Pathogenic and Protective Correlates of T Cell Proliferation in AIDS

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Abstract

To investigate the association of antigen specific CD4 T cell activation with HIV disease progression and AIDS-related central nervous system damage, T cell proliferation responses to HIV, CMV, and HSV were evaluated in infected individuals. CD4 T cell loss and neurocognitive impairment were assessed at 6-mo intervals.

Individuals with known times of seroconversion who responded to more HIV peptides were at greater risk of progressing to < 200 CD4 T cells ($P = 0.04$) and dying ($P = 0.03$) than those with responses to fewer peptides. A positive correlation (0.52) was seen between the breadth of the HIV proliferation response and HIV plasma RNA levels. Higher proliferation responses to CMV and HSV were also associated with more rapid CD4 loss ($P = 0.05$).

HLA phenotyped individuals ($n = 150$) with two HLA-DR alleles associated with response to more HIV peptides and CMV (DR-2,5,w6,10) were less likely to develop neurocognitive ($P = 0.002$) and neurologic impairment ($P = 0.04$), but were not protected from CD4 loss and death. Thus, the ability to generate a greater T cell proliferation response to HIV and opportunistic herpes viruses may lead to resistance to central nervous system damage, but also risk of more rapid HIV disease progression. (*J. Clin. Invest.* 1996. 98:731–740.) Key words: HIV • CD4 T cell proliferation • human • T cell activation • immune response

Introduction

In HIV infection, the positive and negative consequences of in vivo CD4 T cell activation are still largely undefined. Activation of CD4 T cells is thought to be necessary for generation and maintenance of CD8 T cells and specific neutralizing antibody. In retroviral models, CD4 T cells have been shown to be an important component of protection (1, 2). Specifically in AIDS, evidence suggests that, overall, the immune response has some capacity to restrict HIV replication after acute infection and HIV specific CD8 effector cells and antibody (CD4-dependent) can be detected (3–5). However, HIV is known to replicate in activated CD4 T cells and in macrophages which present antigen to T cells (6, 7) raising concern that CD4 T cell activation could also result in amplification of virus, spread, and further destruction of CD4 T cells.

It has been difficult to determine whether the amount of HIV produced after immune activation in vivo is sufficient to have pathogenic consequences for the host and if the CD4 activation and virus production is, in fact, necessarily in direct conflict with positive functions, specifically, immune control of opportunistic pathogens and HIV itself. The amount of HIV generated in vivo and extent of CD4 mortality resulting from antigen specific activation is likely partially balanced by the ability of other limbs of the immune response (such as CD8 T cells and antibody) to restrict replication and dissemination of HIV. Although the complexity of the immune response and various potential interactions with virus are daunting, practical information is necessary to evaluate the risks and benefits of therapeutic HIV immunization, immunization against opportunistic pathogens as well as both immune enhancing and immunosuppressive therapies.

One recent approach to investigating whether CD4 activation leads to increased HIV replication has been the measurement of plasma HIV RNA levels after influenza immunization of HIV-infected individuals. Transient but significant rises in HIV RNA have been detected (8) and the in vivo rises were most evident in persons who showed high T cell proliferative responses in vitro (9). Whether the in vivo HIV RNA rise is a minor event or if the response to a single pathogen significantly increases the amount of HIV and the number of HIV infected cells, resulting in increased HIV load after subsequent immune activation, is unknown.

To examine long-term correlates of T cell activation, we evaluated CD4 T cell proliferative responses to HIV, cytomegalovirus (CMV),[1] and herpes simplex virus (HSV) in HIV-infected individuals enrolled in a longitudinal study. Participants were HLA typed and were monitored clinically and neuropsychologically, as part of the UCSD-associated HIV Neurobehavioral Research Center. The data collected allowed us to examine the relationship between high and low proliferative responses to HIV and CMV, subsequent disease manifestations of HIV and CMV, and HIV disease progression, as measured by loss of CD4 T cells and death.

Methods

Study population. Study participants come from the HIV Neurobehavioral Research Center (HNRC) (10). The HNRC participant pool consists of ~ 500 men and women who are presently enrolled in a longitudinal investigation of neurobehavioral changes associated with HIV infection. Associated immunologic, virologic, and molecular

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1. *Abbreviations used in this paper:* CMV, cytomegalovirus; CNS, central nervous system; DTH, delayed type hypersensitivity; HSV, herpes simplex virus.

pathogenesis projects contribute to a comprehensive database on the study population. Confidentiality is maintained and the central database is monitored, updated, and checked at regular intervals. Healthy seropositive individuals were initially selected from the community and the US Naval Medical Center, San Diego, some of whom had known dates of seroconversion, within a 1-yr window. For the immune response substudy, a (randomly selected) preliminary group of seropositive individuals was enrolled in 1986-1987 and the majority in 1990 and 1991. After voluntary enrollment, individuals were HLA typed, and proliferative responses were tested at subsequent visits when possible. Visits and neurobehavioral assessments were at 6-mo to 1-yr intervals, depending on stage of disease. 60 of the HLA typed individuals from the second stage of enrollment had known dates of seroconversion and are referred to in the text as "dated seroconvertors." Currently, half of the 150 HLA-typed study participants fall into the "AIDS" category according to the 1993 CDC system (C1-C3, A3, B3), or are deceased. Cell samples for CD4 count and plasma samples later used for RNA levels were done at the same blood collection visit as lymphocytes for immune assay.

T cell proliferation assays. The conditions for T cell proliferation to CMV-infected cells and HIV peptides have been published (11). For isolation of PBMC, blood was collected in lithium heparin coated tubes, underlayered with Histopaque (Sigma, St. Louis, MO), and spun at 2,000 rpm for 25 min at room temperature. The mononuclear band was collected and washed twice in sterile saline. Plasma samples were also frozen and stored. Mononuclear cells were resuspended to 0.5–1 million/ml (lymphocytes from 40 cm^3 of blood were resuspended in 37 ml) in Iscove's media (Gibco Laboratories, Grand Island, NY) with Pen/Strep and 10% human AB serum (seronegative for HIV, HSV, and CMV). Cells were plated at 0.2 ml/well in each well in prepared 96-well tissue culture plates and cultured at 37°C with 7% CO_2. After 6 d of culture, each well was pulsed with 1 μCi of tritiated thymidine overnight and plates were then harvested and filters counted in a scintillation counter. Multivalent antigens (CMV, HSV) and PHA were assayed in triplicate and counts were averaged. Peptide antigens were tested in triplicate at two different concentrations (six wells), and in most cases, reactivity was seen at both concentrations. Since background counts can vary considerably among HIV-infected individuals, proliferation data are presented as stimulation index. A stimulation index of 2.0 or greater (with $< 25\%$ SEM) was considered a positive response, as is standard for this type of assay. In previous studies, we and others have found a close correlation with presence of antibody to HSV, CMV, and HIV, and a stimulation index of 2 or higher (11).

Assay plates are prepared with mitogens (PHA at 1 μg/ml, final concentration), and viral antigens [heat-inactivated (1 h at 56°C) virus infected cells for CMV (AD-169 grown in human fibroblasts) and HSV (KOS grown in Vero cells)] with final concentration of 3×10^5 pfu (before inactivation). More than 95% of HIV-infected individuals are also infected with CMV and HSV and would be expected to have memory T cells able to respond to these viral lysates (subject to level of immunodeficiency). HIV peptides represent conserved epitopes of gp-41, gp-120, gag, and pol proteins of HIV and were assayed at two different concentrations as described previously (11). HIV peptides are used since lysates of HIV-infected cells do not activate T cells, possibly due to the presence of suppressive epitopes on gp-120. The HIV peptides represent a variety of structures, lengths (12–26 amino acids), and motifs predicted by several different algorithms for T and B cell recognition (12–14).

HLA typing. HLA phenotypes were determined by the UCSD Immunogenetics Laboratory. Standard tissue typing techniques and Terasaki plates (One Lambda Inc., Irvine, CA) were used for all HLA-A and B loci and most DR typing. Some DR types were evaluated by DNA genotyping.

Statistics and data analysis. Databases were maintained by the HNRC and investigators. Analyses on shared databases were initiated by the investigators and verified by statistics staff. The proportions in empirical survival functions (Figs. 1–3) were compared using Fisher's exact test. Although using Kaplan Meier statistics was an option, we chose to present simple plots based on actual numbers observed, which was more conservative. The results of Kaplan Meier calculations were more decisive in each case than those presented for the step graphs (for example, the *P* value for the data in Fig. 2 is 0.02 by Kaplan Meier for survival graph, but 0.03 by Fisher's exact test for proportions in the step graph). Fisher's exact test was also used for comparison of rates of neurologic and neuropsychologic impairment in two groups (using "ever impaired" criteria according to the complete history of that participant, see description of tests below).

Analysis of continuous variables, such as immune responses and CD4 levels in different patient groups, was done using Student's *t* test. While the *t* test is ideally suited to verifiably normal data, there is a moderate to large sample justification for the *t* test, based on the approximate normality of the sample mean (even when the parent distribution is not normal), which in this case renders the quoted *P* values approximately correct, and in fact more conservative than if a Z test had been used. An application of the Wilcoxon rank test corroborated the findings.

For examination of longitudinal data (without seroconversion dates), we presented the slope of the CD4 T cell decline as a measure of progression. CD4 measurements were made at 6-mo intervals for the duration of seropositivity after entry to study (no extrapolation was made to baseline level), calculations were based on the square root of the CD4 count to minimize small fluctuations, a minimum of four points was available for each individual, and dampening features were added to minimize contributions of single points. Only cumulative slopes (using all points to date) were used in tables (and one value per individual) so that problems with short-term effects were minimal.

Detection of HIV genomic RNA in plasma. Aliquots of plasma were separated from whole blood and stored at −80°C for subsequent batch testing. Quantitative RT-PCR for HIV genomic RNA was performed by National Genetics Institute (Culver City, CA) using a modification of a reported method (15). Briefly, cDNA was obtained using random priming and reverse transcriptase with MuLV-RT for 1 h at optimum temperature. PCR amplification of each sample was performed in four different cycle sets. The amplified products were resolved by Southern blot analysis using ultraviolet cross-linking, digoxigenin-labeled probes, and alkaline phosphatase–conjugated antidigoxigenin antibody. Quantitation was obtained by measuring sample band images for area and mean density, and comparing these with a standard curve run in parallel. The assay has a range of sensitivity of detection of 100 to 2 million copies of HIV-1 RNA per ml.

Neurocognitive and neurologic exams. All subjects underwent comprehensive neuropsychological testing and a structured neurological examination, as described fully elsewhere (16). In brief, the neuropsychological examination consisted of tests that assessed eight ability areas: attention/speeded information processing, verbal/language skills, abstraction ability/executive functions, memory (learning of new information, recall of previously learned information), complex perceptual/motor abilities, simple motor skills, simple sensory functioning. The results of this battery of tests, which took 4–6 h to complete, were then blindly rated by an expert (Robert K. Heaton, Ph.D.), using a methodology that was previously shown to be reliable (17), as well as a valid indicator of brain disease (18). Beyond rating the various ability areas, the neuropsychologist then assigned an overall, or global, rating of impairment using a 9-point scale ranging from 1 for above average neurocognitive functioning to 9 for severely impaired functioning (10). For the purposes of this report a score of 5 or greater was used to classify a subject as at least mildly neurocognitively impaired.

The structured neurological examination was performed by a research neurologist or trained research nurse, and covered the following areas: mental status, cranial nerves, sensory abnormality, motor strength, coordination, gait, abnormal movements, tendon reflexes, and release signs (primitive reflexes) (16). As for the neuropsychological evaluation, the neurological results were summarized as a glo-

bal rating of neurological abnormality, on a 4-point scale, ranging from 0 for no abnormality to 3 for multiple severe abnormalities. For this report a score of 1 or more was considered as evidence of at least mild neurological abnormality (10, 19). In this report, we reasoned that the important question was whether the study participants in each group experienced neurologic or neurocognitive impairment at any time of disease, not simply at the dates when immune response and CD4 levels were measured. Therefore, data in tables derived from the highest ever impaired (most impaired) score for each individual from their complete history. To reduce the likelihood that a few very high scores might bias results, the scores were not analyzed as continuous variables, but rather compared by the number of individuals with scores above the impairment thresholds described above (percentage ever impaired).

Results

Proliferation response to HIV and HIV disease progression. T cell response to HIV, CMV, and HSV was measured by PBMC proliferation, using uptake of tritiated thymidine as an indicator of DNA synthesis. This type of assay, using large peptides or proteins, is primarily an indication of CD4 T cell activation. The HIV peptides varied widely with respect to structure and charge, no peptides were recognized in > 30% of assays, patterns of recognition changed over time, and responses to individual peptides have shown no specific association with protection. Given this heterogeneity for both response patterns and structure, we made the assumption that the number of peptides recognized by each individual in the vitro assay was representative of the number of HIV epitopes recognized (the breadth of the response) in vivo.

We initially approached the data by asking: What were the long-term correlates of low proliferation response to HIV? From careful study of the data, it appeared that a substantial proportion of individuals had few or no responses to HIV peptides at all times tested while others frequently responded to many peptides. We reasoned that none, one, or two peptide responses (stimulation index < 3) out of 31 peptides, on a consistent basis, represented a minimal response, and responses to more peptides represented a significant response. This also separated two groups fairly equally, which is preferable from a statistical perspective. If an individual responded to at least three peptides on one of two occasions, the individual was placed in the high response group since this indicated potential for higher proliferation response and each individual is represented in one group or the other. There was no correlation between the number of assays for a given individual and likelihood of a response to three or more peptides, so that there was no bias toward individuals who had been tested more frequently to be in the high responder group.

To best compare HIV disease progression, we focused on the subgroup of HIV seropositive individuals in our population for whom the date of seroconversion was known (within 1 yr) and for whom T cell proliferation was tested when they had CD4 T cell counts > 300. Although 300 CD4 T cells may appear low as a criteria for response competency, our cross-sectional data for the whole population suggest that likelihood of response to protein antigens in HIV infected individuals is relatively constant above 300 CD4 T cells, and falls below 200 CD4 T cells.

The data are shown in Fig. 1. Those individuals with responses to more HIV peptides when CD4 counts were > 300 had a greater chance of progressing to < 200 CD4 T cells than those who consistently responded to fewer or no HIV peptides. Mean time seropositive at time of assay was shorter for the high responders because low responders had CD4 counts > 300 for a longer interval and were more likely to be sampled at later times after seroconversion. To examine survival of low and high responders and to determine whether the data in Fig. 1 were critically dependent on the CD4 sampling interval chosen, survival was plotted for high and low responders where criteria for low response was less than three peptides, as before, but no CD4 range for immune assay was specified. Resulting data are shown in Fig. 2. Consistent with disease progression results, those HIV-infected individuals with responses to more HIV peptides were at greater risk of progression to death. Mean CD4 levels at time of assay were comparable.

Response to CMV and HSV and disease progression. The relation of HIV disease progression and immune response to antigens of CMV and HSV, two herpes viruses, was also examined. More than 95% of all HIV-infected individuals are also latently infected with CMV and HSV and both viruses reactivate occasionally even in healthy individuals, although most CMV reactivations remain subclinical. However, as immunoimpairment worsens in AIDS, endogenous CMV reactivations are often clinically severe. Retinitis caused by CMV develops in ~ 30% of all HIV-infected individuals and can

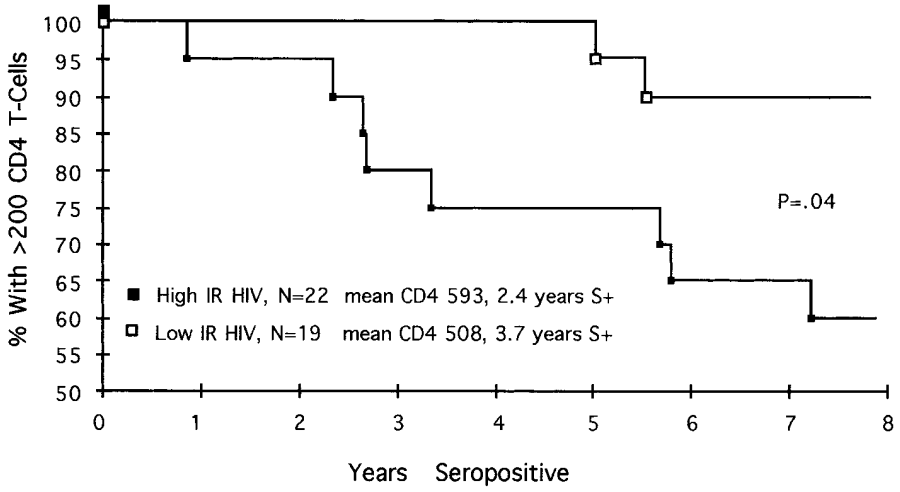

Figure 1. CD4 T cell loss in dated seroconvertors with high and low proliferation responses to HIV. High response was defined as a stimulation index of two or more to three or more HIV peptides (out of 31) and a low response as stimulation index of two or more to less than three peptides, at all times.

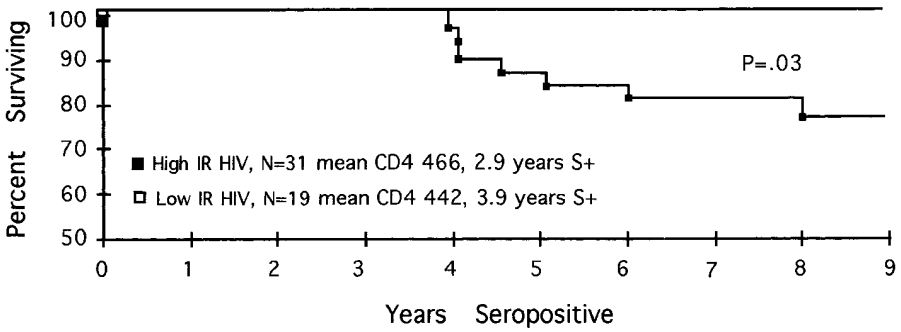

Figure 2. Survival of dated seroconvertors with high and low proliferation responses to HIV. High response was defined as a stimulation index of two or more to three or more HIV peptides (out of 31) and a low response as stimulation index of two or more to less than three peptides.

progress to blindness and encephalitis (20, 21). We reported recently that high response to CMV antigens correlated with decreased likelihood of developing CMV-related disease. Specifically, individuals with higher T cell proliferative responses to CMV (infected cell lysate preparations) were less likely to develop CMV retinitis when their CD4 levels dropped, compared with those with low proliferative responses (22).

Given the data shown in Fig. 1 for HIV, we asked how T cell response, tested when CD4 levels were $> 300/mm^3$ (as for Fig. 1 above), related to subsequent HIV disease progression by examining CD4 T cell loss in high and low CMV and HSV responders with known dates of seroconversion. Because HSV and CMV antigen preparations are multivalent, high response to CMV or HSV was defined as stimulation index of ≥ 4.9. Individuals were separated into two groups: those with high responses to both CMV and HSV, or a low response to both. Like the previous analysis, individuals for which data from two time points existed, one showing a response > 4.9 and a second < 4.9, were included once, in the high responder category.

As seen with response to HIV peptides, a high proliferation response to both endogenous pathogens, at CD4 $> 300/mm^3$ was associated with more rapid loss of CD4 T cells to levels $< 200/mm^3$ (Fig. 3). In contrast, low responders to both herpes viruses were at less risk of progressing to < 200 CD4 T cells. Mean CD4 T cells at time of testing were comparable, and again, time from seroconversion to testing was slightly longer for low responders. Survival was 73% in the high response group and 83% in the low response group. Mitogen (PHA) responses, an indication of nonspecific potential activation, were not different in the two groups (data not shown).

Immune responses in progressors and nonprogressors. To determine whether the association of proliferation response with disease progression would be observed if the data were examined from the opposite perspective, progressors and nonprogressors were defined in this same group of dated seroconvertors and proliferation responses and clinical data were then compared. To achieve a median split for this small group (yielding equal numbers of progressors and nonprogressors) a threshold of 300 CD4 T cells was used. Progressors were defined as those HIV-infected individuals who advanced to < 300 CD4 T cells within 5 yr of seroconversion. Those individuals who did not fall below 300 CD4 T cells, but had been followed for at least 5 yr, were defined as nonprogressors. Average immune responses and clinical data are shown for the two groups in Table I. Immune response data, cell numbers, and beta 2 correspond to the date of immune assay. Neurobehavioral data were ever impaired from complete history to date and slope values are cumulative and include all measurements to date.

Consistent with the previous analysis, mean CMV and HSV proliferation responses were higher in the progressors. CD4 counts were significantly lower, even at earlier stages of disease. PHA mitogen responses were not different in progressors and nonprogressors. Mean responses to HIV peptides were higher in the progressors, although the difference was not statistically significant ($P = 0.08$). This was not inconsistent with data in Fig. 1, since individual longitudinal analysis of high and low responders to HIV (data not shown) suggested that while some progressed rapidly, others did not progress at all in 5 yr. In contrast, those with early high responses to CMV and HSV had average, and significantly, lower T cell numbers at

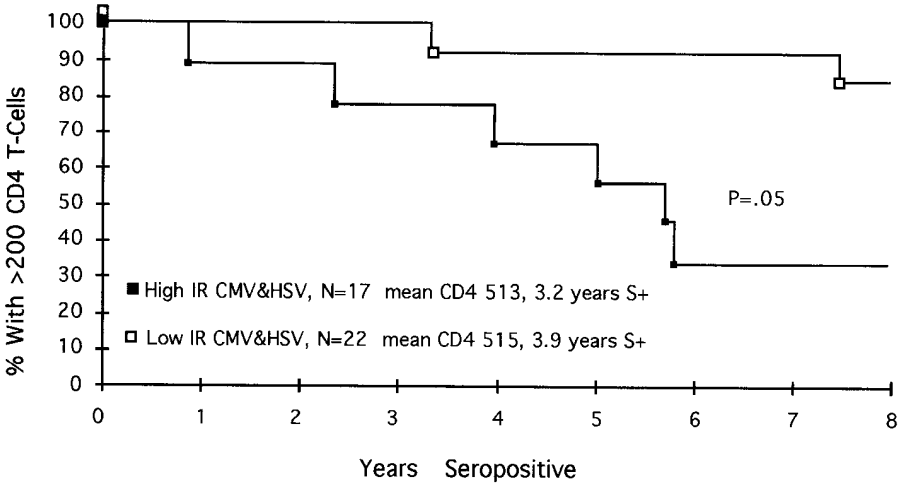

Figure 3. CD4 T cell loss in dated seroconvertors with high and low proliferation responses to CMV and HSV. High response was defined as a stimulation index ≥ 4.9 to both CMV and HSV virus lysates and low response as a stimulation index of < 4.9 for both CMV and HSV, when individuals had > 300 CD4 T cells.

Table I. Comparison of Early Mean Immune Responses of Dated Seroconvertors Who Progressed or Did Not Progress within 5 Years

	Mean T cell proliferation*					Clinical status						
Group	*N*	PHA SI	CMV SI	HSV SI	HIV #pep	CD4 cells	CD8 cells	Slope CD4	N.Psych %Imp‡	Neurol %Imp‡	Beta2§	Years Sero+
Progressors‖	13	21.3	8.5	6.8	3.2	429	1151	−3.2	29%	0%	2.5	3.06
Nonprogressors	14	23.8	4.5	4.1	2.0	578	1282	−0.78	33%	33%	2.1	4.17
P value		0.40	0.05	0.05	0.08	0.002	0.19	< 0.001	0.32	0.03	0.13	

*T cell proliferation responses expressed as stimulation index for (SI) for PHA, CMV, and HSV. HIV response is expressed as average number of peptides (of 31) which elicited a stimulation index of 2 or more. ‡ Percent impaired as determined by a Neuropsychologic or Neurologic test battery. §Serum Beta 2 levels (mg/liter). ‖Progressors were defined as those who fell below 300 CD4 T cells within 5 yrs of seroconversion.

early stages of disease. Low mean CD4 counts in the early years after seroconversion may indicate that high responders to endogenous pathogens have irreversible CD4 loss during the acute stage of HIV infection (4, 23, 24). The results shown in Table I suggest that trends between higher CD4 proliferation and disease progression can be detected from examining the data from different perspectives and using different criteria for progression (< 200 in Figs. 1 and 3, < 300 in Table I, and death in Fig. 2).

Plasma HIV RNA quantitation. Plasma HIV RNA levels were determined in a subset of study participants (16) with known dates of seroconversion and existing T cell proliferation data. Plasma samples had been collected at the same time or 6 mo from the date of immune assay and stored at −70°C. All but three of the individuals had been undergoing treatment with antiretroviral agents (AZT or ddI, range of doses 400–800 mg/d) for > 8 mo at the time samples were collected and there was no correlation between antiviral treatment and RNA levels. HIV RNA was detected in all samples, ranging from 600 to 400,000 copies/ml.

HIV RNA levels were correlated with clinical data and proliferation responses from the 16 individuals. Consistent with data from other studies, a negative correlation was seen between CD4 slope and HIV RNA load (Table II). A significant positive correlation was seen between the number of HIV peptides (out of 31) which elicited a proliferation response in that person and HIV plasma RNA levels. No significant correlations for HIV plasma RNA and responses to HSV and CMV were detected. The low R value is negative, but the lack of significance precludes interpretation. We also compared HIV RNA levels for progressors or nonprogressors (as defined for Table I, falling below 300 CD4 T cells within 5 yr). Because they progressed within such a short time, few samples were available for rapid progressors, reducing our sample size considerably, but progressors did have higher HIV RNA levels (mean of 3 was 134,000) than nonprogressors (mean of 6 was 7,000) despite similar mean CD4 levels (391 for progressors and 418 for nonprogressors).

Immunogenetic control of T cell proliferation response. To further investigate potential interaction between the T cell proliferative response and HIV disease, we used observations from our analysis of HLA-DR phenotypes and HIV peptide responses (a more extensive discussion of those data will be presented in a future report). From examining which individuals with each specific DR type (heterozygous and homozygous combined) responded to specific peptides and viewing summary population data, it appeared that DR types 1,3,4,7 were associated with responses to fewer HIV peptides on average in each assay (< 3) and were also "nonresponders" for between 5 and 11 of the 31 peptides assayed. In contrast, individuals heterozygous or homozygous for 2,5,w6, and 10 appeared to be able to respond to all 31 peptides (on a population basis) on one occasion or another and had a mean response of > 3.5 peptides per assay. Assuming that the 31 peptides are representative of HIV sequences overall, then individuals with low or "selective" HLA alleles DR 1,3,4,7 would be predicted to be responding to fewer HIV epitopes in vivo than those with high, "nonselective" alleles DR 2,5,w6, and 10. If higher CD4 immune response has both protective and pathogenic components, it would be predicted that those with the nonselective, high response HLA types might have overall higher immune responses and could be protected from pathogen-mediated CNS damage, but not HIV disease progression. To examine the high and low extreme population groups, we compared individuals who had combinations of DR1,3,4,7 with those who had combinations of 2,5,w6, and 10.

Few of the individuals (only 3 out of 62) with two selective or nonselective response alleles overlapped with the group with known dates of seroconversion. Although this was unfortunate since seroconversion dates provide a fixed basis for comparison, it was useful to examine a different group of HIV-infected individuals to ensure that trends identified in Figs. 1–3 and Table I were not artifacts of the one group sampled. Therefore, we did a cross-sectional analysis for genetically separated groups of 29 and 33 individuals, with approximately two assays per individual. The results are shown in Table III. Group mean immune responses and clinical values were calculated as for Table I. CD4 slopes were cumulative, to date, and neurologic and neuropsychologic values were ever impaired

Table II. Correlation of Levels of HIV RNA Plasma with CD4 Slope and Proliferation Responses

Host variables	Correlation	*P* value
Slope CD4* vs. HIV RNA‡	−0.69	0.003
HIV proliferation (#pep)§ vs. HIV RNA	0.52	0.04
CMV proliferation (SI) vs. HIV RNA	−0.22	0.42
PHA proliferation (SI) vs. HIV RNA	−0.18	0.5

*Square root of slope of CD4 counts determined from a minimum of four time points. ‡ HIV plasma RNA levels, copies/ml. § T cell proliferation responses. Stimulation index (*SI*) was used for PHA, CMV, and HSV. HIV response is number of peptides (of 31) which elicited a stimulation index of 2 or more.

from the complete history available for that participant and only one value per individual was represented in calculations.

As predicted by our initial analysis of each allele, individuals with DR types consisting of combinations of 1,3,4, or 7, responded to significantly fewer HIV peptides than those with combinations of 2,5,w6, and 10. When immune responses to other antigens were compared in these two groups, it was observed that proliferative responses to CMV and HSV were also higher for those with 2,5,w6, and 10, although the difference was only significant for CMV. This suggested that the observed selective (DR-1,3,4,7) versus nonselective (DR-2,5,w6,10) pattern was not limited to HIV epitopes. Mean CD4 counts and PHA (T cell mitogen) responses were comparable in the two groups. The HIV proliferation response profiles of the two genetically separated in Table III were also examined graphically (not shown), with number of peptides which elicited a response as a function of CD4 number at time of assay. It appeared that the low responders (as a group) had mean highest levels of response to HIV peptides at fairly low CD4 counts (peak was at 200 CD4 T cells). In contrast, the high responders had highest responses to HIV at $\sim$ 400 CD4 T cells and their peak is twice as high as the low responder peak.

Neuropsychologic and neurologic impairment in genetically separated high and low proliferation response groups. Since the study participants underwent extensive neurologic and neuropsychologic testing and the most common causes of CNS impairment in AIDS are HIV and CMV (HIV is present in 48% and CMV present in 29% of AIDS brains at autopsy), we examined the percentage of patients impaired in the high and low proliferation allele response groups (Table III). The high HIV/CMV immune responder DR types had significantly lower frequency of both neurologic ($P = 0.04$) and neurocognitive impairment ($P = 0.002$, proportions ever impaired, by Fisher's exact test), suggesting that higher immune responses to CMV and HIV could be associated with protection from tissue damage by these agents. This is consistent with our previous findings for CMV showing that those with high responses to CMV were less susceptible to CMV retinitis (22).

HIV disease progression in genetically defined high and low proliferation response groups. With respect to HIV disease progression, comparison of CD4 slopes of persons with two high or low proliferation response DR alleles indicated that high DR proliferation response individuals had a slightly steeper slope (−2.4 vs. −2.0, Table III). Also, more deaths occurred in the high response group. Although increases in slope and death rate were not statistically significant, the opposite trend for both measures would be expected if proliferation response were solely beneficial. Overall, the immune response and progression trends noted for the genetically divided groups in Table III are similar to the progressor, nonprogressor groups shown in Table I. It would have been of interest to also examine the dated seroconvertor group according to high and low response DR types, but as mentioned above, only three of the dated seroconvertor group had two high or low alleles (most had a mix) so that such an analysis was not possible. Minimally, it can be concluded from these two genetically separated groups that a high proliferative immune response to HIV is associated with a higher response to other antigens such as CMV and with protection from CNS impairment, but not protection from CD4 loss and death.

Discussion

At the initiation of this study, our hypothesis was that a higher proliferation response to HIV and opportunistic pathogens would be indicative of protection against both HIV disease progression and opportunistic infections. The findings do indeed indicate that high proliferation to HIV and the latent pathogen CMV correlate with reduced CNS impairment which is often caused by these agents. However, results also suggest that a proportion of HIV infected individuals with higher levels of immune recognition of HIV, CMV, and HSV antigens have a greater chance of rapid HIV disease progression than individuals with low proliferation responses.

Given the complex pathogenesis of HIV, this is not altogether surprising. The most direct explanation for this apparent paradox would relate to the enhanced ability of HIV to replicate within activated CD4 T cells and macrophages such that CD4 T cell activation resulted in increased production of virus. Although CD4 cell activation and expansion is thought to be a prerequisite for effective immune response, it would also provide a cellular environment conducive to HIV spread. Because HIV antigens are present throughout infection and herpes viruses commonly reactivate, high proliferation responses detected in vitro may well reflect high in vivo responses, which would provide more host cells permissive for HIV replication and hence more virus and cell destruction. This interpretation of the results is also consistent with the observed lack of correlation between HIV disease progression and mitogen responses since, unlike HIV, CMV, and HSV, PHA is not representative of an endogenous antigen.

Prospective data collection with known times of seroconversion for the first cohort was an important aspect of this study since it allowed analysis of the consequences of previously detected high and low immune responses. Cross-sec-

Table III. Immune and Clinical Profiles of HIV-infected individuals with Low (1,3,4,7) and High (2,5,w6,10) DR Response Alleles

	Mean T Cell Proliferation*					Clinical status					
Host HLA-DR	*N*	PHA SI	CMV SI	HSV SI	HIV #pep.	CD4 cells	CD8 cells	Slope CD4	N. Psych. % Imp. ‡	Neurol .%Imp. §	Deaths
1,3,4,7 ‖	29	19.5	4.2	4.5	2.7	380	941	−2.00	41%	36%	6
2,5,w6,10	33	16.9	8.3	7	4.2	386	962	−2.40	16%	18%	11
P value		0.65	0.02	0.2	0.01	0.91	0.82	0.21	0.0002	0.04	0.1

*T cell proliferation responses expressed as stimulation index (SI) for PHA, CMV, and HSV. HIV response is expressed as average number of peptides (of 31) which elicited a stimulation index of 2 or more. ‡ Percent impaired as determined by a Neurologic test battery. §Percent impaired as determined by a Neuropsychologic test battery. ‖ Individuals with any combination of two of the indicated alleles.

tional analysis of immune response in HIV disease generally indicates that healthier individuals have higher detectable immune responses to most antigens. This translates to a general positive correlation between CD4 level and immune response (25–29). Our prospective data show similar trends when analyzed in this type of cross-sectional manner. For instance, positive responses to CMV antigen are seen in 72% of those HIV-infected individuals with $>$ 200 CD4 T cells per mm^3, 52% in those with 100–200, and 27% in those with $<$ 100 CD4 T cells. Overall, the correlation coefficient between immune response and CD4 level is 0.35. Thus, the applied approach to data analysis is critical to the prognostic influences of early immune responses and subsequent HIV disease progression. Also, the difference between the cross-sectional and prospective analysis indicates that prognosis correlated with CD4 activation is independent of the subsequent loss of CD4 responsiveness resulting from advanced immunosuppression.

Correlates for T cell proliferation shown here differ from those reported for delayed type hypersensitivity (DTH) responses to mumps and tetanus toxoid where response declined with HIV disease progression and absence of DTH response also predicted progression (30). While CD4 T cells are involved in both responses, functionally, T cell proliferation and DTH responses can diverge.

Although study subgroups were small, inspection of patterns of CD4 loss suggested that high proliferation response to HIV has a slightly different relationship to HIV disease progression than does high response to opportunistic pathogens, CMV and HSV. Individuals demonstrating high proliferation responses to both CMV and HSV during early stages of disease had lower mean CD4 levels at 1–3 yr after seroconversion, implying early rapid loss of CD4 T cells or failure to recover from initial T cell loss during acute HIV infection.

In contrast, the study subgroup with high response to HIV (but not necessarily to CMV and HSV) did not have a lower mean level of CD4 T cells in the few years after seroconversion. Nor did a wider response to HIV peptides predict rapid progression in all individuals with a high HIV response. Rather, a subset of the high responders progressed rapidly: 23% died within 7 yr, compared with none in the low responder subgroup. It would be of considerable interest to determine the factors which can influence such radically different courses in HIV disease progression within a group of individuals that demonstrate good immune responses. Levels of other forms of immunity, such as CD8 cytotoxicity, CD8 mediated suppression of HIV, and neutralizing antibody, could certainly be major effector mechanisms that offset the effects of CD4 cell activation by restricting HIV spread. Early high antibody responses to HIV (especially combined with low levels of nonspecific immune activation) have been shown to predict prolonged asymptomatic intervals before diagnosis of AIDS (23). In depth analysis of several types of immune responses will be required to address this issue.

While there has been little evidence published previously to indicate that specific T cell proliferation responses might have long-term pathogenic consequences, a number of studies have clearly suggested that nonspecific immune activation, represented by factors such as beta 2 microglobulin and neopterin, correlates with more rapid HIV disease progression (10, 23, 31, 32). Also, secondary diseases at the time of seroconversion (24) and CMV infection later in disease (33) are predictors of rapid HIV disease progression. However, it is difficult to interpret these coinfection data since it is necessary to consider the effects of combined immunosuppression caused by multiple pathogens (33) and transcriptional interactions (34–36), as well as HIV produced as a result of CD4 activation in response to each agent.

As expected, there was a strong negative correlation of plasma levels of HIV RNA with CD4 cell slope and a weak negative correlation with absolute CD4 cell count. However, a positive correlation was found between plasma HIV RNA levels and T cell proliferative response to HIV. The latter correlation is consistent with our findings that: (*a*) proliferative response to HIV in infected individuals is associated with more rapid disease progression; and (*b*) relation of early proliferative response to disease progression is independent of increased HIV production due to advanced immunosuppression. Whether greater initial levels of HIV induce a high proliferative response or whether more active CD4 proliferation causes the higher HIV RNA levels cannot be determined from this study. However, the association of specific HLA-DR alleles with higher immune responsiveness indicates a clear contribution of a host characteristic (the capacity to generate a broad immune response to HIV) to manifestations of HIV disease. It would not be expected that this particular genetic characteristic would completely predict the pattern of HIV progression since the replicative ability and host cell preference of specific HIV strains and initial virus inoculum, as well as efficacy of other immune responses, also likely contribute to disease course. No significant correlations were detected for CMV or HSV with HIV plasma RNA levels in this small sample. The finding that individuals with higher levels of HIV RNA progress more rapidly is consistent with a number of recent reports on this topic (24, 37, 38).

The longitudinal assessment of neurocognitive and neurologic impairment in this study population allowed us to follow central nervous system (CNS) impairment as a discrete form of HIV disease. From our study database, and consistent with others' findings, HIV and CMV are the most common CNS pathogens in AIDS brains at autopsy and the HNRC group has described mild to severe forms of neurocognitive and neurologic impairment in HIV disease which correlate with HIV, and possibly CMV infection, and are not attributable to other opportunistic agents (10, 39). HIV can be found in the cerebrospinal fluid at relatively early times of infection (40, 41), but severe impairment usually does not occur until advanced stages, suggesting that an intact immune system has a role in restricting HIV either within the brain parenchyma or the periphery. CMV encephalitis also develops at advanced stages of immunodeficiency. We have shown previously that low T cell proliferation response to CMV indicates predisposition to CMV retinitis (42) which usually precedes CMV encephalitis (21) and the results presented here indicate that higher proliferation responses to both HIV and CMV are associated with less risk of neurologic and neurocognitive impairment, clearly protective correlates of the CD4 proliferation response. HIV- and CMV-mediated impairment were not analyzed separately since differential diagnosis cannot always be made in life and both pathogens are present in many cases at autopsy. While pathogens other than CMV and HIV also cause CNS damage in AIDS, correlations shown in this study were likely significant for HIV and CMV either because of relatively greater prevalence of these two viruses or because immunity to other pathogens (such as Toxoplasma) follows similar patterns.

HLA-DR types were used in this study as host-specific, disease stage–independent criteria for examining the correlates of high and low T cell proliferation responses. The results of our studies on HLA associations with proliferation to HIV peptides suggested that individuals with certain DR types, specifically 2,5,w6, and 10, generally have the capacity to respond to a wider range of HIV epitopes, and tend to respond to more peptides, than individuals with DR 1,3,4, and 7. This grouping partially correlates with the DR 52/53 specificities, suggesting some evolutionary basis (43). With respect to individual alleles, DRw6 (currently split to 13,14) was associated with highest responses to CMV, HIV, and HSV, and DR 7 was associated with the lowest. We have reported previously that individuals with HLA-DR7, a low response allele, are significantly more at risk for development of CMV retinitis (22).

Some trends we identified for HLA-DR are supported by results from other HIV studies. Our "low proliferation response" allele (DR-7) was associated both with multiple opportunistic infections in one report and slower disease progression in another (44, 45). These two observations reflect the dual nature of our findings with respect to low proliferation being related to susceptibility to opportunistic infection and slow HIV disease progression. Our hypothesis would be that tissue infection and damage by HIV or opportunistic agents (both within and external to the CNS) is more likely to develop in infected individuals who experience slow disease progression with prolonged survival at moderate to severe levels of immunodeficiency. Individuals with more rapid disease progression would have less opportunity to develop opportunistic infections and CNS damage. However, it should be noted that while a correlation was seen between low immune response and neurobehavioral impairment, when CNS infection does occur late in disease, indicators of nonspecific immune reactivity (such as beta 2 microglobulin) are typically elevated and the mechanism of CNS damage probably involves cytokines, again illustrating the complexity of prospective and cross-sectional results in HIV disease (10, 46).

Concerning the HLA-DR alleles most consistently associated with high proliferation responses in our system, Kaslow et al. recently reported that, in two large study populations, DR13,14 (splits of DRw6) are the DR types significantly associated with increased risk of progression to AIDS (47). Complete DR haplotypes were examined and the findings suggested that it was important to consider adjacent loci (such as TAP) alleles in linkage disequilibrium with the markers being examined, but unfortunately we have no data on this locus. Other HLA associations for HIV disease progression have been reported (48). A common Celtic haplotype (A1B8DR3) has been found to predispose to rapid progression, but there were too few individuals with these alleles (and we did not determine haplotypes) to examine this group in our data set.

Results from other infectious disease systems suggest that some trends discussed here for DR associations (specifically high and low extremes of DRw6 and DR7) likely extend beyond HIV and are unlikely to be epitope specific. We are well aware that there is little precedent for the idea that different DR alleles generate a relatively lower CD4 response or are more selective than others, but such trends would not have been apparent in studies of fine specificity involving one or two epitopes and class II alleles. The influence of the immune response proteins on an immune response or protection from disease (usually in the context of HLA class I) was thought to be regulated by the ability of a specific HLA type to present a specific or protective epitope of that pathogen or protein (49–52). However, given data (ours and others) which suggest trends extending among pathogens which do not share specific determinants, it seems logical to propose a DR allele–specific influence on response level, existing in addition to epitope-specific control. For instance, DR-13 (split of w6) is associated with resistance to severe malaria (53) as well as high proliferation responses to HIV peptides, HSV antigens, and CMV antigens reported here. Individuals with DRw6 were able to respond to all 31 of our HIV peptides and it is the only common DR type for which no motif has been published, possibly due to the "promiscuity" of binding.

In contrast, we found DR7 to be associated with low T cell responses for HIV, HSV, and CMV and others have reported associations for DR-7 and susceptibility to severe malaria (53), hepatitis (54), herpes simplex type II reactivations (55), CMV infections after transplantation (56, 57), and EBV (58), but virtually no autoimmune diseases (43), suggesting a general relationship between this allele, low T cell response, and susceptibility to microbial agents. DR7 appears to bind certain peptides well (59, 60) so mechanisms other than poor affinity are likely involved (possibly selectivity, combined with low expression or poor interaction with T cell receptor). Assigning high/low response or selectivity characteristics to DR alleles is an attractive idea in the context of HIV pathogenesis because it would also explain how individual pathogen-specific responses could collectively be associated with long-term higher HIV RNA load, since any single high response detected (such as to influenza immunization) in an individual with high immune recognition would be representative of the general CD4 proliferative response level for that individual.

Overall, our evidence from two different study populations, viewed from several perspectives, indicates that there are both long-term protective and pathogenic aspects associated with CD4 T cell activation occurring in HIV-infected individuals. Further investigation will be required to evaluate the relative hazards of selective immunoenhancing therapies to combat HIV and opportunistic agents, to examine the abilities of other immune responses in restricting HIV replication, and to identify characteristics of different HIV strains which interact with the host to influence disease course.

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