is possible that activated CD4+ lymphocytes from HIV-infected subjects are susceptible to programmed cell death following recognition by CD8+ CTL.

Our studies suggest that in HIV infection, polymorphic antigens on CD4+ lymphocytes become targets of autoimmune CTL activity. The lack of HLA-restriction of this activity suggests a possible explanation we are currently investigating. Although non-HLA-restricted TCR-mediated recognition by CTL is unusual, there are well documented examples of such recognition (45-47). Exposure to foreign antigens clearly elicits HLA-restricted T cells specific for that antigen. However, recognition between T cells is sometimes restricted to loci outside the MHC (48, 49) and has been postulated to occur via complementary T cell receptors (50). Evidence for this type of T cell recognition was reviewed by Coutinho (51) and is exemplified by the non-HLA restricted protection from GVHD induced by transfer of parental lymphocytes into F1 offspring obtained by prior exposure to low numbers of parental lymphocytes (52). In HIV infection, it is possible that CTL recognizing idiotypic determinants specific to HLA class-II restricted TCRs lyse autologous activated CD4+ lymphocytes. This would explain the observed specificity of the CTL and variation in activity over time, since the TCR is extensively polymorphic and elimination of CD4+ lymphocytes in HIV infection may be selective according to TCR V region determinants (11). Through receptor complementarity, the CTL themselves could provide the requisite activation signal to sensitize CD4+ target cells for lysis.

The idea that autoimmunity is involved in the pathogenesis of AIDS is not new, but the finding of CTL which specifically lyse uninfected CD4+ lymphocytes is nonetheless remarkable. The action of these CTL in the pathogenesis of AIDS is consistent with the observed increases in activated T cells (28-30) and soluble markers of lymphocyte activation, such as β-2 microglobulin (53), CD4 (54), CD8 (55) and IL-2 receptor (56). We detected CTL against autologous CD4+ lymphocytes in a minority of HIV+ subjects, however, this may reflect the dynamic nature of the process as specific targets are eliminated and periods of equilibrium last until the CTL infringe upon more of the CD4+

T cell repertoire. The result of this activity would be gradual selective diminuition of the CD4+ T cell repertoire with decreasing CD4+ T cell numbers over time, which has been reported by Imberti *et al.* (11). In their study, the selective depletion of CD4+ T cells was attributed to an HIV encoded superantigen, but autoimmune CTL recognizing specific CD4+ TCR idiotypes could also explain the selective depletion. It is not clear how HIV induces these CTL, but the normal regulatory features of the immune system must be compromised. This could involve HIV induced cross-reactive immunity against regulatory T cell idiotypes (4, 10) or specific infection of regulatory T cells. Further research is necessary to better understand the origin, specificity and role of these CTL in HIV-infected individuals.

# REFERENCES


1. **Harper, M. E., L. M. Marsalle, R. C. Gallo and F. Wong-Staal.** 1986. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization. *Proc. Natl. Acad. Sci. USA 83:772.*

2. **Brinchmann, J. E., I. Albert and F. Vartdal.** 1991. Few infected CD4+ T cells but a high proportion of replication-competent provirus copies in asymptomatic human immunodeficiency virus type 1 infection. *J. Virol. 65:2019.*

3. **Hsia, K. and S. A. Spector.** 1991. Human immunodeficiency virus DNA is present in a high percentage of CD4+ lymphocytes of seropositive individuals. *J. Infect. Dis. 164:470.*

4. **Hoffmann, G. W., T. A. Kion and M. D. Grant.** 1991. An idiotypic network model of AIDS immunopathogenesis. *Proc. Natl. Acad. Sci. USA 88:3060.*

5. **Ameisen, J. C. and A. Capron.** 1991. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. *Immunol. Today 12:102.*

6. **Habeshaw, J. A. and Dalgleish, A.** 1989. The relevance of HIV/envCD4 interactions to the pathogenesis of acquired immune deficiency syndrome. *J. Acq. Immun. Def. Synd. 2:457.*

7. **Sheppard, H. W. and M. S. Ascher.** 1991. The relationship between AIDS and immunologic tolerance. *J. Acq. Immun. Def. Synd. 5:143.*

8. **Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory and E. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4+ cell depletion. *Cell 54:561.*

9. **Janeway, C.** 1991. Mls: makes a little sense. *Nature 349:459.*

10. **Grant, M. D.** 1991. HIV and idiotypic T cell regulation. *Immunol. Today 12:171.*

11. **Imberti, L, A. Sottini, A. Bettinardi, M. Puotti and D. Primi.** 1991. Selective depletion in HIV-infection of T cells that bear specific T cell receptor Vβ sequences. *Science 254:860.*

12. **Pantaleo G., S Koenig, M. Baseler, H. C. Lane and A. S. Fauci.** 1990. Defective clonogenic potential of $CD8^+$ T lymphocytes in patients with AIDS. *J. Immunol. 144:1696.*

13. **Groux, H., G. Torpier, D. Monte, Y. Mouton, A. Capron and J. C. Amiesen.** 1992. Activation-induced death by apoptosis in $CD4^+$ T cells from human immunodeficiency virus-infected individuals. *J. Exp. Med. 175:331.*

14. **Susal, C., V. Daniel, H. H. Oberg, P. Terness, A. Huth-Kuhne, R. Zimmerman and G. Opelz.** 1992. Striking inverse association of IgG-anti-$Fab_\gamma$ antibodies and CD4 cell counts in patients with acquired immunodeficiency syndrome (AIDS)/Aids-related complex. *Blood 79:954.*

15. **Kion, T. A. and G. W. Hoffmann.** 1991. Anti-HIV and anti-anti-MHC antibodies in alloimmune and autoimmune mice. *Science 253:1138.*

16. **Grant, M. D., M. S. Weaver, C. Tsoukas, and G. W. Hoffmann.** 1990. Distribution of antibodies against denatured collagen in AIDS risk groups and homosexual AIDS patients suggests a link between autoimmunity and the immunopathogeneis of AIDS. *J. Immunol. 144:1241.*

17. **Golding, H., G. M. Shearer, K. Hillman, P. Lucas, J. Manischewitz, R. A. Zajac, M. Clerici, R. E. Gress, R. N. Boswell and B. Golding.** 1989. Common epitope on human immunodeficiency virus (HIV) 1-GP41 and HLA Class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV-1-infected individuals. *J. Clin. Invest. 83:1430.*

18. **Israel-Biet, D., A. Venet, K. Beldjord, J. M. Andrieu, and P. Even.** 1990. Autoreactive cytotoxicity in HIV-infected individuals. *Clin. exp. Immunol. 81:18.*

19. **Zarling, J. M., J. A. Ledbetter, J. Sias, P. Fultz, J. Eichberg, G. Gjerset and P. Moran.** 1990. HIV-infected humans, but not chimpanzees, have circulating cytotoxic T lymphocytes that lyse uninfected CD4+ cells. *J. Immunol. 144: 2992.*

20. **Fahey, J. L., H. Prince, M. Weaver, J. Groopman, B. Visscher, K. Schwartz and R. Detels.** 1984. Quantitiative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immune deficiency syndrome from other immune subset disorders. *Am. J. Med. 76:95.*

21. **Vanham, G., L. Kestens, P. Gigase, R. Colebunders, M. Vandenbruane, L. Brijs and J. L. Ceuppens.** 1990. Evidence for circulating activated cytotoxic T cells in HIV-infected subjects before the onset of opportunistic infections. *Clin. exp. Immunol. 82:3.*

22. **Vanham, G., Kestens L., Penne, G., Goilav, C., P. Gigase, R. Colebunders, M. Vandenbruane, J. Goeman, G. Van Der Groen and J. L. Ceuppens.** 1991. Subset markers of CD8(+) cells and their relation to enhanced cytotoxic T-cell activity during human immunodeficiency virus infection. *J. Clin. Immunol. 11:345.*

23. **Pantaleo, G., A. DeMaria, S. Koenig, L. Butini, B. Moss, M. Baseler, H. C. Lane and A. S. Fauci.** 1990. CD8+ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of human immunodeficiency-virus-specific cytotoxicity. *Proc. Natl. Acad. Sci. USA 87:4818.*

24. **Autran, B., C. M. Mayaud, M. Raphael, F. Plata, M. Denis, A. Bourguin, J. Guillon, P. Debre and G. Akoun.** 1988. Evidence for a cytotoxic T-lymphocyte alveolitis in human immunodeficiency virus-infected patients. *AIDS 2:179.*

25. **Itescu, S., L. J. Brancato, J. Buxbaum, P. K. Gregersen, C. C. Rizk, T. S. Croxson, G. E. Solomon and R. Winchester.** 1990. A diffuse infiltrative CD8 lymphocytosis syndrome in human immunodeficiency virus (HIV) infection; a host immune response associated with HLA-DR5. *Ann. Intern. Med. 112:3.*

26. **Calabrese, L. H., M. Estes, B. Yen-Lieberman, M. R. Proffitt, R. Tubbs, A. Fishleder and K. H. Levin.** 1989. Systemic vasculitis in association with human immunodeficiency virus infection. *Arthritis Rheum. 32:569.*

27. **Sethi, K. K., H. Naher, and I. Strochmann.** 1988. Phenotypic heterogeneity of cerebrospinal fluid-derived HIV-specific and HLA-restricted cytotoxic T-cell clones. *Nature 335:178.*

28. **Prince, H. E., L. Arens and S. H. Kleinman.** 1987. CD4 and CD8 subsets defined by dual color cytofluorometry which distinguish symptomatic from asymptomatic blood donors seropositive for human immunodeficiency virus. *Diag. clin. Immunol. 5:188.*

29. **Giorgi, J. V. and R. Detels.** 1989. T-cell subset alterations in HIV-infected homosexual men: NIAID multicenter AIDS cohort study. *Clin. Immunol. Immunopathol. 52:10.*

30. **Levacher, M., S. Tallet, M. C. Dazza, E. Dournon, B. Rouveix and J. J. Pocidalo.** 1990. T activation marker evaluation in ARC patients treated with AZT. Comparison with CD4+ lymphocyte count in non-progressors and progressors towards AIDS. *Clin. exp. Immunol. 81:177.*

31. **Anderson, R. E., S. C. Shiboski, R. Royce, N. P. Jewell, W. Lang and W. Winkelstein Jr.** 1991. CD8+ T lymphocytes and progression to AIDS in HIV-infected men: some observations. *AIDS 5:213.*

32. **Walker, B. D., S. Chakrabarti, B. Moss, T. J. Paradis, T. Flynn, A. G. Durno, R. S. Blumberg, J. C. Kaplan, M. S. Hirsch and R. T. Schooley.** 1987. HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature 328:345.*

33. **Gotch, F. M., D. F. Nixon, N. Alp, A. J. McMichael and L. K. Borysiewicz.** 1990. High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals. *Int. Immunol. 2:707.*

34. **Koup, R. A., J. L. Sullivan, P. H. Levine, D. Brettler, A. Mahr, G. Mazzara S. McKenzie and D. Panicali.** 1989. Detection of major histocompatibility complex class I-restricted HIV-specific cytotoxic T lymphocytes in the blood of infected hemophiliacs. *Blood 73:1909.*

35. **Terasaki, P. I., D. Bernoco, M. S. Park, G. Ozturk, and Y. Iwaki.** 1978. Microdroplet testing for HLA-A, -B, -C and -D antigens. *Am. J. Clin. Pathol. 69:103.*

36. **Tung, K. S., K. F. Foster, D. C. Bernstein, P. W. Kriebel, S. M. Payne and G. M. Shearer.** 1985. Elevated allogeneic cytotoxic T lymphocyte activity in peripheral blood leukocytes of homosexual men. *J. Immunol. 135:3163.*

37. **Flexner, C., S. S. Broyles, P. Earl, S. Chakrabarti and B. Moss.** 1988. Characterization of human immunodeficiency virus gag/pol gene products expressed by recombinant vaccinia viruses. *Virol. 166:339.*

38. **Chakrabarti, S., M. Robert-Guroff, F. Wong-Staal, R. C. Gallo and B. Moss.** Expression of the HTLV-III envelope gene by a recombinant vaccinia virus. *Nature 320:535.*

39. **Ozols, D. Y., D. G. Harnish, W. E. Rawls and K. L. Rosenthal.** 1990. Assessment of the specificity of cytotoxic T lymphocytes for the nucleoprotein of pichinde virus using recombinant vaccinia viruses. *Arch Virol . 115:209.*

40. **Nixon, D. F., A. R. M. Townsend, J. G. Elvin, C. R. Rizza, J. Gallwey and A. J. McMichael.** 1988. HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature 336:484.*

41. **Bensussan, A., V. David, E. Vilmer, G. Leca and L. Boumsell.** 1990. Immunodeficiency after bone marrow transplantation can be associated with autoreactive T-cell receptor γδ-bearing lymphocytes. *Immunol. Rev. 116:5.*

42. **Autran, B., F. Triebel, C. Katlama, W. Rozenbaum, T. Hercend and P. Debre.** 1989. T cell receptor γ/δ+ lymphocyte subsets during HIV infection. *Clin. exp. Immunol. 75:206.*

43. **De Paoli, P., D. Gennari, P. Martelli, G. Basaglia, M. Crovatto, and S. Battistin.** 1991. A subset of γδ lymphocytes is increased during HIV-1 infection. *Clin. exp. Immunol. 83:187.*

44. **Russell, J. H.** 1983. Internal disintegration model of cytotoxic lymphocyte-induced target damage. *Immunol. Rev. 72:97.*

45. **Schmidt, R. E., G. T. Bartley, S. S. Lee, J. F. Daley, H. D. Royer, H. Levine, E. L. Reinherz, S. F. Schlossman and J. Ritz.** 1986. Expression of the NKa clonotype in a series of human natural killer clones with identical cytotoxic specificity. *J. Exp. Med. 163:812.*

46. **Wilson, A., A. J. T. George, C. A. King and F. K. Stevenson.** 1990. Recognition of a B cell lymphoma by anti-idiotypic T cells. *J. Immunol. 145:3937.*

47. **Barnd, D. L., M. S. Lan, R. S. Metzgar and O. J. Finn.** 1989. Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells. *Proc. Natl. Acad. Sci. (USA) 86:7159.*

48. **Tada, T., W. Uracz and R. Abe.** 1986. Are there unique I region controlled determinants on T cells? In *Paradoxes in Immunology* , G. W. Hoffmann, J. G. Levy and G. T. Nepom, eds. CRC Press, Boca Raton, Florida, p. 253.

49. **Man-Sun, S. and B. Benacerraf.** 1988. Suppressor T cells, immunoglobulin and Igh restriction. *Immunol. Rev. 101:133.*

50. **Sim, G. K., I. A. MacNeil and A. A. Augustin.** 1986. T helper cell receptors: idiotypes and repertoires. *Immunol. Rev. 90:49.*

51. **Pereira, P., A. Bandeira, A. Coutinho, M.-A. Marcos, M. Toribio and C. Martinez-A.** 1989. V-region connectivity in T cell repertoires. *Ann. Rev. Immunol. 7:209.*

52. **Kimura, H. and D. B. Wilson.** 1984. Anti-idiotypic cytotoxic T cells in rats with graft-versus-host disease. *Nature 308:463.*

53. **Zolla-Pazner, S., D. William, W. El-Sadr, M. Marmor and R. Stahl.** 1984. Quantification of b-2 microglobulin and other immune characteristics in a prospective study of men at risk for acquired immune deficiency syndrome. *JAMA 251:2951.*

54. **Peakman, M., G. Senaldi, N. Foote, T. J. McManus and D. Vergani.** 1992. Naturally occurring soluble CD4 in patients with human immunodeficiency virus infection. *J. Infect. Dis. 165:799.*

55. **Reddy, M. M., M. Lange and M. H. Grieco.** 1989. Elevated soluble CD8 levels in sera of human immunodeficiency virus-infected populations. *J. Clin. Microbiol. 27:257.*

56. **Kloster, B. E., P. A. John, L. E. Miller, L. A. Rubin, D. L. Nelson, D. C. Blair and R. H. Tomar.** 1987. Soluble interleukin 2 receptors are elevated in patients with AIDS or at risk of developing AIDS. *Clin. Immunol. Immunopathol. 45:440.*

## FOOTNOTES

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TABLE I

*Lysis of autologous and heterologous CD4+ lymphocytes from HIV-infected and uninfected individuals is mediated by CD3+ CD8+ cytotoxic T lymphocytes from HIV-infected individuals*

| CD8+ Effectors | CD4+ Target Source | E:T | Percent specific lysis 50 | 25 | 12.5 | 50+OKT3[a] | 50+OKT8 |
|---|---|---|---|---|---|---|---|
| HIV+ autologous targets: | | | | | | | |
| 119 | 119 | | 26 | 25 | 21 | 0 | |
| 119+OKT3[b] | 119 | | 0 | 0 | 0 | | |
| 139 | 139 | | 17 | 11 | 10 | | 1 |
| 139+OKT3 | 139 | | 0 | 0 | 0 | | |
| 140 | 140 | | 15 | 16 | 13 | 6 | |
| 152 | 152 | | 43 | 33 | 20 | 1 | |
| 167 | 167 | | 35 | 25 | 19 | 8 | |
| 188 | 188 | | 41 | 23 | 14 | | 0 |
| 188+OKT3 | 188 | | 2 | 0 | 0 | | |
| 203 | 203 | | 15 | 4 | 5 | 0 | |
| 203+OKT3 | 203 | | 0 | 0 | 0 | | |
| HIV+ heterologous targets: | | | | | | | |
| 112 | 140 | | 23 | 20 | 13 | 8 | |
| 182 | 140 | | 14 | 14 | 11 | 0 | |
| 119 | 136 | | 15 | 8 | 7 | 1 | |
| 136 | 72 | | 45 | 27 | 19 | 4 | 6 |
| 136 | 139 | | 40 | 14 | 10 | 1 | 18 |
| 142 | 140 | | 14 | 6 | 0 | 0 | |
| HIV- heterologous targets: | | | | | | | |
| 119 | KR | | 45 | 30 | 12 | 2 | |
| 119+OKT3 | KR | | 1 | 3 | 1 | | |
| 119 | DS | | 27 | 11 | 2 | 9 | 0 |
| 119+OKT3 | DS | | 7 | 3 | 0 | | |
| 139 | KR | | 31 | 27 | 19 | 0 | |
| 155 | KR | | 29 | 9 | 0 | | 5 |
| 152 | DS | | 48 | 26 | 30 | 11 | |
| 123 | DS | | 21 | 10 | 7 | 6 | |
| 203 | DS | | 44 | 24 | 18 | 7 | |
| HIV- autologous targets | | | | | | | |
| KR | KR | | 3 | 0 | 2 | | |
| CP | CP | | 0 | 0 | 0 | | |
| DS | DS | | 0 | 0 | 0 | | |
| SP | SP | | 0 | 0 | 0 | | |
| MW | MW | | 0 | 0 | 0 | | |
| AA | AA | | 0 | 0 | 0 | | |
| AG | AG | | 0 | 0 | 0 | | |
| SG | SG | | 0 | 0 | 0 | | |

TABLE I. legend

CD4-depleted effector cells stimulated with Con A/IL-2 were tested for cytotoxicity against purified autologous PHA activated $CD4^+$ lymphocytes, activated heterologous $CD4^+$ lymphocytes from HIV-infected individuals and uninfected activated $CD4^+$ lymphocytes from HIV-seronegative individuals.

[a]Lysis in the presence of OKT3 or OKT8 was measured at an E:T ratio of 50:1 by adding 200 µl hybridoma supernatant to test wells in place of medium for a final volume of 300 µl.

[b]In some cases, effector cells alone were incubated with 1 ml OKT3 hybridoma supernatant for 30 min immediately prior to the assay. Unbound OKT3 was removed by 2 washes in PBS containing 1% FCS.

TABLE II

*CTL from HIV-infected individuals lyse CD4+ but not CD8+ T cells from heterologous HIV-infected and uninfected individuals*

| CD8+ Effectors | HIV+ Targets | Percent specific lysis (E:T=50, 25, 12.5) CD4+ | | | CD8+ | | |
|---|---|---|---|---|---|---|---|
| 112 | 140 | 23 | 20 | 13 | 0 | 0 | 0 |
| 182 | 140 | 14 | 14 | 11 | 0 | 0 | 0 |
| 119 | 136 | 15 | 8 | 7 | 0 | 0 | 0 |
| 136 | 139 | 40 | 14 | 10 | 0 | 0 | 0 |
| 136 | 72 | 45 | 27 | 19 | 2 | 2 | 0 |
| 142 | 140 | 14 | 6 | 0 | 0 | 0 | 0 |
| 119 | 140 | 65 | 56 | 56 | 7 | 0 | 1 |
| 139 | 140 | 48 | 49 | 46 | 0 | 0 | 0 |
| 207 | 188 | 58 | 45 | 35 | 0 | 0 | 5 |
| | HIV- Targets | | | | | | |
| 119 | KR | 45 | 30 | 12 | 9 | 6 | 1 |
| 139 | KR | 31 | 27 | 19 | 4 | 1 | 0 |
| 28 | KR | 34 | 33 | 25 | 7 | 4 | 0 |
| 123 | DS | 21 | 10 | 7 | 4 | 0 | 0 |
| 152 | DS | 48 | 26 | 30 | 2 | 1 | 0 |
| 203 | DS | 44 | 24 | 18 | 0 | 0 | 0 |
| 132 | MW | 19 | 15 | 12 | 0 | 0 | 0 |
| 207 | MW | 20 | 14 | 9 | 0 | 0 | 0 |
| 168 | MW | 11 | 8 | 4 | 0 | 0 | 0 |
| | Anti-allo HLA CTL Controls[a] | | | | | | |
| SP | KR | 31 | 15 | 5 | 30 | 15 | 10 |
| KR | SP | 22 | 9 | 0 | 27 | 16 | 7 |
| AG | SG | 31 | 14 | 2 | 26 | 10 | 0 |

TABLE II. legend

Effector cells were tested for cytotoxicity against purified heterologous PHA-stimulated $CD4^+$ and Con A-stimulated $CD8^+$ lymphocytes from HIV-infected ($HIV^+$) and uninfected ($HIV^-$) individuals.

[a]Anti-alloantigen specific CTL were generated following 6 day MLR using a 1:10 stimulator:responder ratio.

TABLE III

*Activation of autologous or heterologous CD4+T lymphocytes is required for susceptibility to lysis by CTL from HIV-infected individuals*

| CD8+ | CD4+ | % Specific Lysis | | |
|---|---|---|---|---|
| Effectors | Targets | E/T 50 | 25 | 12.5 |
| 119 | 119 non-activated | 0 | 3 | 1 |
| | 119 PHA/IL-2 | 25 | 18 | 16 |
| 152 | 152 non-activated | 8 | 0 | 0 |
| | 152 PHA/Il-2 | 82 | 77 | 66 |
| 185 | KR non-activated | 16 | 9 | 9 |
| | KR PHA/IL-2 | 61 | 55 | 50 |
| 214 | KR non-activated | 19 | 15 | 6 |
| | KR PHA/IL-2 | 78 | 63 | 56 |
| 221 | KR non-activated | 4 | 5 | 1 |
| | KR PHA/IL-2 | 51 | 37 | 28 |
| 252 | KR non-activated | 43 | 25 | 19 |
| | KR PHA/IL-2 | 69 | 51 | 40 |

TABLE III. legend

Effector cells from HIV-infected individuals were tested for cytotoxicity against non-activated or $CD4^+$ lymphocytes activated with PHA and IL-2. $CD4^+$ lymphocytes were positively selected from freshly isolated PBMC from HIV-infected subjects 119 and 152 by magnetic bead separation. $CD4^+$ cells were removed from the beads by vigorous pipetting and used as targets after 24 h of culture in lymphocyte medium. Lysis of non-activated $CD4^+$ cells from an HIV-seronegative donor (KR) was measured against CD4 cells enriched from PBMC by depletion of $CD8^+$ T cells with OKT8-coated magnetic beads and depletion of monocytes by adherence to plastic.

TABLE IV

*Anti-HLA class I A, B and C mAb does not inhibit lysis of autologous or heterologous CD4+ lymphocytes by CD8+ CTL from HIV-infected individuals*

| CD8+ Effector cells | CD4+ Targets | Percent Specific Lysis E:T 50 | 25 | 12.5 | 50+PA2.6[a] |
|---|---|---|---|---|---|
| 188 | 188 | 60 | 54 | 43 | |
| 188 | 188+PA2.6[b] | 73 | 62 | 48 | |
| 139 | 139 | 17 | 11 | 10 | |
| 139 | 139+PA2.6 | 17 | 16 | 17 | |
| 119 | 119 | 26 | 25 | 21 | |
| 119 | 119+PA2.6 | 34 | 36 | 30 | |
| 119 | KR | 50 | 38 | 19 | |
| 119 | KR+PA2.6 | 41 | 33 | 13 | |
| 155 | KR | 29 | 9 | 0 | 25 |
| 185 | KR | 70 | 55 | 50 | 71 |
| 214 | KR | 78 | 63 | 56 | 75 |
| 221 | KR | 51 | 37 | 28 | 40 |
| 252 | KR | 69 | 51 | 40 | 67 |
| Anti-viral and anti-allogeneic HLA Controls | | | | | |
| 119 anti-HIV CTL | Vacc-env | 60 | 45 | 28 | 14 |
| 123 anti-HIV CTL | Vacc-pol | 54 | 27 | 13 | 28 |
| 152 anti-EBV CTL | 152 BLCL | 70 | 54 | 26 | 8 |
| KR anti-SP CTL | SP BLCL | | 27 | | 6 |
| SP anti-KR CTL | KR BLCL | 59 | 38 | 22 | 19 |

TABLE IV. legend

The ability of PA2.6, a mAb against HLA class I A, B and C, to inhibit lysis of $CD4^+$ lymphocytes by CTL from HIV-infected individuals was tested. Recombinant vaccinia viruses expressing the pol (Vacc-pol Vcf40) (37) and env (Vacc-env VSc25) (38) genes of HIV were used in anti-HIV CTL studies. These viruses were obtained from Dr. B. Moss (NIH) and used to infect autologous BLCL at a multiplicity of infection of 15 for 16 h before their use as target cells. A vaccinia recombinant containing the nucleoprotein of Pichinde virus (VVNP) was constructed in our laboratory (39) and used as a control for these studies.

[a]In some cases, 200µl hybridoma supernatant was added to microtitre plates in place of medium to attain a final volume of 300 µl at an E:T ratio of 50.

[b]In other cases, target cells alone were incubated for 30 min with 1 ml hybridoma supernatant and unbound antibody removed by 2 washes in PBS containing 1% FCS.

TABLE V

*CD8+ CTL from HIV-infected individuals lyse HLA-A and B class I-mismatched CD4+ lymphocytes*

| CD8+ | HLA[a] | | | | CD4+ | HLA | | | | Percent specific lysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effectors | A | | B | | Targets | A | | B | | E:T | 50 | 25 | 12.5 |
| 119 | 2 | 28 | 44 | 18 | 140 | 1 | 3 | 7 | w57 | | 65 | 56 | 56 |
| 139 | 2 | 31 | 7 | 51 | DS | 1 | 23 | 8 | 44 | | 25 | 21 | 13 |
| 139 | | | | | KR | 32 | w33 | 14 | 44 | | 31 | 27 | 19 |
| 140 | 1 | 3 | 7 | w57 | KR | | | | | | 62 | 52 | 33 |
| 152 | 1 | 26 | 18 | 49 | KR | | | | | | 44 | 36 | 26 |
| 155 | 1 | 3 | 7 | 35 | KR | | | | | | 29 | 9 | 0 |
| 155 | | | | | CP | 31 | | 13 | 39 | | 49 | 43 | 26 |
| 111 | 2 | 28 | 51 | 44 | CP | | | | | | 52 | 40 | 29 |

Effector CTL from HIV-infected individuals were tested for cytotoxicity against purified CD4+ target cells from HLA-class I A and B typed HIV-infected (140) and uninfected (KR, DS and CP) individuals.

[a]HLA types were determined using standard microcytotoxicity assays (35).

TABLE VI

*CD8+ CTL -mediated lysis of CD4+ T cells discriminates between CD4+ T cells from different HIV+ or HIV- individuals*

| CD8+ Effectors | E:T | CD4+ Targets | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 136 | | AA | MW | 139 | 72 | 119 | 140 | 136 |
| | 50 | 14 | 3 | 40 | 45 | 8 | 8 | 3 |
| | 25 | 16 | 4 | 14 | 27 | 5 | 4 | 5 |
| | 12.5 | 6 | 0 | 10 | 19 | 4 | 3 | 2 |
| 137 | | DS | KR | SP | 137 | | | |
| | 50 | 45 | 0 | 6 | 9 | | | |
| | 25 | 42 | 0 | 2 | 9 | | | |
| | 12.5 | 34 | 0 | 3 | 5 | | | |
| 207 | | CP | DS | KR | SP | 188 | 207 | |
| | 50 | 11 | 11 | 0 | 0 | 58 | 2 | |
| | 25 | 0 | 0 | 0 | 0 | 45 | 0 | |
| | 12.5 | 0 | 6 | 0 | 0 | 35 | 0 | |
| 188 | | CP | DS | KR | 136 | 188 | | |
| | 50 | 14 | 0 | 1 | 14 | 38 | | |
| | 25 | 4 | 0 | 0 | 8 | 30 | | |
| | 12.5 | 1 | 0 | 0 | 8 | 21 | | |
| 127 | | DS | 126 | 232 | 127 | | | |
| | 50 | 12 | 8 | 19 | 0 | | | |
| | 25 | 13 | 7 | 10 | 2 | | | |
| | 12.5 | 5 | 0 | 6 | 1 | | | |

Effector cells from HIV-infected individuals were tested for cytotoxicity against a panel of purified CD4+ cells from HIV-infected (numbered) or uninfected (initials) individuals.

TABLE VII

*CTL mediating non-HLA-restricted lysis of CD4+ lymphocytes are TCR αβ +T cells*

| CD8+ | CD4+ | Percent Specific Lysis | | |
|---|---|---|---|---|
| Effectors | Targets | E:T = 50 | 25 | 12.5 |
| 119 | KR | 28 | 19 | 8 |
| 119+OKT3 | KR | 0 | 0 | 0 |
| 119+BMA-031 | KR | 0 | 0 | 0 |
| 119 | DS | 24 | 14 | 9 |
| 119+OKT3 | DS | 7 | 3 | 0 |
| 119+BMA-031 | DS | 2 | 0 | 1 |
| 139 | KR | 32 | 18 | 8 |
| 139+OKT3 | KR | 15 | 11 | 8 |
| 139+BMA-031 | KR | 14 | 14 | 4 |
| 140 | DS | 28 | 20 | 9 |
| 140+OKT3 | DS | 7 | 5 | 1 |
| 140+BMA-031 | DS | 5 | 1 | 2 |
| 203 | KR | 30 | 25 | 18 |
| 203+OKT3 | KR | 8 | 2 | 4 |
| 203+BMA-031 | KR | 8 | 4 | 3 |
| 203 | 203 | 15 | 4 | 5 |
| 203+OKT3 | 203 | 0 | 1 | 0 |
| 203+BMA-031 | 203 | 0 | 0 | 0 |
| 214 | KR | 53 | 44 | 39 |
| 214+OKT3 | KR | 5 | 5 | 0 |
| 214+BMA-031 | KR | 10 | 6 | 3 |
| 221 | KR | 18 | 17 | 14 |
| 221+OKT3 | KR | 0 | 0 | 0 |
| 221+BMA-031 | KR | 0 | 0 | 0 |
| 252 | KR | 39 | 24 | 11 |
| 252+OKT3 | KR | 0 | 0 | 0 |
| 252+BMA-031 | KR | 12 | 2 | 4 |

TABLE VII. legend

Immediately prior to assay, effector cells were incubated with either 1 ml of OKT3 hybridoma supernatant, 1μg of anti-TCR αβ framework mAb BMA-031 in 1 ml medium or 1 ml of medium alone for 30 min. Effector cells were then washed 2 times in PBS containing 1% FCS and tested for cytotoxicity.

TABLE VIII

*Stimulation with Con A or HIV, but not EBV, generates CTL which lyse CD4+ lymphocytes*

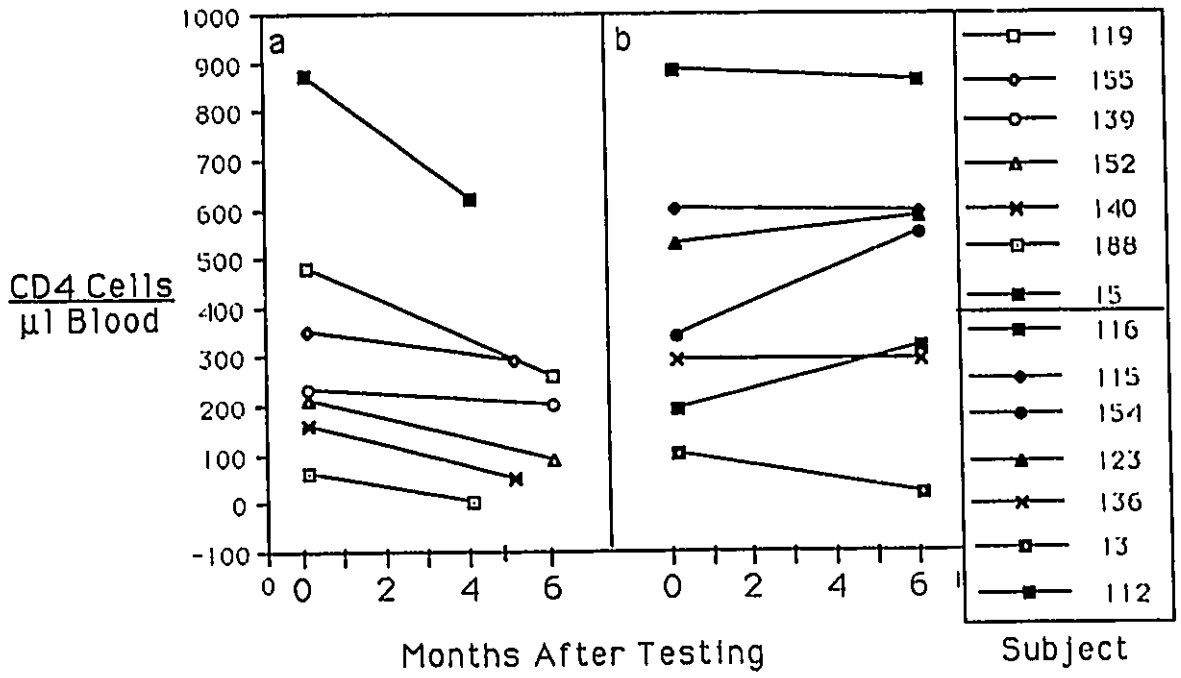
| Effectors | Stimulation | Target | E:T | Percent Specific Lysis 50 | 25 | 12.5 | 50+OKT3[a] |
|---|---|---|---|---|---|---|---|
| 232 | Con A | 232 CD4+ | | 13 | 7 | 5 | 0 |
| 232 | Con A | 126 CD4+ | | 44 | 31 | 22 | 7 |
| 232 | HIV | 232 CD4+ | | 24 | 7 | 1 | 0 |
| 232 | HIV | 126 CD4+ | | 66 | 46 | 31 | 17 |
| 136 | HIV | 72 CD4+ | | 36 | 30 | 14 | 3 |
| 136 | Con A | 72 CD4+ | | 45 | 27 | 19 | 4 |
| 123 | EBV | 123 BLCL | | 64 | 38 | 30 | 43 |
| 123 | EBV | 123 CD4+ | | 19 | 11 | 11 | 23 |
| 123 | EBV | DS CD4+ | | 33 | 23 | 22 | 41 |
| 123 | HIV | 123 BLCL | | 13 | 8 | 7 | 12 |
| 123 | HIV | 123 CD4+ | | 13 | 7 | 5 | 3 |
| 123 | HIV | DS CD4+ | | 21 | 10 | 7 | 6 |
| 119 | EBV | 119 BLCL | | 57 | 48 | 36 | 13 |
| 119 | EBV | 119 CD4+ | | 5 | 5 | 3 | 3 |
| 119 | EBV | KR CD4+ | | 10 | 3 | 0 | 0 |
| 119 | HIV | 119 BLCL | | 29 | 21 | 12 | 12 |
| 119 | HIV | 119 CD4+ | | 0 | 4 | 0 | 6 |
| 119 | HIV | KR CD4+ | | 30 | 16 | 9 | 0 |
| 119 | Con A | 119 BLCL | | 27 | 22 | 12 | 31 |
| 119 | Con A | 119 CD4+ | | 0 | 0 | 0 | 0 |
| 119 | Con A | KR CD4+ | | 35 | 26 | 11 | 0 |

TABLE VIII. legend

The ability of Con A, HIV and EBV to stimulate CTL from HIV-infected individuals against CD4+ lympocytes was compared. Effector cells were generated *in vitro* by stimulation with Con A, PHA-stimulated autologous PBMC (HIV) or autologous EBV-transformed BLCL (EBV). Target cells were purified CD4+ cells or BLCL from HIV-infected individuals (numbered) and CD4+ cells from uninfected (initials) individuals.

[a]In order to show that cytotoxicity was mediated by T cells, 200 µl OKT3 hybridoma supernatant was added in place of medium to test wells with an E:T ratio of 50:1.

Fig. 1




$\bar{X}_a$ $t_0$=337±271 $\bar{X}_b$ $t_0$=419±269

$\bar{X}_a$ $t_6$=216±209 p<.01 $\bar{X}_b$ $t_6$=459±271 ns

$\Delta\bar{X}_a$= -121±84 $\Delta\bar{X}_b$=40±99 p<.01

*Figure 1.* HIV+ subjects initially with CTL activity against autologous activated CD4+ T cells demonstrate a loss of CD4 cells over the 6 month period following testing. This study compared subjects who at time 0 had >50 CD4+ T cells/µl blood and for whom CD4 counts at appropriate times were available. Fig. 1a subjects all had >15% OKT3 inhibitable lysis of autologous CD4+ lymphocytes after *in vitro* stimulation while Fig. 1b subjects had <5% lysis of autologous CD4+ lymphocytes under identical conditions of stimulation. For group a, the mean CD4 count fell significantly (36%) over the 6 months following testing (p<.01) while for group b, no significant change in the mean CD4 count occurred (Wilcoxon Signed Rank Test). The mean change in CD4 count was also significantly greater in group a (p<.01) (Student's *t* test).

TABLE IX

*Detection of CTL activity against autologous CD4+ lymphocytes in HIV-infected individuals precedes loss of CD4 +cells in vivo and loss of sensitivity of autologous CD4+ lymphocytes to lysis*

| $CD8^+$ CTL | Time Months | CD4 Count | Percent Specific Lysis (E:T=50, 25, 12.5) Autologous CD4s | | | KR CD4s | | |
|---|---|---|---|---|---|---|---|---|
| 140 | 0 | 160 | 20 | 16 | 14 | 35 | 32 | 10 |
| 140 | 8 | 10 | 0 | 0 | 0 | 39 | 31 | 20 |
| 139 | 0 | 230 | 16 | 12 | 7 | 31 | 27 | 19 |
| 139 | 8 | 200 | 2 | 0 | 0 | 28 | 19 | 8 |
| 119 | 0 | 480 | 25 | 18 | 16 | 23 | 21 | 9 |
| 119 | 6 | 260 | 2 | 0 | 0 | 45 | 30 | 12 |
| 152 | 0 | 210 | 82 | 77 | 66 | ND | | |
| 152 | 6 | 90 | 0 | 0 | 0 | ND | | |
| 15 | 0 | 870 | 18 | 11 | 8 | ND | | |
| 15 | 4 | 590 | 1 | 3 | 3 | ND | | |
| 199 | 0 | 530 | 14 | 12 | 10 | ND | | |
| 199 | 2 | 310 | 0 | 0 | 0 | ND | | |

CD4-depleted effector cells taken from HIV-infected individuals at different times were stimulated with Con A and IL-2 and tested for lysis of concurrently isolated autologous activated $CD4^+$ lymphocytes. Lysis of $CD4^+$ lymphocytes from an HIV-seronegative individual (KR) was also measured for some subjects at the diiferent time points for comparison.

# Changes in the Cytotoxic T cell Repertoire of HIV-1 Infected Individuals: Relationship to Disease Progression

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The repertoire of antigen specific receptors expressed on T lymphocytes is shaped by fixed genetic and variable environmental selective pressures. Recent technological advances have enabled the analysis of T cell receptor (TCR) expression in the context of selective pressures arising through normal immune system development and also through pathological features of disease. The pathological features of acquired immune deficiency syndrome (AIDS) are reflected by selective depletion of particular T lymphocyte subsets and expansion of others. An important question concerning the immunopathogenesis of AIDS is whether or not the perturbation of the $CD4^+$ and $CD8^+$ T cell subsets following infection with human immunodeficiency virus (HIV) is selective based on TCR variable (V) region gene expression. To address this question, we have functionally analyzed TCR V gene expression on $CD8^+$ cytotoxic T lymphocytes from HIV-1-infected individuals. This was done using monoclonal antibodies against individual TCR V regions to trigger redirected cytolysis in $^{51}Cr$ release assays. The percent specific lysis induced by each antibody functionally measures the representation of the TCR V region gene product it is specific for. Relative to non-HIV-infected controls and asymptomatic HIV-infected individuals with only moderate CD4 lymphocyte depletion, HIV-infected individuals with low CD4 lymphocyte counts exhibited skewed patterns of TCR V region representation. Therefore, the perturbation within the $CD8^+$ cytotoxic T lymphocyte repertoire in HIV-infection appears to be selective based on TCR V region usage, increasingly so as disease progresses. The TCR V genes affected varied between different HIV-infected individuals and skewing detected in functional assays was not always apparent by flow cytometric analysis. These results suggest that HIV-infection causes generalized effects on the T cell repertoire which are reflected in the relative TCR V gene representation of the $CD8^+$ cytotoxic T lymphocyte population in peripheral blood.

## INTRODUCTION

T cell antigen receptors are encoded by rearranged genes assembled from dispersed segments within the genome. Much of the extensive diversity is drawn from multiple genomic α and β V gene segments out of which one per individual rearranged α and β chain gene is chosen to encode the most common TCR (Davis and Bjorkman, 1988). Once receptors are expressed on the thymocyte surface, positive and negative selection of individual receptors in the thymus produces a peripheral repertoire of T lymphocytes expressing characteristic proportions of the V genes represented in the genome (Baccala et. al., 1991). Further selective events in the periphery may alter the proportions of individual V genes expressed. Under normal conditions, the proportions of individual V gene expression are maintained within relatively narrow limits (Grunewald et al., 1991a), even across varied ethnic backgrounds (Ramakrishnan et al., 1992). When the immune system is subjected to unusual pressures such as exposure to superantigens, chronic dysregulation, or malignancy, there may be marked effects on the proportions of individual TCR V genes expressed. This is reflected in highly skewed V region representation in the T cell repertoire in certain types of autoimmunity (Kay et al., 1991; Grunewald et al., 1991b; Davies et al., 1991), lymphoma (Wen et al., 1990; Janson et al., 1991) and following superantigenic challenge (Webb et al., 1990; MacDonald et al., 1991).

The characteristics of skewing of the T cell repertoire in pathological states can provide insight into the etiology of the condition. Oligoclonal T cell populations in the joints of subjects with rheumatoid arthritis are consistent with a highly selective localized antigen driven recruitment and expansion of reactive T cells (Stamenkovic et al., 1988). The demonstration that an endogenous superantigen encoded by a murine retrovirus causes deletion of T cells bearing particular V regions in the thymus (Marrack et al., 1991; Woodland et al. 1991; Dyson et al. 1991) and in the periphery (Webb et al., 1990;

MacDonald et al., 1991) led to speculation that HIV encodes a superantigen (Janeway, 1991). Support for this suggestion appeared in the report of consistent deletion of certain V region expressing T cells in AIDS patients (Imberti et al., 1991). This deletion was demonstrated by reverse transcription of T lymphocyte mRNA and quantitative polymerase chain reaction (PCR) amplification of TCR V regions. In addition, elevated levels of $CD4^+$ T cells with particular V regions have been detected by flow cytometry in asymptomatic HIV-infected individuals (Dalgleish et al., 1992). Expansion could reflect the period of superantigen-mediated expansion of T cells with particular V regions preceding T cell deletion in murine systems (Webb et al., 1990; MacDonald et al., 1991). Although interpreting changes in the T cell repertoire in HIV-infection as due to superantigens has been questioned, it is clear that the T cell repertoire changes markedly during HIV-infection (Imberti et al., 1991).

A prominent feature of HIV-infection is systemic activation of the immune system and an increase in activated HLA-$DR^+$ $CD8^+$ T lymphocytes in the peripheral blood (Prince et al., 1987; Giorgi and Detels, 1989; Levacher et al., 1990). Some of these activated lymphocytes are cytotoxic T lymphocytes (CTL) which recognize HIV-infected cells (Walker et al., 1987, Grant et al., 1992), others are autoreactive CTL (Israel-Biet et al., 1990; Zarling et al., 1990), and many are of unknown specificity. In order to understand AIDS, it is important to characterize the selective pressures operating on the T cell repertoire during HIV infection. V region gene representation in the T cell repertoire of HIV-infected individuals is therefore an area of intense investigation. We have adapted a redirected cytolysis assay (Leeuwenberg et al., 1985) to functionally quantitate V region gene utilization by $CD8^+$ CTL in HIV-infected subjects. This assay allows rapid comparative analysis of TCR V region representation. Results of these studies suggest that HIV has a generalized effect on the T cell repertoire distinct from the effects of nominal antigens or superantigens and that this effect on the T cell repertoire relates to disease progression.

## MATERIALS AND METHODS

### Subjects

HIV-1-infected individuals were recruited through the Chedoke-McMaster Special Immunology Services Clinic. Infection with HIV-1 was determined by ELISA and confirmed by Western blot. Subjects were clinically assessed and $CD4^+$ and $CD8^+$ lymphocyte counts determined at 3 month intervals whenever possible. These studies received local ethical approval and informed consent was obtained from all individuals involved. Non HIV-infected volunteers were recruited from hospital and laboratory personnel.

### Lymphocyte Separations

Blood was collected in heparinized vacutainers, diluted with an equal volume of phosphate buffered saline (PBS), layered over Ficoll-paque gradient separation medium (Pharmacia Chemicals, Dorval, Que.) and centrifuged at 400xg for 30 min. Interface cells were collected, washed 3 times in PBS containing 1% fetal calf serum (FCS), and counted. In order to remove $CD4^+$ lymphocytes, cells were resuspended in PBS containing 0.1% bovine serum albumin (BSA) at $3x10^6$/ml with enough OKT4 coated magnetic beads (DYNAL Inc., Great Neck, NY) for a 5:1 bead-to-target cell ratio and rotated together for 1 h in a 15 ml conical tube at 4°C. After magnetic displacement of beads, unbound cells were removed by pipetting. To positively select $HLA\text{-}DR^+$ lymphocytes, $1x10^7$ peripheral blood mononuclear cells (PBMC) were incubated for 30 min with 5µg monoclonal anti-HLA-DR (L243, Becton-Dickinson, Mountain View, CA), washed twice in PBS with 0.1% BSA, and selected as above using goat anti-mouse IgG coated magnetic beads (DYNAL Inc., Great Neck, NY).

**Lymphocyte Stimulation**

$CD4^+$ lymphocyte depleted PBMC or positively selected HLA-$DR^+$ PBMC were resuspended at approximately $10^6$/ml in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% FCS (Bocknek, Burlington, Ont.), 10 mM hepes, 2mM L-glutamine, 1% penicillin-streptomycin, (all from Gibco) and $2x10^{-5}$ M 2-mercaptoethanol (Sigma Chemical Co. St. Louis MO). Cultured cells were stimulated with 10 µg/ml concanavalin A (Con A) (Difco, BDH, Toronto, Ont., Canada) for 3 days after which 5 U/ml recombinant interleukin-2 (rIL-2) (Genzyme, Cambridge, MA) was added. After a total of 7 days these cultures contained >90% $CD8^+$ T cells measured by flow cytometry (data not shown). Magnetic beads were removed from stimulated HLA-$DR^+$ cells by 3 cycles of vigorous pipetting, magnetic displacement of beads, and washing with PBS 0.1% BSA. For stimulation with HIV, 10% of the freshly isolated PBMC were cultured with 5 µg/ml phytohemagglutinin-purified (PHA-P) (Wellcome Diagnostics, Dartford, England) for 3 days to activate replication of endogenous HIV, washed, irradiated at 5000 Rad, and added to the remaining 90% of the PBMC which had been maintained in unsupplemented medium. After 3 days of coculture, 5 U/ml of rIL-2 was added. Stimulated cells were routinely tested for cytotoxicity following 7 days incubation in rIL-2.

**Redirected Cytotoxicity Assay**

Approximately $2x10^6$ Fc receptor positive P815 murine mastocytoma cells (American Type Culture Collection, Rockville, MD) were incubated for 60 min with 200 µCi $Na^{51}CrO_4$ (New England Nuclear, Boston, MA), washed 3 times in PBS containing 1% FCS, counted, and resuspended in lymphocyte medium at $1.5x10^6$/ml. $1.5x10^5$ $^{51}Cr$-labeled P815 cells were transferred to separate tubes. These cells were incubated for 45 min at 37°C with 1µg of one of 6 different monoclonal antibodies, including OKT3 (anti-CD3), 1C1 (anti-Vβ 5.2 and 5.3) OT145 (anti-Vβ 6.7) 16G8 (anti-Vβ8) S511 (anti-Vβ12) and F1 (anti-Vα.2.3). In some assays OKT8 (anti-CD8) was also used as a

negative control antibody. OKT3 and OKT8 were from Coulter Immunology (Hialeah, FL) and all other antibodies were from T-Cell Sciences (Cambridge, MA). Target cells were then resuspended without further washing at $1x10^5$/ml and 50 µl added to each test well of round bottom 96 well microtitre plates (Nunclon, Roskilde, Denmark). Effector cytotoxic cells were counted and resuspended at $5x10^6$/ml (control HIV seronegative subjects) or $2.5x10^6$/ml (HIV-infected subjects) and 50 µl added to each test well of microtitre plates for effector cell:target cell ratios (E:T) of 50:1 ($HIV^-$) or 25:1 ($HIV^+$). Medium was added to each well to obtain a final volume of 300 µl. For each set of antibody-coated P815 cells, triplicate wells containing target cells and medium alone to determine spontaneous $^{51}Cr$ release and target cells and 1N hydrochloric acid (HCl) to determine maximum $^{51}Cr$ release were included. All sets of effector cells were also tested in triplicate for lysis of P815 cells redirected by each individual antibody. Following incubation at 37°C in a humidified 5% $CO_2$ incubator for 5 hours, 100 µl supernatant was removed from each well and counted in a $\gamma$ counter. Percent specific redirected lysis mediated by each of the monoclonal antibodies was calculated by the following formula: (Test $^{51}Cr$ release-Spontaneous release)÷ (Maximum $^{51}Cr$ release-Spontaneous release) x 100. Results are expressed as the mean of triplicate tests. In all assays, spontaneous $^{51}Cr$ release was <15% of total $^{51}Cr$ release. Assays were considered invalid if redirected lysis mediated by OKT3 or by the 5 anti-TCR monoclonals in total was <50%. Additionally, if lysis of the OKT8 treated P815 cells was >10%, the assay was considered invalid, since OKT8 binds to a non-triggering molecule expressed on the surface of $CD8^+$ CTL. Depletion of $CD8^+$ cells immediately before assays reduced the redirected lysis mediated by OKT3 by >95% in 6/6 cases (data not shown), demonstrating that $CD8^+$ cells account for the overwhelming majority of CTL detected by redirected cytolysis.

**Flow Cytometry**

In order to phenotypically determine levels of TCR V region representation, T cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-TCR V region antibodies and phycoerythrin (PE)-conjugated OKT8 as previously described (Silver et al., 1992) and counted by two color direct immunofluorescence flow cytometry (FACScan flow cytometer, Becton-Dickinson, Mountain View, CA)

**Data Normalization**

The anti-TCR monoclonal antibodies used in this study together cover approximately 20% of the normal human T-cell repertoire (Janson et al., 1991). In order to compare the relative use of each individual TCR V region within this 20% window between different subjects, the redirected lysis mediated by all 5 antibodies was totalled and lysis mediated by individual antibodies expressed as a percentage of this total. Results from flow cytometric analyses were treated similarly so that the number of lymphocytes bearing each individual V region gene was expressed as a percentage of the total number of lymphocytes expressing any of the 5 V region genes detected by the antibodies.

## RESULTS

### Functional TCR V region gene representation of CD8+ cytotoxic T lymphocytes from controls

Results of redirected lysis assays done with CD8+ lymphocytes from 6 normal control donors are shown in table 1. A normal range for TCR V region gene representation in CTL was estimated from these results based on the mean percentage representation of each TCR V region gene for the 6 control individuals±3 standard deviations (SD). Notably consistent within the control group was the functional eminence of Vβ5 and Vα2; ranking 1st or 2nd in level of representation in 12/12 cases. These results generally agree with published data on physical representation of TCR V region genes in controls (Janson et al., 1991), except we found Vβ8 functionally underrepresented on CD8+ T cells relative to its reported physical level of representation.

### Functional TCR V region gene representation of CD8+ cytotoxic T lymphocytes from HIV-infected individuals

The cytotoxic activity of lymphocytes from HIV-infected subjects was much higher than that of lymphocytes from uninfected controls, therefore an E:T ratio of 25:1 instead of 50:1 was used in the analysis of V region gene representation of CTL from HIV-infected subjects. Results from redirected lysis assays done with CD8+ lymphocytes from 6 asymptomatic HIV-infected individuals with CD4+ lymphocyte counts ≥400/μl blood are presented in table 2. In this group of 6 subjects, least affected by HIV, there is only 1 case in which functional representation of a TCR V region gene falls outside the normal range. This instance is the relative overrepresentation of Vβ6 by the cytotoxic CD8+ lymphocytes of subject 115. As in the control group, the functional eminence of Vβ5 and Vα2 is consistent (1st or 2nd ranking in 11/12 cases), except for subject 115, where the eminence of Vα2 fell to 3rd coincident with overrepresentation of Vβ6.

Table 3 shows the results of redirected lysis asays done with lymphocytes from 6 HIV-infected individuals with $CD4^+$ lymphocyte counts between 200 and 400/μl blood. These individuals were also clinically asymptomatic, although their CD4 counts indicated progressive disease. In this group, there were 6 cases in which the functional representation of a TCR V region gene fell outside the normal range, involving 4 of the 6 individuals. These included 3 examples of diminished representation: in subject 155, of Vβ5, in subject 16 of Vα2, and in subject 112 of Vβ6; and 3 examples of overrepresentation: in subject 155 of Vβ6, in subject 132 of Vβ8 and in subject 112 of Vβ12. The eminence of Vβ5 is less consistent in this group, as Vβ5 is undetectable in subject 155 and ranks 3rd in subjects 132 and 112. Despite a low representation relative to controls, Vα2 ranks a distant 2nd, comparable with Vβ12 in subject 16.

Table 4 shows the results of redirected lysis assays done with lymphocytes from 6 HIV-infected individuals with <200 $CD4^+$ lymphocytes/μl blood. All but subjects 152 and 122 had suffered AIDS defining illnesses prior to testing, but at the time of testing, only subject 13 was symptomatic. This group demonstrated 9 cases of functional V region gene representation outside the normal range, involving 5 of the 6 individuals. These included 3 cases of diminished representation: in subjects 140, 185 and 13 all of Vβ5; and 6 cases of overrepresentation: subjects 140, 152, 185 and 13 all Vβ6 and subjects 122 and 13 Vβ12. Vβ5 was not at all eminent in this group (ranked 1st or 2nd in 0/6 cases) and the eminence of Vα2 was less than in the control group (ranked 1st or second in 4/6 cases). The number of individuals affected per group (1 vs 4 vs 5) and the total number of TCR V region genes affected per group (1 vs 6 vs 9) increased with each stratification of decreasing $CD4^+$ lymphocyte counts. This consistent trend towards an abnormal $CD8^+$ repertoire as $CD4^+$ lymphocyte counts decrease suggests that distortion of the functional CTL repertoire is a component of progressive HIV disease.

**Comparison of functional and phenotypic analysis of the CD8+ T cell repertoire**

Table 5 shows the results of flow cytometric analysis of TCR V region gene expression by the CD8+ lymphocytes of 6 HIV-infected individuals for whom concurrent analysis by redirected cytotoxicity was done. There were 3 cases where phenotypic TCR V region gene representation by CD8+ lymphocytes fell outside the normal range. Subjects 232, 152 and 140 all showed elevated levels of Vβ12 relative to published control levels (Janson et al., 1991). These elevations were not accompanied by functional overrepresentation. Conversely, in none of the 4 cases where functional TCR V region gene representation fell outside the normal range (132 Vβ8, 140 Vβ5 and Vβ6 and 152 Vβ6) were phenotypic levels outside the normal range. In fact, the relative levels of expression for each TCR V region gene were different when measured functionally or phenotypicically, as was the ranking of V region gene representation. It therefore appears these methods measure different things and that phenotypic and functional representation of TCR V region genes on CD8+ T cells are not necessarily directly related.

An obvious discrepancy between the functional and phenotypic representation of Vβ8 ($p=.007$, Student's $t$ test), in that it was physically abundant, but functionally subdued, can be seen in table 5. In the 6 HIV-infected individuals for whom both measurements are available, Vβ8 physically accounted for a mean±SD of 29±3.8% of the portion of the T cell repertoire covered by the anti-V gene antibodies used. Functionally, Vβ8 accounted for only 15±9.7% of the same portion of the repertoire, suggesting a population of cytotoxically silent Vβ8+CD8+ T lymphocytes. Since this discrepancy was apparent in controls as well as HIV-infected individuals, it appears unrelated to HIV infection.

**Functional TCR V region gene representation of distinct CD8+ T cell subsets from the same individual**

An advantage of functional analysis of the T cell repertoire is access to diagnostic subsets of T cells which may be too sparse for study by three color flow cytometry or indistinguishable by PCR analysis. By positively selecting HLA-DR+ cells from fresh peripheral blood, expanding this population with Con A, and analyzing by redirected cytolysis, we can determine TCR V region gene representation specifically within circulating activated CTL. Since the original activation occurs *in vivo*, the repertoire expressed by these cells may be more diagnostic for disease related changes than the general repertoire. Results shown in figure 1 for two HIV-infected individuals support this possibility. Comparison of the functional repertoire of HLA-DR+ cells and HLA-DR- cells from HIV-infected individuals 139 and 152, shows selective expansion of Vβ6+ cytotoxic T cells in the HLA-DR+ population. One year later, this activated population was selectively expanded in the general repertoire. Thus skewing of the activated repertoire, especially for subject 152, predicted later changes in the repertoire. Analysis of activated subsets may therefore identify disease related changes in the repertoire earlier, or actually predict changes. The time between analyses for both subjects was nearly a year, over which the CD4 count of subject 139 fell from 230 to 170 and that of subject 152 from 210 to 80. Interestingly, the more dramatic fall in CD4+ lymphocytes occurred in the individual with TCR V region gene representation values outside the normal range. We have observed a similar trend in other individuals in that a stable T cell repertoire with V region gene representation within the normal range is associated with clinical stability. However, more subjects must be followed longtitudinally to document this association and determine any prognostic significance of repertoire skewing.

**Stimulation of differential functional V region gene representation by antigen or mitogen**

Another application of V region gene analysis by redirected lysis is to look at the effect of antigenic stimulation on V gene use by cytotoxic T cells. We compared lymphocytes from HIV-infected individuals after stimulation with Con A or HIV and were surprised by the ability of HIV to stimulate broad representation of TCR V genes (Fig. 2). There were, however, several cases where certain V genes selectively responded to, or selectively ignored antigen specific stimulation with HIV. For example, stimulation with HIV reduced the usage of Vβ8 by cytotoxic lymphocytes of subject 132 to within the normal range, but also reduced the use of Vβ5 to below the normal range compared to Con A stimulation. Stimulation with either Con A or HIV induced Vβ6 utilization at a level outside the normal range by cytotoxic T lymphocytes from subject 115. The levels of V region gene use by cytotoxic lymphocytes from subject 188 were similar with either stimulation. Overall, the pattern of V region gene representation induced by stimulation with HIV was not markedly distinct from that induced by stimulation with Con A. The reasons for this are not clear, but could relate to the way lymphocytes are stimulated with HIV and an immunopathogenic mechanism of CD4 depletion proposed in the discussion.

## DISCUSSION

This study introduces the application of redirected lysis assays with monoclonal antibodies against TCR V region gene products to the analysis of the cytotoxic T cell repertoire. V region analysis by this method allows comparison of functional and physical levels of V region gene expression, which may be quite different. Results obtained with lymphocytes from HIV-infected individuals support the concept that progressive distortion of V region gene representation of CTL relative to controls occurs as $CD4^+$ lymphocyte numbers fall. The number of cases of V gene utilization outside the normal range increased successively in 3 groups of HIV-infected individuals stratified according to $CD4^+$ lymphocyte counts. It should be emphasized that these cases were detected with antibodies covering approximately one-fifth of the repertoire only, so the actual perturbation of the T cell repertoire in HIV-infected individuals is probably far greater than detected in this study. This type of finding has been attributed to superantigens, but our observation that increases detected by function are not always reflected by physical increases suggests that not all lymphocytes expressing a certain V region are affected. This is more consistent with antigens rather than superantigens driving the expansion or deletion of CTL expressing particular V region genes. In several cases, changes in the total T cell repertoire were detected earlier by analyzing the repertoire of T cells activated *in vivo*. If this is a general finding, then processes associated with HIV infection which selectively activate T cells may impact on the whole repertoire and are an integral component of pathogenesis. HIV alone may not be directly responsible for the skewing of the repertoire, since stimulation with HIV obscured at least one case of V gene overrepresentation which was apparent after polyclonal stimulation with Con A.

The major question regarding the skewing of the T cell repertoire in HIV infection is whether it reflects an integral component of the immunopathology of AIDS or whether it merely reflects the presence of opportunistic pathogens whose impact parallels disease progression. Although there is not yet a definitive answer to this question, there are

reasons to think that the changes in the repertoire are not due to opportunistic pathogens. One reason is that the success of the opportunistic pathogen, by definition, depends directly upon a weakened immune response to that pathogen. Hence, once the opportunistic pathogen is established, it has the least impact on the immune system. An alternative scenario is that constrictive changes in the repertoire favor the establishment of opportunistic pathogens by eliminating T cells which would normally be involved in the response to the pathogens. The idea that opportunistic pathogens have superantigens which stimulate the dilapidated immune system without inducing immunity to themselves is another possibility which can be investigated by assessing the diversity of TCRs sharing the same overrepresented V region gene. The level of diversity may indicate whether the signal for expansion is antigenic (low diversity) or superantigenic (high diversity). In particular cases, the question of diversity within TCRs sharing V region genes can also be addressed by redirected cytolysis. For instance, if Vα2 was found to be expanded in an individual, Vα2$^+$ cytotoxic T cells could be selected, expanded and analyzed by redirected cytolysis for Vβ region expression. Now that an expanded panel of anti-Vβ TCR monoclonals are available, about 50% of the repertoire can be investigated in this way. Diversity of Vβ expression by the expanded Vα2$^+$ cells over that 50% of the repertoire should indicate if Vα2 expansion is polyclonal (superantigenic) or oligoclonal (antigenic). These methods are also amenable to the characterization of TCR V region gene use by antigen-specific cytotoxic T cell clones.

The idea that skewing of the T cell repertoire is an integral component of the pathogenesis of HIV infection centers around 2 possibilities. One is that HIV itself is a superantigen. The lack of consistent expansion of the same V region genes in different HIV-infected individuals seems to discredit this idea, but there is now evidence of selective growth of HIV in Vβ12$^+$ cells (Laurence et al., 1992). This evidence suggests that HIV directly activates Vβ12$^+$ cells, thereby favoring selective replication in CD4$^+$ Vβ12$^+$ cells following infection. It is interesting that by flow cytometry, but not

redirected lysis, we observed an increase in Vβ12 levels relative to controls in 3/6 HIV-infected individuals. By flow cytometry, only Vβ12 was found outside the normal range of representation.

Another possibility for HIV-induced skewing of the T cell repertoire, more consistent with all the data, is that HIV does not selectively stimulate on the basis of just V gene expression, but induces autoimmunity against conformational determinants of TCRs. This would tend to selectively eliminate some V region genes and favor others, but the pattern could differ in unrelated individuals and only a subset of T cells expressing any particular V region gene would be affected. Autoimmunity against conformational determinants of TCRs has been previously proposed to explain the selective depletion of $CD4^+$ T cells in HIV infection (Hoffmann et al., 1991, Grant, 1991) and crossreactivity between HIV gp120 and Vβ4 has recently been demonstrated (Primi, 1992). This model of HIV pathogenesis is consistent with clinical and immunological features of HIV-infection. We and others have shown that $CD8^+$ CTL from HIV-infected persons selectively kill uninfected $CD4^+$ lymphocytes (Zarling et al., 1990, Israel-Biet et al., 1990). Although the autoimmune CTL cells are phenotypically normal ($CD3^+CD8^+$ TCR $\alpha\beta^+$), they are not HLA-restricted, suggesting recognition of a conformational determinant (Grant et al., submitted for publication). Increases in $CD8^+$ lymphocyte number and activity and a correlation between disease progression and percentage of circulating activated $CD8^+$ T cells are very consistent features of HIV-infection (Vanham et al., 1991). If HIV-induced autoimmunity drives this expansion, then the distortions in the CTL repertoire would reflect activation against certain $CD4^+$ lymphocytes and progress in parallel with depletion of $CD4^+$ lymphocytes. Also, if the target of the cytotoxic lymphocytes is $CD4^+$ lymphocytes, this would explain why stimulation with HIV induces a broad representation of TCR V regions, similar to polyclonal stimulation. HIV stimulation relies upon expression of HIV antigens by activated $CD4^+$ lymphocytes, so the

$CD4^+$ lymphocytes themselves may also activate cytotoxic cells highly represented in the functional repertoire .

In summary, we feel that redirected cytolysis with antibodies against TCR V region gene products is a useful new technique for analysis of the cytotoxic T cell repertoire. Our results corroborate those of others, obtained mainly by PCR techniques, suggesting that distortion of the T cell repertoire accompanies progressive HIV-infection. In light of the possibility that autoimmune $CD8^+$ CTL are involved in the depletion of $CD4^+$ lymphocytes, it may be much more meaningful to focus on the functional cytotoxic T cell repertoire, rather than on all the T cells. Whether these changes in the repertoire are a cause or effect of disease progression is an important and controversial issue for further examination.

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## REFERENCES

Baccala, R., Kono, D.H., Walker, S., Balderas, R.S., and Theofilopoulos, A.N. 1991. Genomically imposed and somatically modified human thymocyte Vβ gene repertoires. Proc. Natl. Acad. Sci. USA 88:2908-2912.

Dalgleish, A.G., Wilson, S., Gompels, M., Ludlam, C., Gazzard, B., Coates, A.M., and Habeshaw, J. 1992. T-cell receptor variable gene products and early HIV-1 infection. Lancet 339:824-828.

Davies, T.F., Martin, A., Concecion, E.S., Graves, P., Cohen, L., and Ben-nun, A. 1991. Evidence of limited variability of antigen receptors on intrathyroidal T cells in autoimmune thyroid disease. N. Engl. J. Med. 325:238-244.

Davis, M.M., and Bjorkman, P.J. 1988. T-cell antigen receptor genes and T-cell recognition. Nature 334:395-400.

Dyson, P.J., Knight, A.M., Fairchild, S., Simpson, E., and Tomonari, K. 1991. Genes encoding ligands for deletion of Vβ11 T cells cosegregate with mamary tumour virus genomes. Nature 349:531-532.

Georgi, J.V., and Detels, R. 1989. T-cell subset alterations in HIV-infected homosexual men: NIAID multicenter AIDS cohort study. Clin. Immunol. Immunopatol. 52:10-19.

Grant, M.D. 1991. HIV and idiotypic T cell regulation. Immunol. Today 12:171-172.

Grant, M.D., Smaill, F.M., Singhal, D.P., and Rosenthal, K.L. 1992. The influence of lymphocyte counts and disease progression on circulating and inducible anti-HIV-1 cytotoxic T cell activity in HIV-1$^+$ humans. AIDS: in press.

Grunewald, J., Ahlberg, R., Lefvert, A.K., DerSimonian, H., Wigzell, H., and Janson, C.H. 1991a. Abnormal T-cell expansion and V-gene usage in myasthenia gravis patients. Scand. J. Immunol. 34:161-168.

Grunewald, J., Janson, C.H., and Wigzell, H. 1991b. Biased expression of individual T cell receptor V gene segments in CD4$^+$ and CD8$^+$ human peripheral blood T lymphocytes. Eur. J. Immunol. 21:819-822.

Heffmann, G.W., Kion, T.A., and Grant, M.D. 1991. An idiotypic network model of AIDS immunopathogenesis. Proc. Natl. Acad. Sci. USA 88:3060-3064.

Imberti, L., Sottiini, A., Bettinardi, A., Puotti, M., and Primi, D. 1991. Selective depletion in HIV-infection of T cells that bear specific T cell receptor Vβ sequences. Science 254:860-863.

Israel-Biet, D., Venet, A., Beldjord, K., Andrieu, J.M., and Even, P. 1990. Autoreactive cytotoxicity in HIV-infected individuals. Clin. exp. Immunol. 81:18-24.

Janeway, C. 1991. Mls: makes a little sense. Nature 349:459-461.

Janson, C.H., Grunewald, J., Osterborg, A., DerSimonian, H., Brenner, M.B., Mellstedt, H., and Wigzell, H. 1991. Predominant T cell receptor V gene usage in patients with abnormal clones of B cells. Blood 77:1776-1780.

Kay, R.A., Hay, E.M., Dyer, P.A., Dennett, C., Green, L.M., Bernstein, R.M., Holt, P.J.L., Pumphrey, R.S.H., Boylston, A.W., and Ollier, W.E.R. 1991. An abnormal T cell repertoire in hypergammaglobulinaemic primary Sjogrens syndrome. Clin. exp. Immunol. 85:262-264.

Laurence, J., Hodtsev, A.S., and Posnett, D.N. 1992. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature 358:255-259.

Leeuwenberg, J.F.M., Spits, H., Tax, W.J.M., and Capel, P.J.A. 1985. Induction of non-specific cytotoxicity by monoclonal anti-T3 antibodies. J. Immunol. 134:3770-3775.

Levacher, M., Tallet, S., Dazza, M.C., Dournon, E., Rouveix, B., and Pocidalo, J.J. 1990. T activation marker evaluation in ARC patients treated with AZT. Comparison with $CD4^+$ lymphocyte count in non-progressors and progressors towards AIDS. Clin. exp. Immunol. 81:177-182.

Marrack, P., Kushnir, E., and Kappler, J. 1991. A maternally inherited superantigen encoded by a mammary tumour virus. Nature 349:524-526.

MacDonald, H.R., Baschieri, S., and Lees, R.K. 1991. Clonal expansion precedes anergy and death of $V\beta8^+$ peripheral T cells responding to staphylococcal enterotoxin B *in vivo*. Eur. J. Immunol. 21:1963-1966.

Primi, D. 1992. Oral presentation, round table discussion on mechanisms of CD4 depletion. VIII$^{th}$ International AIDS Conference, Amsterdam.

Prince, H.E., Arens, L., and Kleinman, S. H. 1987. CD4 and CD8 subsets defined by dual color cytofluorometry which distinguish symptomayic from asymptomatic blood donors seropositive for human immunodeficiency virus. Diag. clin. Immunol. 5:188-195.

Ramakrishnan, N.S., Grunewald, J., Janson, C.H., and Wigzell, H. 1992. Nearly identical T-cell receptor V-gene usage at birth in two cohorts of distinctly different ethnic origin: influence of environment in the final maturation in the adult.
Scand. J. Immunol. 36:71-78.

Gulwani-Akollar, B., Posnett, D.N., Janson, C.H., Grunewald, J.,Wigzell, H., Akolkar, P., Gregersen, P.K., and Silver, J. 1991. T cell receptor V-segment frequencies in peripheral blood T cells correlate with human leukocyte antigen type.
J. Exp. Med. 134:1139-1146.

Stamenkovic, I., Stegagno, M., Wright, K.A., Krane, S.M., Amento, E.P., Colvin, R.B., Duquesnoy, R.J., and Kurnick, J.T. 1988. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc. Natl. Acad. Sci. USA 85:1179-1183.

Vanham, G., Kestens, L., Penne, G., Goilav, C., Gigase, P., Colebunders, R., Vandenbruane, M., Goeman, J., Van Der Groen, G., and Ceuppens, J.L. 1991. Subset markers of CD8(+) cells and their relation to enhanced cytotoxic T-cell activity during human immunodeficiency virus infection. J. Clin. Immunol. 11:345-356.

Walker, B.D., Chakrabarti, S., Moss, B., Paradis, T.J., Flynn, T., Durno, A.G., Blumberg, R.S., Kaplan, J.C., Hirsch, M.S., and Schooley, R.T. 1897. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature 328:345-348.

Webb, S., Morris, C., and Sprent, J. 1990. Extrathymic tolerance of mature T cells:

clonal elimination as a consequence of immunity. Cell 63:1249-1258.

Wen, T., Mellstedt, H., and Jondal, M. 1990. Presence of clonal T cell populations in chronic B lymphocytic leukemia and smoldering myeloma. J. Exp. Med. 171:659-668.

Woodland, D.L., Hap, M.P., Gollob, K.J., and Palmer, E. 1991. An endogenous retrovirus mediating deletion of αβ T cells. Nature 349:529-530.

Zarling, J.M., Ledbetter, J.A., Sias, J., Fultz, P., Eichberg, J., Gjerset, G., and Moran, P. 1990. HIV-infected humans, but not chimpanzees, have circulating cytotoxic T lymphocytes that lyse uninfected $CD4^+$ cells. J. Immunol. 144:2992-2998.

**Table 1.** Functional T cell Receptor V Region Representation of Cytotoxic T cells From non-HIV-infected Controls

| Subject | Percent Specific Lysis Mediated by Antibody | | | | | | Percentage of Total Anti-V Region Antibody Mediated Lysis Mediated by Individual Antibodies[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | OKT3 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 |
| 1 | 100 | 40 | 10 | 28 | 11 | 57 | 27 | 7 | 19 | 8 | 39 |
| 2 | 94 | 56 | 24 | 13 | 14 | 59 | 34 | 14 | 8 | 8 | 36 |
| 3 | 78 | 15 | 11 | 11 | 15 | 20 | 21 | 15 | 15 | 21 | 28 |
| 4 | 82 | 14 | 4 | 2 | 3 | 24 | 30 | 9 | 4 | 6 | 51 |
| 5 | 55 | 13 | 8 | 6 | 0 | 23 | 26 | 16 | 12 | 0 | 46 |
| 6 | 82 | 16 | 4 | 2 | 2 | 14 | 42 | 11 | 5 | 5 | 37 |
| | | | | | Mean±SD | | 30±7 | 13±3 | 11±6 | 8±7 | 40±8 |
| | | | | | Normal Range (Mean±3 SD) | | 9-51 | 4-22 | 0-29 | 0-29 | 16-64 |

[a]To normalize the data to allow comparison between individuals, the percent lysis mediated by each individual antibody was divided by the total of the lysis mediated by the 5 anti-V region antibodies. This gives a percentage expressing the relative level of representation of each V region within the total portion of the repertoire covered by the anti-V region antibodies.

**Table 2.** Functional T cell Receptor V Region Representation of Cytotoxic T cells From HIV-infected Subjects With >400 $CD4^+$ T Lymphocytes/μl Blood

| Subject | Percent Specific Lysis Mediated by Antibody | | | | | | Percentage of Total Anti-V Region Antibody Mediated Lysis Mediated by Individual Antibodies[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | OKT3 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 |
| 116 | 85 | 44 | 33 | 22 | 16 | 46 | 27 | 20 | 14 | 10 | 29 |
| 232 | 69 | 23 | 8 | 9 | 5 | 26 | 32 | 11 | 13 | 7 | 37 |
| 28 | 76 | 9 | 7 | 8 | 7 | 25 | 29 | 11 | 12 | 11 | 38 |
| 115 | 73 | 22 | 20 | 6 | 12 | 12 | 31 | 28[b] | 8 | 17 | 17 |
| 119 | 93 | 22 | 4 | 10 | 13 | 15 | 34 | 6 | 16 | 20 | 23 |
| 190 | 97 | 65 | 44 | 36 | 32 | 55 | 28 | 19 | 16 | 14 | 24 |

[a]To normalize the data to allow comparison between individuals, the percent lysis mediated by each individual antibody was divided by the total of the lysis mediated by the 5 anti-V region antibodies. This gives a percentage expressing the relative level of representation of each V region within the total portion of the repertoire covered by the anti-V region antibodies.

[b]Underlined values are those which fall outside the normal range established for controls (>3 standard deviations above or below control mean).

**Table 3.** Functional T cell Receptor V Region Representation of Cytotoxic T cells From HIV-infected Subjects With 200-400 CD4+ T Lymphocytes/μl Blood

| Subject | Percent Specific Lysis Mediated by Antibody | | | | | | Percentage of Total Anti-V Region Antibody Mediated Lysis Mediated by Individual Antibodies[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | OKT3 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 |
| 139 | 93 | 31 | 11 | 17 | 9 | 42 | 28 | 10 | 15 | 8 | 38 |
| 155 | 100 | 0 | 28 | 0 | 14 | 40 | 0[b] | 34 | 0 | 17 | 49 |
| 111 | 88 | 64 | 20 | 20 | 10 | 55 | 38 | 12 | 12 | 6 | 33 |
| 132 | 87 | 25 | 12 | 61 | 15 | 92 | 12 | 6 | 30 | 7 | 45 |
| 16 | 68 | 40 | 7 | 9 | 12 | 12 | 50 | 9 | 11 | 15 | 15 |
| 112 | 81 | 19 | 0 | 8 | 21 | 22 | 27 | 0 | 11 | 30 | 31 |

[a]To normalize the data to allow comparison between individuals, the percent lysis mediated by each individual antibody was divided by the total of the lysis mediated by the 5 anti-V region antibodies. This gives a percentage expressing the relative level of representation of each V region within the total portion of the repertoire covered by the anti-V region antibodies.

[b]Underlined values are those which fall outside the normal range established for controls (>3 standard deviations above or below control mean).

**Table 4.** Functional T cell Receptor V Region Representation of Cytotoxic T cells From HIV-infected Subjects With <200 $CD4^+$ T Lymphocytes/μl Blood

| Subject | Percent Specific Lysis Mediated by Antibody | | | | | | Percentage of Total Anti-V Region Antibody Mediated Lysis Mediated by Individual Antibodies[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | OKT3 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 |
| 181 | 69 | 6 | 9 | 12 | 13 | 21 | 10 | 15 | 21 | 21 | 34 |
| 140 | 98 | 0 | 21 | 3 | 7 | 21 | 0[b] | 40 | 6 | 13 | 40 |
| 122 | 76 | 14 | 5 | 5 | 27 | 18 | 20 | 7 | 7 | 39 | 26 |
| 152 | 82 | 13 | 34 | 4 | 4 | 40 | 14 | 36 | 4 | 4 | 42 |
| 185 | 87 | 4 | 20 | 3 | 16 | 14 | 7 | 35 | 5 | 28 | 25 |
| 13 | 73 | 6 | 20 | 11 | 27 | 17 | 7 | 25 | 14 | 33 | 21 |

[a]To normalize the data to allow comparison between individuals, the percent lysis mediated by each individual antibody was divided by the total of the lysis mediated by the 5 anti-V region antibodies. This gives a percentage expressing the relative level of representation of each V region within the total portion of the repertoire covered by the anti-V region antibodies.

[b]Underlined values are those which fall outside the normal range established for controls (>3 standard deviations above or below control mean).

**Table 5.** Phenotypic T cell Receptor V Region Representation of CD8+ Lymphocytes From HIV-infected Subjects

| Subject | Percentage of CD8+ Lymphocytes Expressing Individual TCR V Region | | | | | Percentage of CD8+ Lymphocytes Expressing Individual TCR V Region Total[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vα2 |
| 232 | 20 | 5.7 | 2.4 | 5.6 | 2.3[b] | 3.7 | 29 | 4.1 | 28 | 12 | 19 |
| 132 | 19 | 4.4 | 2.2 | 5.3 | 2.1 | 4.8 | 23 | 12 | 28 | 11 | 26 |
| 139 | 15 | 3.5 | 2.0 | 4.8 | 1.9 | 2.3 | 24 | 14 | 33 | 13 | 16 |
| 181 | 18 | 4.9 | 2.9 | 5.8 | 1.8 | 2.9 | 27 | 16 | 32 | 10 | 16 |
| 152 | 20 | 5.3 | 3.8 | 4.6 | 3.4 | 2.5 | 27 | 19 | 23 | 17 | 13 |
| 140 | 21 | 5.4 | 3.9 | 6.9 | 2.6 | 2.4 | 25 | 18 | 33 | 12 | 11 |

[a]To normalize the data to allow comparison between individuals, the percentage of CD8+ cells reacting with each individual antibody was divided by the total percentage of CD8+ cells reacting with the 5 anti-V region antibodies. This gives a percentage expressing the relative level of representation of each V region within the total portion of the repertoire covered by the anti-V region antibodies.

[b]Underlined values are those which fall outside the range observed for CD8+ lymphocytes from 24 HIV-seronegative controls (Janson et al., 1991). The cytolysis data for comparison is presented in tables 2-4: subject 232, >400 CD4s/μl; subjects 132 and 139 200-400 CD4s/μl; and subjects 181, 152, and 140 <200 CD4s/μl.

Fig. 1

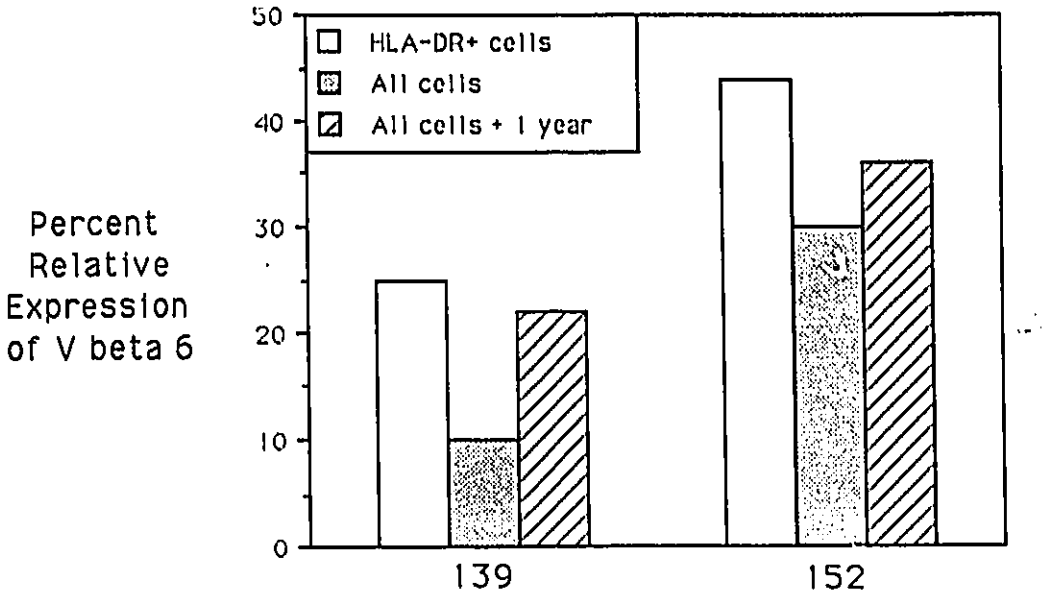


**Fig. 1.** Differences in relative level of Vβ6 expression in *in vivo* activated versus all cytotoxic cells and change in relative Vβ6 expression over time. Functional expression of Vβ6 was compared in cytotoxic T cells derived from *in vivo* HLA-DR$^+$ cells and in all cytotoxic cells for 2 HIV-infected individuals. All cytotoxic cells were retested approximately one year later. Results for Vβ6 alone are shown because the functional expression of this subset either began or went outside the normal range of representation.

Fig. 2

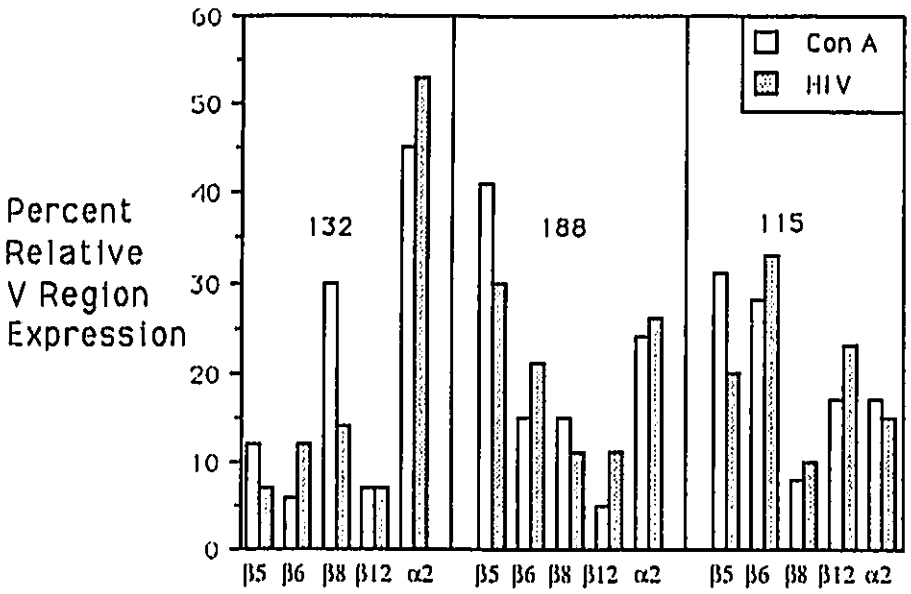


**Fig. 2.** Comparison of relative levels of V region use by cytotoxic T cells stimulated with Con A or HIV. Lymphocytes from 3 separate HIV-infected individuals, 132 (200-400 CD4s/μl), 188 (<200 CD4s/μl), and 115 (>400 CD4s/μl), were stimulated with either HIV or Con A before analysis of V region expression by redirected lysis assay.

# Discussion

This thesis has examined three aspects of CTL activity in the pathogenesis of AIDS: protection from disease progression; autoimmunity against $CD4^+$ T lymphocytes; and the cytotoxic T cell repertoire. Results from these studies will be interpreted in light of our hypothesis that the pathogenesis of AIDS is caused by negative selection of $CD4^+$ T cells in the periphery of HIV-infected individuals by autoimmune CTL activity.

## A. Protection From Disease Progression.

### i. Circulating anti-HIV CTL activity.

Several aspects of the findings on circulating anti-HIV CTL activity are consistent with a role for anti-HIV CTL in protection from disease progression. The presence of detectable circulating anti-HIV CTL activity was associated with $CD8^+$ lymphocyte counts above 900/µl peripheral blood. This demonstrates that at least a portion of the $CD8^+$ lymphocytosis characteristic of HIV infection is caused by activation and expansion of $CD8^+$ anti-HIV CTL. Circulating anti-HIV CTL were also found exclusively in asymptomatic HIV-infected individuals, although only in about 40% of those individuals tested. In addition, there was a lower proportion of HIV-infected individuals who had detectable plasma HIV antigens (1/6 versus 7/14) with circulating anti-HIV CTL activity than without circulating anti-HIV CTL. This is consistent with a lower level of HIV replication in those individuals with circulating anti-HIV CTL activity. Although all HIV-infected individuals with circulating anti-HIV CTL activity had more than 900 $CD8^+$ lymphocytes/µl peripheral blood, these individuals showed a wide variation in $CD4^+$ lymphocyte counts. $CD4^+$ lymphocyte counts in this group ranged from approximately

normal (880/μl) to extremely low (90/μl) and there was no significant difference in the mean $CD4^+$ lymphocyte number in groups with or without circulating anti-HIV CTL activity. In contrast, there was a highly significant difference in mean $CD8^+$ lymphocyte number in the two groups. These results can be interpreted to suggest that absolute $CD8^+$ lymphocyte count is a critical determinant of the presence of detectable circulating anti-HIV CTL and that the presence of this activity is practically independent of $CD4^+$ lymphocyte counts. Since circulating anti-HIV CTL activity is associated with a decreased incidence of HIV antigenemia but not with higher $CD4^+$ lymphocyte counts, this suggests a dissociation between viral replication and the loss of $CD4^+$ lymphocytes, although production of p24 does not necessarily correlate with production of infectious HIV. Even though circulating anti-HIV CTL may inhibit HIV replication *in vivo*, they do not appear to protect aginst $CD4^+$ lymphocyte loss.

**ii. Inducible anti-HIV CTL activity .**

In contrast to the clear association of circulating anti-HIV CTL activity in asymptomatic HIV-infected individuals with $CD8^+$ lymphocyte count above 900/μl, inducible anti-HIV CTL activity was found in all HIV-infected individuals tested, regardless of clinical stage or laboratory parameter measurements (ie. $CD4^+$ and $CD8^+$ lymphocyte counts. This suggests that inducible anti-HIV CTL precursors persist through all stages of disease. Even in individuals with AIDS, the anti-HIV CTL precursors showed evidence of activation in the form of HLA-DR expression. There was however, a heirarchy of potential defects seen in terms of the requirements for anti-HIV CTL activation, in that exogenous IL-2 and a longer period of *in vitro* stimulation of CTL precursors was required for the detection of anti-HIV CTL activity in some individuals. This could be interpreted as a functional decrease in CTL precursor activity with or without a decrease in actual numbers of anti-HIV CTL precursors. This interpretation is

consistent with the reported finding of suppression of anti-HIV CTL activity in symptomatic HIV-infected individuals (20, 61, 62). Since considerable anti-HIV CTL activity was inducible *in vitro* in all HIV-infected individuals, it is difficult to relate changes in anti-HIV CTL precursor frequency to disease progression. However, the accumulation of successively more stringent requirements for the activation of anti-HIV CTL suggests that some aspects of disease progression may have a negative effect upon the efficacy of anti-HIV CTL activity.

In terms of the general hypothesis, the most significant finding in these studies was that circulating anti-HIV CTL activity may inhibit the *in vivo* replication of HIV without conferring protection against $CD4^+$ lymphocyte loss. This potential dissociation of HIV replication from $CD4^+$ lymphocyte depletion implies that indirect effects of HIV-infection are at least partially responsible for the loss of $CD4^+$ lymphocytes.

For this reason, we decided to test HIV-infected individuals for the presence of autoimmune CTL capable of lysing uninfected $CD4^+$ lymphocytes. CTL with this specificity were reported in the circulation of HIV-infected individuals and were not found in HIV-infected chimpanzees (41), which don't develop AIDS following HIV-infection. The reported specificity of these CTL and their absence in HIV-infected chimpanzees suggested that these CTL might play a role in $CD4^+$ lymphocyte depletion in human infection with HIV.

## B. Autoimmune CTL Against Uninfected $CD4^+$ Lymphocytes.

### i. Presence in HIV-infected individuals.

HIV-infected individuals were shown to possess Con A inducible CTL activity capable of lysing autologous $CD4^+$ lymphocytes, heterologous $CD4^+$ lymphocytes from HIV-infected individuals and surprisingly, heterologous $CD4^+$ lymphocytes from non-

HIV-infected individuals. This CTL activity was not detectable in uninfected controls. Levels of this activity against autologous cells varied, but was often extremely high against $CD4^+$ lymphocytes from uninfected individuals. These results suggested that the CTL may eliminate their own autologous targets *in vivo*.

**ii. Phenotype of autoimmune CTL.**

Lysis of $CD4^+$ lymphocytes by autoimmune CTL was shown to be inhibitable by anti-CD3 antibodies and anti-CD8 antibodies when the antibodies were included in the assay. Also, when effector cells alone were treated with anti-CD3 antibodies, killing of $CD4^+$ cells was markedly inhibited. This argues against a veto phenomenon since inhibition of $CD4^+$ cell death by treating the $CD8^+$ effectors with anti-CD3 demonstrates that the $CD8^+$ cells are delivering the "lethal hit" to the target cells in contrast to the veto phenomenon where autoimmune target cells recognize self MHC class I on $CD8^+$ lymphocytes and commit suicide (63). Inhibition with anti-CD3 and anti-CD8 identified the autoimmune CTL as $CD3^+CD8^+$ T lymphocytes, the classical phenotype of CTL. Inhibition with anti-CD3 and also with anti-$\alpha\beta$ TCR antibodies demonstrated the use of the antigen specific TCR in recognition of the $CD4^+$ targets and suggested a specific cellular immune response against $CD4^+$ T lymphocytes.

**iii. Specificity of the autoimmune CTL.**

In contrast to their classical phenotype and use of the TCR to recognize their targets, these CTL have unusual characteristics. The most obvious is their recognition of uninfected $CD4^+$ lymphocytes. Thus, these CTL are not virus-specific. In addition, these CTL show no evidence of HLA-restriction which is an almost universally accepted constraint of $\alpha\beta$ $TCR^+$ T cell recognition. Although at least some $\gamma\delta$ $TCR^+$ T cells are not

HLA-restricted, we showed that the autoimmune CTL were at least predominantly αβ TCR+ T lymphocytes. Despite these uncharacteristic features, the CTL display specificity for an antigen expressed on a subset of uninfected activated CD4+ lymphocytes and recognition of antigenic polymorphism. This was shown in the failure of the autoimmune CTL to lyse autologous or heterologous CD8+ lymphocytes and the ability of the CTL to lyse CD4+ T lymphocytes from particular donors preferentially, when tested against a panel of CD4+ lymphocytes from multiple HIV-infected or uninfected donors. This was not due to an inherent resistance of CD4+ lymphocytes from certain donors to lysis since CD4+ lymphocytes from every donor were lysed by CTL from at least one HIV-infected individual.

These cells can be activated *in vitro* by polyclonal stimulation with Con A or in the same way as anti-HIV CTL are specifically activated. The activity was not generated by stimulation with another virus (EBV) known to induce a strong CD8+ CTL response. Although stimulation with EBV in some cases induced cells which lysed uninfected CD4+ lymphocytes, lysis in these cases was not inhibitable with antibodies against CD3. Therefore, the activity induced by stimulation with EBV was different from the autoimmune CTL activity and did not represent a specific CTL response against uninfected CD4+ lymphocytes.

**iv. Role of Autoimmune CTL in CD4+ lymphocyte depletion.**

Several aspects of the autoimmune CTL activity suggest it might play a role in the pathogenesis of CD4+ lymphocyte depletion and AIDS. The most obvious is the presence of this activity in HIV-infected individuals, but not in non-HIV-infected individuals. These CTL were also absent in HIV-infected chimpanzees (41), which despite being susceptible to HIV-infection do not develop AIDS.

When tested at a single time point, not all HIV-infected individuals showed CTL activity against their own CD4+ lymphocytes. We followed the CD4+ lymphocyte counts of 14 individuals, seven of which lysed >15% of their own CD4+ lymphocytes and seven of which lysed <5% of their own CD4+ lymphocytes, for a six month period after tesing for autoimmune CTL activity. As a group, the individuals with >15% lysis of their own CD4+ lymphocytes lost more CD4+ lymphocytes over the six month follow-up period($p<.01$). There was no significant change in the mean CD4 count of the group without autoimmune CTL activity, while 7/7 of those with autoimmune CTL activity lost >15% of their CD4+ lymphocytes (a mean loss of 36%) over the six month follow-up. This is consistent with a role for autoimmune CTL activity in the *in vivo* depletion of CD4+ lymphocytes. The only subject without detectable autoimmune CTL activity who lost CD4+ lymphocytes over the follow-up period was receiving chemotherapy for an AIDS-associated malignancy .

In a number of HIV-infected individuals who initially had autoimmune CTL activity, we found that this activity became undetectable against autologous CD4+ lymphocytes coincident with an *in vivo* decrease in CD4+ lymphocyte count. However, when tested against CD4+ lymphocytes from heterologous non-HIV-infected individuals, CTL activity against CD4+ lymphocytes was maintained. Since the CD4+ lymphocyte count and repertoire of the uninfected individual are unlikely to change drastically, the same CTL target antigens should be expressed over time. In contrast, the HIV-infected individual has lost a significant number of CD4+ lymphocytes over the same time period and the same CTL target antigens appear to no longer be expressed. One interpretation of this finding is that CD4+ lymphocytes expressing relevant target antigens recognized by the autoimmune anti-HIV CTL activity are eliminated *in vivo* in HIV-infected individuals and that this elimination is responsible for the loss of CD4+ lymphocytes. This finding is consistent with a direct role for the autoimmune CTL in the *in vivo* depletion of CD4+ lymphocytes.

Selective elimination of certain CD4+ lymphocytes over time in HIV-infection by autoimmune CTL suggests that changes in the CD4+ lymphocyte population will be mirrored by changes in the CD8+ cytotoxic T cell population. Different subsets of CD4+ lymphocytes will stimulate different sets of autoimmune CTL. If autoimmune CTL cause selective elimination of CD4+ lymphocytes, there should be selective expansion of CD8+ CTL with different specificities as CD4+ lymphocyte depletion progresses. Since specificity is related to V gene usage, we decided to look at the effects of HIV infection on the CTL repertoire by functionally measuring V gene usage in relation to progressive CD4+ lymphocyte depletion.

**C. Cytotoxic T Cell Repertoire in HIV Infection.**

We examined effects on the cytotoxic T cell repertoire in HIV-infected individuals by comparing the functional level of expression of five TCR V regions in non-HIV-infected individuals and in HIV-infected individuals stratified into three groups based on degree of CD4+ lymphocyte depletion. The most obvious difference between the two groups was the higher level of T cell-mediated cytotoxicity expressed by CD8+ lymphocytes from HIV-infected individuals than by CD8+ lymphocytes fron uninfected controls. This suggests that *in vivo*, in HIV-infected persons, there is either positive selection of a subset of CD8+ T lymphocytes with greater cytotoxic potential or that CD8+ lymphocytes in general are activated for cytotoxicity by HIV infection. In either case, the expansion of CD8+ lymphocytes seen in HIV infection includes the expansion of functionally activated cytotoxic CD8+ T cells.

Analysis of the TCR repertoire of the cytotoxic T cells activated in HIV infection showed that there was selective activation based on the V region expressed by the CD8+ lymphocytes. This is apparent from the skewing of the repertoire reflected in over or

underutilization of particular V regions by CTL from HIV-infected individuals relative to controls. The number of examples of detectable skewing increased from 1 in the group of HIV-infected individuals with >400 $CD4^+$ lymphocytes to 6 in the group of HIV-infected individuals with 200-400 $CD4^+$ lymphocytes to 9 in the group of HIV-infected individuals with <200 $CD4^+$ lymphocytes. The number of affected individuals in the same three groups increased from 1/6 to 4/6 to 5/6. While this skewing could occur in response to HIV or other pathogens favored by the immunodeficiency associated with HIV-infection, it might also reflect the autoimmune CTL response against autologous $CD4^+$ lymphocytes. The association between the degree of skewing and $CD4^+$ lymphocyte count is consistent with either possibility.

$CD8^+$ lymphocyte counts are often highest in HIV-infected individuals with high $CD4^+$ lymphocyte counts. These individuals make a strong anti-HIV CTL response, but tend not to exhibit skewing of the CTL repertoire. This suggests that the skewing is not a direct result of the immune response to HIV. Individuals with high $CD4^+$ lymphocyte counts also rarely possess detectable autoimmune CTL activity.

*In vitro* stimulation with HIV uses PHA activated autologous lymphoblasts as stimulators and in some HIV-infected individuals, this also activates autoimmune CTL against autologous $CD4^+$ lymphocytes. This is probably due to the presence of activated $CD4^+$ lymphocytes in the stimulating population. In the individuals tested, this form of stimulation induced broad representation of TCR V regions on activated CTL, very similar to that induced by polyclonal stimulation with Con A. Con A also effectively stimulated autoimmune CTL in HIV-infected individuals. Since Con A stimulation selectively expands cells already activated *in vivo* (64), this suggests that the autoimmune CTL are present at a high frequency in the peripheral blood and together with the broad response induced by autologous lymphoblasts as stimulators, suggests that the accumulation of autoimmune CTL may be a key factor in the skewing of the CTL repertoire. The association between the skewing of the CTL repertoire and the loss of

$CD4^+$ lymphocytes may reflect the direct role of autoimmune CTL in $CD4^+$ lymphocyte depletion for which evidence was presented in the previous section.

Some investigators have suggested that the skewing of the repertoire seen in HIV infection results from the action of superantigens encoded either by HIV or other pathogens (46-48). Superantigens stimulate T cells which express a particular Vβ gene segment and this can result in expansion and subsequent deletion of T cells expressing that gene product (52). Superantigenic stimulation non-selectively effects all T cells expressing the relevant Vβ gene. When we compared the phenotypic level of expression of TCR V regions measured by flow cytometry to the functional level of expression measured by redirected cytolysis, we saw little if any relationship between phenotypic and functional levels. The fact that functional skewing occurred for certain V regions without concurrent phenotypic skewing can be interpreted as evidence that not all T cells with a certain V region have been expanded, but only a specific subset of CTL with that V region have been expanded. Therefore, the skewing we have detected functionally does not suggest the action of a superantigen. It is also unlikely that opportunistic pathogens, which thrive due to a reduced host response, could at the same time influence the T cell repertoire to the extent seen.

**D. The Pathogenesis of AIDS.**

Throughout the clinically asymptomatic phase of HIV infection, the immune system shows signs of massive activation. While direct detection of HIV requires sensitive analytical techniques, the indirect effects of HIV infection are obvious. The hypothesis behind our work is that this immune system activation, rather than the infection of smal numbers of $CD4^+$ lymphocytes is the real pathogenic effect of HIV infection.

In any immune response there is clonal selection of reactive cells. In parallel, there may be selection of variants of the immunogen. The longer and more pronounced the immune response, the more stringent selection becomes. With HIV infection, a massive immune response is mounted against the $CD4^+$ lymphocytes themselves. A way in which HIV may accomplish this is by stimulating $CD4^+$ cells independently of HLA-restricted antigen presentation. This would impair the selection of dominant $CD4^+$ HLA-class II restricted anti-HIV helper cells and drive the activation of polyclonal populations of $CD4^+$ cells. If an idiotypic network of T cells exists, this will subsequently result in the activation of T cells anti-idiotypic to $CD4^+$ cells. At least three ways in which HIV could activate T cells independently of HLA-presentation have been proposed. The first way is via an HIV-encoded superantigen, which could selectively activate T cells with particular V regions (48). Continued mutation of the putative HIV-encoded superantigen could result in activation of T cells with other V regions (48). Evidence for this type of HLA-non-restricted presentation has been presented in two recent reports (46, 47). A second way by which HIV may activate T cells without HLA presentation has also been reported by two groups (65-67). Binding of HIV gp120 to the CD4 molecule of T cells, followed by crosslinking of gp120 by anti-gp120 antibodies has been shown to anergize T cells while inducing the expression of a number of activation antigens and activating regulatory protein kinases (65, 66). Thirdly HIV may activate T cells through molecular mimicry of HLA antigens themselves (68) or mimicry of T cell receptors with a high level of connectivity to the receptors on $CD4^+$ lymphocytes (69). Sequence homology and immunological cross reactivity have been demonstrated between HIV and HLA antigens (27-29) and between HIV and TCR V region genes (70). The third possibility offers the most complete explanation for the immunological effects of HIV infection. In this scenario, the immune response against HIV might cross-react with the receptors activated by HIV in a non-HLA-restricted fashion. Two opposed responses would result which would select against activated $CD4^+$ lymphocytes and select for particular clones

of T cells reacting against the CD4+ lymphocytes. The longer the activation occurred, the more stringent the selection process would become. Eventually, the result would be the elimination of nearly all CD4+ T cells and selection of dominant clones of CD8+ T cells anti-idiotypic to the remaining antigenic CD4+ T cells. This would explain the progressive skewing we observed in the CD8+ CTL repertoire in association with CD4 depletion and the V region selective diminuition of the CD4+ T cell repertoire (47).

The results of this thesis provide three pieces of evidence supporting the hypothesis that immune activation is involved in the pathogenesis of AIDS. The anti-HIV CTL response which appears to limit viral replication does not seem to protect against CD4+ lymphocyte depletion. This suggests there is no direct association between viral replication and CD4+ lymphocyte depletion. Autoimmune CTL, which specifically lyse activated uninfected CD4+ lymphocytes were demonstrated in HIV infection. The characteristics of these cells reflect a specific immune response against CD4+ lymphocytes and natural history studies suggest this response plays a role in the *in vivo* depletion of CD4+ lymphocytes. The characteristics of the autoimmune CTL are consistent with recognition of polymorphic determinants of TCR idiotypes specific to CD4+ lymphocytes. However, even if the autoimmune CTL recognize activation molecules distinct from the TCR, the same sort of repertoire skewing would occur as long as the activation of the CD4+ lymphocytes was V region dependent. Finally, the observed skewing of the CTL repertoire in parallel with CD4+ depletion is consistent with the interdependence of these two processes.

Over the ten years since the recognition of AIDS tremendous amounts of research and resources have been directed at better understanding this syndrome. Despite the knowledge obtained about HIV, AIDS remains an untreatable infection. At a scientific level, the impact of billions of dollars into antiviral research has been negligible. At a medical level, progress has offered HIV-infected individuals an extra year or so of higher quality life. Ongoing clinical trials with new antivirals are designed to establish whether

these drugs have any effect at all, not whether they offer something more than palliative effects. The only real breakthroughs have come in the prophylaxis and treatment of secondary infections associated with AIDS.

The most important contemporary question facing everyone involved in AIDS research is whether this failure can be overcome by the current approach or whether new areas of research must be championed. The results obtained in this thesis suggest that autoimmunity and specifically autoimmune CTL against $CD4^+$ lymphocytes may be promising targets for future therapeutic strategies in HIV infection.

Bibliography

1. Zinkernagel, R. M., E. Haenseler, T. Leist, A. Cerny, H. Hengartner and A. Althage. (1986). T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class-I restricted virus-specific cytotoxic T cells as a physiological correlate of the $^{51}$Cr-release assay. *J. Exp. Med.* 164:1075.

2. Zinkernagel, R. M., C. J. Pfau, H. Hengartner and A. Althage. 1985. A model for MHC-disease associations: sensitivity to murine lymphocytic choriomeningitis maps to class I MHC genes and correlates wiith LCMV-specific cytotoxic T cell-activity. *Nature* 316:814.

3. Fahey, J. L., H. Prince, M. Weaver, J. Groopman, B. Visscher, K. Schwartz and R. Detels. 1984. Quantitiative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immune deficiency syndrome from other immune subset disorders. *Am. J. Med.* 76:95.

4. Lash, R. H., R. R. Tubbs and R. H. Calabrese. 1988. Paracortical immunoregulatory subpopulations in lymph nodes from homosexual men with persistent generalized lymphadenopathy. *Hum. Pathol.* 19:419.

5. Cheesman, S. H. 1988. Infectious mononucleosis. *Seminars in Hematology* 3:261.

6. Giorgi, J. V. and R. Detels. 1989. T-cell subset alterations in HIV-infected homosexual men: NIAID multicenter AIDS cohort study. *Clin. Immunol. Immunopathol.* 52:10.

7. Walker, B. D., S. Chakrabarti, B. Moss, T. J. Paradis, T. Flynn, A. G. Durno, R. S. Blumberg, J. C. Kaplan, M. S. Hirsch and R. T. Schooley. 1987. HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* 328:345.

8. Plata, F., B. Autran, L. Pedroza-Martins, S. Wain-Hobson, M. Raphael, C. Mayaud, M. Denis, J. M. Guillon and P. Debre. 1987. AIDS-virus specific cytotoxic T lymphocytes in lung disorders. *Nature* 328:348.

9. Nixon, D. F. and A. J. McMichael. 1991. Cytotoxic T-cell recognition of HIV proteins and peptides. *AIDS* 5:1049.

10. Walker, C. M., D. J. Moody, D. P. Stites and J. A. Levy. 1986. CD8+ lymphocytes can control HIV infection *in vitro* by suppressing virus replication. *Science* 234:1563.

11. Kannagi, M., T. Masuda, T. Hattori, T. Kanoh, K. Nasu, N. Yamamoto and S. Harada. 1990. Interference with human immunodeficiency virus (HIV) replication by $CD8^+$ T cells in peripheral blood leukocytes of asymptomatic HIV carriers *in vitro*. *J. Virol.* 64:3399.

12. Mackewicz, C. E., H. W. Ortega and J. A.. Levy. 1991. $CD8^+$ cell anti-HIV activity correlates with the clinical state of the infected individual. *J. Clin. Invest.* 87:1462.

13. Walker, C. M., A. L. Erickson, F. Hseuh and J. A. Levy. 1991. Inhibition of HIV replication in acutely infected CD4+ cells does not involve cytotoxicity. *J. Virol.* 65:5921.

14. Tsubota, H., C. I. Lord, D. I. Watkins, C. Morimoto and N. L.Letvin. 1989. A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes. *J. Exp. Med.* 169:1421.

15. Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. M. Bangham, C. R. Rizza and A. J.

McMichael. 1991. Human immunodeficiency virus genetic variants that can escape cytotoxic T cell recognition. *Nature* 354:453.

16. Meyerhans, A., G. Dadaglio, J. Vartanian, P. Langlade-Demoyen, R. Frank, B. Asjo, F. Plata and S. Wain-Hobson. 1991. *In vivo* persistence of a HIV-1-encoded HLA-B27-restricted cytotoxic T lymphocyte epitope despite specific *in vitro* reactivity. *Eur. J. Immunol.* 21:2637.

17. Pantaleo, G., A. DeMaria, S. Koenig, L. Butini, B. Moss, M. Baseler, H. C. Lane and A. S. Fauci. 1990. CD8+ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of human immunodeficiency-virus-specific cytotoxicity. *Proc. Natl. Acad. Sci. USA* 87:4818.

18. Vanham, G., L. Kestens, P. Gigase, R. Colebunders, M. Vandenbruane, L. Brijs and J. L. Ceuppens. 1990. Evidence for circulating activated cytotoxic T cells in HIV-infected subjects before the onset of opportunistic infections. *Clin. exp. Immunol.* 82:3.

19. Hoffenbach, A., P. Langlade-Demoyen, G. Dadaglio, E. Vilmer, F. Michel, C. Mayaud, B. Autran and F. Plata. 1989. Unusually high frequencies of HIV-specific cytotoxic T lymphocytes in humans. *J. Immunol.* 142:452.

20. Joiy, P., J. Guillon, C. Mayaud, F. Plata, I. Theodorou, M. Denis, P. Debre, and B. Autran. 1989. Cell-mediated suppression of HIV-specific cytotoxic T lymphocytes. *J. Immunol.* 143:2193.

21. Riviere, Y., F. Tanneau-Salvadori, A. Regnault, O. Lopez, P. Sansonetti, B. Guy, M. Kieny, J. Fournel and L. Montagnier. 1989. Human immunodeficiency virus-specific cytotoxic responses of seropositive individuals: distinct types of effector cells mediate killing of targets expressing gag and env proteins. *J. Virol.* 63:2270.

22. Redfield, R. R., D. L. Birk, N. Ketter, E. Tramont, V. Polonis, C. Davis, J. F. Brundage, G. Smith, S. Johnson, A. Fowler, T. Wierzba, A. Shafferman, F. Volvovitz, C. Oster and D. Burke. 1991. A phase I evaluation of the safety and immunogenicity of immunization with recombinant gp160 in patients with early human immunodeficiency virus infection. *N. Engl. J. Med.* 321:1621.

23. Tsoukas, C., J. Sampalis, P. Gold, M. O'Shaughnessey, M. Wainberg, G. Smith and F. Volvovitz. 1992. Specific active immunotherapy in asymptomatic HIV disease using recombinant gp160. Oral Abstract TuB 0560, VIII[th] International Conference on AIDS, Amsterdam, the Netherlands.

24. Levine, A. Killed HIV vaccines. 1990. Address, 5[th] Annual Conference on Clinical Immunology Specialized meeting on "The Status of Immune-based Therapies in HIV Infection and AIDS" Chicago, Ilinois.

25. Koenig, S., R. van Kuyk, G. Jones, B. Torbett, D. Mosier, Y. A. Brewah, S. Leath, V. J. Davey, J. Yanelli, S. Rosenberg, A. S. Fauci and H. C. Lane. 1992. Adoptive transfer of a nef-specific CTL clone into hu-PBL-scid mice and an HIV-infected patient. Oral Abstract ThA 1573, VIII[th] International Conference on AIDS, Amsterdam, the Netherlands.

26. Ho, D. D. 1992. Address, Wellcome Foundation Commercial Satellite Meeting, VIII[th] International Conference on AIDS, Amsterdam, the Netherlands.

27. Young, J. A. T. 1988. HIV and HLA similarity. *Nature* 333:215.

28. Golding, H., G. M. Shearer, K. Hillman, P. Lucas, J. Manischewitz, R. A. Zajac, M. Clerici, R. E. Gress, R. N. Boswell and B. Golding. 1989. Common epitope on human immunodeficiency virus (HIV) 1-GP41 and HLA Class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV-1-infected individuals. *J. Clin. Invest.* 83:1430.

29. Vega, M. A., R. Guigo and T. F. Smith. 1990. Autoimmune response in AIDS. *Nature* 345:26.

30. Gotch, F. M., D. F. Nixon, N. Alp, A. J. McMichael and L. K. Borysiewicz. 1990. High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals. *Int. Immunol.* 2:707.

31. Walker, B.D., C. Flexner, T. J. Paradis, T. C. Fuller, M. S. Hirsch, R. T. Schooley and B. Moss. 1988. HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. *Science* 240:64.

32. Zinkernagel, R. M. 1988. Virus-triggered AIDS: a T-cell-mediated immunopathology. *Immunol. Today.* 9:370.

33. O'Hara, C. J. 1989. Lymphoid system. pp. 136-183 In "Pathology and pathophysiology of AIDS and HIV-related disease" Harawi, S. J. and C. J. O'Hara eds. London, Chapman and Hall Medical

34. Rosenberg, Y. J., P. M. Zack, E. C. Leon, G. A. Eddy and M. G. Lewis. 1992. A role for activated CD45RAlo CD8+ cells in the decline of CD4+ cells in LN of SIV infected macaques and in AIDS onset. Abstract 82, International Conference on Cellular and Molecular Aspects of Self-reactivity and Autoimmune Disorders, Taormina, Italy. *Autoimmunity* 12S:46.

35. Calabrese, L. H., M. Estes, B. Yen-Lieberman, M. K. Proffitt, R. Tubbs, A. Fishleder and K. H. Levin. 1989. Systemic vasculitis in association with human immunodeficiency virus infection. *Arthritis Rheum.* 32:569.

36. Autran, B., C. M. Mayaud, M. Raphael, F. Plata, M. Denis, A. Bourguin, J. Guillon, P. Debre and G. Akoun. 1988. Evidence for a cytotoxic T-lymphocyte alveolitis in human immunodeficiency virus-infected patients. *AIDS* 2:179.

37. Itescu, S., L. J. Brancato, J. Buxbaum, P. K. Gregersen, C. C. Rizk,

T. S. Croxson, G. E. Solomon and R. Winchester. 1990. A diffuse infiltrative CD8 lymphocytosis syndrome in human immunodeficiency virus (HIV) infection: a host immune response associated with HLA-DR5. *Ann. Intern. Med.* 112:3.

38. Sethi, K. K., H. Naher, and I. Strochmann. 1988. Phenotypic heterogeneity of cerebrospinal fluid-derived HIV-specific and HLA-restricted cytotoxic T-cell clones. *Nature* 335:178.

39. Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman and S. Abrignani. 1988. T cells can present antigens such as HIV gp120 targeted to their own surface molecules. *Nature* 334:530.

40. Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory and E. Reinherz. 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for $CD4^+$ cell depletion. *Cell* 54:561.

41. Zarling, J. M., J. A. Ledbetter, J. Sias, P. Fultz, J. Eichberg, G. Gjerset and P. Moran. 1990. HIV-infected humans, but not chimpanzees, have circulating cytotoxic T lymphocytes that lyse uninfected $CD4^+$ cells. *J. Immunol.* 144, 2992.

42. Anderson, R. E., S. C. Shiboski, R. Royce, N. P. Jewell, W. Lang and W. Winkelstein Jr. 1991. $CD8^+$ T lymphocytes and progression to AIDS in HIV-infected men: some observations. *AIDS* 5:213.

43. Woodland, D.L., M. P. Hap, K. J. Gollob and E. Palmer. 1991. An endogenous retrovirus mediating deletion of $\alpha\beta$ T cells. *Nature* 349:529.

44. Marrack, P., E. Kushnir and J. Kappler J. 1991. A maternally inherited superantigen encoded by a mammary tumour virus. *Nature* 349:524.

45. Dyson, P.J., A. M. Knight, S. Fairchild, E. Simpson and K. Tomonari, K. 1991. Genes encoding ligands for deletion of $V\beta11$ T cells cosegregate with mamary tumour virus genomes. *Nature* 349:531.

46. Imberti, L, A. Sottini, A. Bettinardi, M. Puotti and D. Primi. 1991. Selective depletion in HIV-infection of T cells that bear specific T cell receptor Vβ. sequences. *Science* 254:860.

47. Laurence, J., A. S. Hodtsev and D. N. Posnett. 1992. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. *Nature* 358:255.

48. Janeway, C. 1991. Mls: makes a little sense. *Nature* 349:459.

49. Hoffmann, G. W., T. A. Kion and M. D. Grant. 1991. An idiotypic network model of AIDS immunopathogenesis. *Proc. Natl. Acad. Sci. USA* 88:3060.

50. Grant, M. D. 1991. HIV and idiotypic T cell regulation. *Immunol. Today* 12:171.

51. Heath, W. R., M. E. Hurd, F. R. Carbone, and L. A. Sherman. (1989). Peptide-dependent recognition of H-2K$^b$ by alloreactive cytotoxic T lymphocytes. *Nature* 341:749.

52. White, H., A. Herman, A. M. Pullen, R. Kubo, J. W. Kappler and P. Marrack. (1989). The Vβ specific superantigen staphylococcal enterotoxin B; stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 56:27-35.

53. Meuer, S. C., J. C. Hogdon, R. E. Hussey, J. P. Protentis, S. F. Schlossman and E. L. Reinherz. (1983). Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988.

54. Cohly, H. H. P., D. R. Morrison and M. Z. Atassi. (1988). Conformation-dependent recognition of a protein by T-lymphocytes; apomyoglobin-specific T-cell clone recognizes conformational changes between apomyoglobin and myoglobin. *Immunol. Invest.* 17:337.

55. Schmidt, R. E., G. T. Bartley, S. S. Lee, J. F. Daley, H. D. Royer, H. Levine, E. L. Reinherz, S. F. Schlossman and J. Ritz. (1986) Expression of the NKa clonotype in a series of human natural killer clones with identical cytotoxic specificity. *J. Exp. Med.* 163:812.

56. Diamond, D. J., P. Szalay, D. Symer, P. Hao, H. S. Shin, R. Z. Dintzis, H. M. Dintzis, E. L. Reinherz and R. F. Siliciano. (1991). Major histocompatibility complex independent T cell receptor-antigen interaction: functional analysis using fluorescein derivatives. *J. Exp. Med.* 174:229.

57. Wilson, A., A. J. T. George, C. A. King and F. K. Stevenson. (1990). Recognition of a B cell lymphoma by anti-idiotypic T cells. *J. Immunol.* 145:3937.

58. Barnd, D. L., M. S. Lan, R. S. Metzgar and O. J. Finn. (1989). Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells. *Proc. Natl. Acad. Sci. (USA)* 86:7159.

59. Tada, T., W. Uracz and R. Abe. (1986). Are there unique I region controlled determinants on T cells? In "*Paradoxes in Immunology*" (G. W. Hoffmann, J. G. Levy and G. T. Nepom, Eds.), CRC Press, Boca Raton, Florida, pp. 253-265.

60. Man-Sun, S. and B. Benacerraf. (1988). Suppressor T cells, immunoglobulin and Igh restriction. *Immunol. Rev.* 101:133.

61. Autran, B., V. Leblond, B. Sadat-Sowti, E. Lefranc, P. Got, L. Sutton, J. Binet and P. Debre. (1991). A soluble factor released by $CD8^+CD57^+$ lymphocytes from bone marrow transplanted patients inhibits cell-mediated cytolysis. *Blood* 77:2237.

62. Sadat-Sowti, B., P. Debre, T. Idziorek, J. Guillon, F. Hadida, E. Okzenhendler, C. Katlama, C. Mayaud and B. Autran. (1991). A lectin-binding soluble factor released by $CD8^+CD57^+$ lymphocytes from AIDS patients inhibits T cell cytotoxicity. *Eur. J. Immunol.* 21:737.

63. Sambhara, S. R. and R. G. Miller. (1991). Programmed cell death of T cells signaled by the T cell receptor and tha $\alpha_3$ domain of Class I MHC. *Science* 252:1424.

64. Mor, F., A. W. Lohse, N. Karin and I. R. Cohen. (1990). Clinical modeling of T cell vaccination against autoimmune disease in rats: selection of antigen-specific T cells using a mitogen. *J. Clin. Invest.* 85:1594.

65. Fortin, J., J. Bernier, T. Gregory, S. Meloche and R. Sekaly. (1992). Anergy induced by gp120: biochemical aspects. *Can. J. Infect. Dis.* 3S:31A.

66. Bernier, J., J. Fortin, T. Gregory, S. Meloche and R. Sekaly. (1992). Anergy induced by gp120: cellular aspects. *Can. J. Infect. Dis.* 3S:31A.

67 Finkel, T. H., D. K. Kurahara, J. Bernier, R. Kurrie, N. Haigwood, R. Sekaly and N. K. Banda. (1991). Crosslinking CD4 by HIV gp120 primes T cells for activation-induced apoptosis. *J. Leuk. Biol.* S2:73.

68. Habeshaw, J. A. and Dalgleish, A. (1989). The relevance of HIV/envCD4 interactions to the pathogenesis of acquired immune deficiency syndrome. *J. Acq. Immun. Def. Synd.* 2:457.

69. Hoffmann, G. W. (1988). On I-J, a network centre pole and AIDS. In: *The Semiotics of cellular communication in the immune system.* Sercarz, E., Celada, F., Mitchison, N. A. and Tada, T. eds. Springer Verlag, New York.

70. Primi, D. 1992. Oral presentation, round table discussion on mechanisms of CD4 depletion. V111$^{th}$ International AIDS Conference, Amsterdam, The Netherlands.