Immune predispositions for cytomegalovirus retinitis in AIDS. The HNRC Group.

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Research Article

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Immune Predispositions for Cytomegalovirus Retinitis in AIDS

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Abstract

CMV retinitis develops in ~ 28–35% of all AIDS patients at later stages of disease, often leading to blindness. To determine whether the subset of AIDS patients who developed CMV retinitis (CMV-R) were immunologically predisposed, T cell proliferation responses to CMV were examined prospectively in an HIV infected, HLA typed, longitudinal study population. Individuals who developed CMV-R had significantly lower T cell proliferation responses to CMV, both early and late in disease, compared to CD4 matched controls who have not developed CMV-R.

Since HLA proteins influence T-cell recognition, phenotypes of 21 CMV-R patients were examined to determine whether certain HLA alleles were associated with low immune response and predisposed AIDS patients to CMV-R. HLA DR7 and B44 were at increased (nearly twice the expected) frequency in those with CMV-R. The combined association of either B44, 51 or DR7 with CMV-R was highly significant (P = .008, relative risk of CMV-R = 15) with correction for multiple comparisons. Low immune responses were twice as frequent in those with (61%) compared to those without (30%) predisposing alleles. Thus, AIDS patients with immunogenetically related hyporesponsiveness to CMV antigens may be at increased risk of retinitis. (*J. Clin. Invest.* 1995. 95:1741–1746.) Key words: HIV • human • CMV • HLA • T cell proliferation

Introduction

While up to 70% of healthy adults are infected with Human Cytomegalovirus (CMV),[1] overt disease is rare. However, for individuals whose immune system has been compromised by disease, transplant maintenance therapy, or chemotherapy, CMV is a major cause of serious infection (1, 2). Since transmission patterns for CMV resemble those for HIV, most individuals who acquire HIV are already infected with CMV and often develop symptomatic reactivation disease as immunocompromise progresses. Retinitis is the most common manifestation of CMV, developing in up to one third of all HIV infected individuals by the time CD4 counts fall below 50 (3–5). While retinitis is readily diagnosed with ophthalmologic examination, pathology is often extensive at presentation, particularly if only symptomatic patients are examined (4, 6). Anti-viral therapy is usually effective initially, but reactivations recur periodically during the remaining lifetime of the individual (which may be several years), leading to progressive visual impairment. Identification of individuals who are predisposed to CMV retinitis (CMV-R) could facilitate early diagnosis through close monitoring when CD4 levels decline below 200 (6). Prophylaxis of HIV infected individuals, against a variety of pathogens, is an increasingly common approach to AIDS management. Recent clinical trials have demonstrated that oral ganciclovir can reduce the risk of CMV-R. Given the toxicity and cumulative expense of anti-viral, mycobacterial, and anti-fungal agents, identification of those at high and low risk of CMV-R could limit prophylaxis to those most susceptible, sparing those least susceptible.

The course of HIV disease is highly variable. While some aspects of HIV pathogenicity certainly relate to the infecting strain, individuals who contract the same strain of HIV often exhibit markedly different manifestations of HIV disease and opportunistic infections, implying contribution of host factors. The T cell immune response likely influences the course of HIV disease, particularly with respect to cell associated viruses such as HIV itself, CMV, and HSV. Since T cell antigen recognition is controlled by HLA genes which are highly polymorphic, epitope response patterns vary considerably from person to person and can impact the overall response to a pathogen. Recent investigation of immunogenetics and the immune response to plasmodium falciparum (malaria) suggests that certain epitopes (which are recognized in the context of HLA types: HLA-B53 and DR13) elicit the most protective responses (7, 8) and that individuals with these alleles are comparatively resistant to severe infection. The association of both HLA B and DR (MHC-I and MHC-II) with resistance to severe malaria suggests that both CD8 T cells and CD4 T cells have roles in control of this pathogen.

CMV specific T cell proliferation may be considered an indicator of potential immune response to CMV. Proliferation primarily represents activation of CD4 T cells which are central to the immune response. CD4 T cell activation is necessary for generation of CMV specific antibody and cytokines produced by CD4 T cells support CD8 T cells. In addition, it is known that for CMV, CD8 as well as CD4 T cells proliferate when cultured with CMV infected cells (as antigen) (9, 10). In those immunosuppressed by HIV, Wahren et al. (1987) and others (11, 12, 13) found that, on average, T cells from HIV (and CMV) infected donors had lower levels of T cell proliferation to CMV antigen in vitro than did cells from CMV-infected, HIV seronegative controls. However, within these average

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1. *Abbreviations used in this paper:* CMV, Cytomegalovirus; CMV-E, CMV encephalitis; CMV-R, CMV retinitis.

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lower responses, there is considerable individual variation. Accordingly, we investigated whether CMV-R was related to CMV immune response and immunogenetic inheritance.

Methods

Patient population. The HIV Neurobehavioral Research Center (HNRC) participant pool consists of 473 men and women who are followed longitudinally for neurobehavioral changes associated with HIV infection. The principle sources of HNRC participants are Navy personnel and civilians at high risk for HIV infection. The individuals selected for the immune response and HLA study were HIV seropositive, but were otherwise randomly chosen, after their second visit to the clinic, to select for reliability. Currently, approximately one half of the 150 HLA typed participants fall into the "AIDS" category according to the 1993 CDC system (C1-C3, A3, B3). An additional 16 HNRC patients were HLA phenotyped following onset of CMV retinitis.

T cell proliferation assays. The protocol for T cell proliferation to CMV infected cells and HIV peptides has been published (14). Briefly, peripheral blood mononuclear cells (PBMC) were isolated by collecting blood in lithium heparin coated tubes, under layering with Histopaque (Sigma Chemical Co., St Louis, MO.), and centrifugation at 2,000 rpm for 25 min. at room temperature. The mononuclear band was collected and washed twice in sterile saline. Plasma samples were frozen and stored. Mononuclear cells were resuspended to .5–1 million/ml in Iscoves media (GIBCO BRL, Gaithersburg, MD) with Pen/Strep and 10% human AB serum (seronegative for HIV, Herpes simplex virus (HSV) and CMV). Cells were plated at .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 microcurie of tritiated thymidine overnight and plates were then harvested and filters counted in a scintillation counter. All antigens were assayed in triplicate and counts averaged. Since background counts can vary considerably among HIV infected individuals, proliferation data are presented as stimulation index. 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 (14), which is common for this type of assay. Thus, a stimulation index of 1.95 or greater was considered a positive response (contingent on less that 25% standard error for the mean raw counts of the triplicate wells).

Assay plates were prepared with mitogens (Phytohemagglutinin (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). Virus antigens were prepared by infecting 80% confluent monolayers at an MOI of 1 for CMV and .1 for HSV and cultures were harvested when all cells had detached from the flask. Infected cells were collected by centrifugation and resuspended at 2 million/ml in supernatant. These large stocks were aliquoted and frozen for consistency. Infected cell preparations (rather than protein preparations) have been found by ourselves and others to give reproducible results (12, 14). Over 98% of HIV infected HNRC study participants 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 (14). 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.

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.

Statistical analysis. Analysis of discrete variables (such as HLA) used Fisher's Exact Test and Chi square. Given the number of HLA alleles, we corrected for the likelihood that some alleles would be over-represented by chance. A preferred method is to use an initial observation in a preliminary sample to hypothesize specific allele associations which are then tested in a second group of patients (15, 7). This approach was used for testing the significance of individual and grouped alleles in retinitis patients. Relative risks were calculated as described by Tiwari and Terasaki (15). Individuals for whom only one allele was detected at a certain locus, were not assumed to be homozygous in calculations. Analysis of continuous variables, such as immune responses of different patient groups, was done using Student's *t* Test.

Diagnosis of CMV retinitis. CMV retinitis was diagnosed in life at the AIDS Ocular Research Unit at UCSD based on examination using the indirect ophthalmoscope in conjunction with slit lamp biomicroscopy of any atypical lesions. In all cases, the diagnosis of CMV retinitis was made when an area of grainy white deep and superficial retinal opacification larger than 2,000 μm was present, associated with variable amounts of retinal hemorrhage (16). In cases where lesions were questionable, the lesions were followed for progression (5, 17). Progressive retinal opacification ruled out retinal cotton wool spots, the most common retinal lesion in AIDS patients (18). Other causes of retinitis were ruled out on the basis of the typical appearance of CMV retinitis. In all cases the diagnosis of retinitis was confirmed through reading of 60 degree wide angle fundus photography (4). At autopsy, all eyes were bisected and examined under a dissecting microscope. Areas of retinal opacification as noted above were examined using light microscopy and, where indicated, immunocytochemistry, to confirm the diagnosis of CMV retinitis using methods previously described (19, 20). In addition, any areas of atrophic retina (probable healed CMV retinitis) were also examined by light microscopy to confirm the presence of retinal gliosis and border activity typical of CMV retinitis.

Results

Immune response to CMV in retinitis patients and controls. T cell proliferation responses were compared for 9 patients with CMV retinitis and 22 controls with fewer than 100 CD4 T cells (at risk for CMV-R) (Table I). Data for the nine individuals with retinitis are shown in Table I and the mean proliferation responses for the controls, matched for CD4 count, are shown below. Mean group retinitis and control values are shown at the bottom with *P* values. The retinitis group consists of those tested after diagnosis of retinitis as well as those assayed prior to diagnosis since assay profiles did not differ (CMV responses were low) and history or presence of CMV infection was not known at time of assay. T cell proliferation assays for all antigens and T cell subset counts were done on blood samples drawn the same day.

AIDS patients who developed retinitis had significantly lower responses to CMV than controls without retinitis. The retinitis and control groups did not differ with respect to other immune responses or cell counts, suggesting that there were not major differences in the level of overall immune competence or stage of disease. The low immune responses to CMV in patients with fewer than 100 CD4 T cells who subsequently developed retinitis suggested that the low responses might indicate predisposition to CMV.

To examine whether an early predisposition to CMV-R could be identified, we compared early T cell proliferative responses of individuals who eventually developed retinitis with controls, before development of severe immunosuppression (as defined by less than 200 CD4 T cells). Of those with more than 200 CD4 T cells, 73% of all individuals in our study show positive proliferative responses to CMV and disease is rare. The responses of those individuals with more than 200 CD4 T cells who later developed retinitis were compared with means for controls who have not developed retinitis (Table II). Although

Table I. HIV-infected Individuals Who Develop CMV Retinitis Have Low T Cell Proliferation Responses to CMV

Patient	T. prolif.*	CMV-R‡	PHA (SI)§	CMV (SI)	HSV (SI)	HIV (PC)‖	CD4	CD8
21996	9/19-5/92	5/92	24	.96	1.12	4	51	545
22400	1/92	9/91	0.81	0.93	0.87	0	3	109
11799	1-9/91	10/91	4.5	1.8	1.5	5	49	1092
22528	4/92	1/92	0.78	0.85	0.92	0	8	196
53030	9/92	6/92	5.72	0.40	0.30	2	10	347
22262	8/92	12/92	29.35	0.99	0.88	0	45	1058
21847	5/92	2/93	0.46	0.67	0.97	0	24	434
23285	9/92	7/93	12.45	0.96	0.73	0	15	234
22212	1/92	8/93	8.39	1.43	1.52	3	94	423
Summary		N¶						
Mean	CMV retinitis	9	9.6	1.00	.97	1.56	33	495
Mean	No retinitis**	22	10.38	5.72	2.0	1.57	43	517
	t test‡‡	*P*=	0.84	<0.001	0.22	0.99	0.37	0.85

* Date of T cell proliferation assay. A range indicates that multiple assays were averaged. ‡ Date of diagnosis of CMV-R. § Proliferation response, SI of 1.95 or more considered a positive response. ‖ (peptide count) is the number of HIV peptides which elicited a response of 1.95 or more (out of 31). ¶ N is number of individuals. Individual responses are not shown for controls. ** Patients with <100 CD4 T cells, but without CMV-R. ‡‡ *t* test: 2 tailed, 2 sample, unequal variance.

the mean CD4 counts were comparable, the immune responses to PHA, CMV, and HSV were lower for those who subsequently developed retinitis. Thus, individuals who later develop CMV retinitis show low immune responses to PHA, HSV, and CMV, despite having similar CD4 counts and reactivity to HIV peptides to the control group.

CMV responses were also examined by matching retinitis patient and control responses (retinitis responses: 19, control responses: 118) for mitogen response (PHA) and CD4 levels. In this analysis, mean CMV responses were still lower for the group who eventually developed retinitis (*P* values: HIV = .67, CMV = .001, HSV < .001). With respect to predictive value of low T cell proliferation, analysis is limited to 10 individuals for whom we have both early (> 200 CD4 T cells) and late (< 100 CD4 T cells) observations. 5 of the 10 individuals had negative T cell proliferative responses at early stages and all five have developed CMV-R. Of the five with positive responses, two developed CMV-R and three are currently retinitis free. Based on this small sample, sensitivity of T cell proliferation as a predictor of CMV-R was calculated to be .69 and specificity, .68.

It should be noted that the high percentage of retinitis patients in these and subsequent samples is due to intense following and treatment of retinitis patients, not a higher incidence or retinitis in our overall population. Also, low immune responses in the CMV-R patients were not due to absence of endogenous CMV infection since assay of serum from these early time points indicated presence of CMV antibody.

Table II. Individuals Who Develop CMV Retinitis Show Low Responses before Severe Immunosuppression

Patient	T. prolif.*	CMV-R‡	PHA (SD)§	CMV (SI)	HSV (SI)	HIV (PC)‖	CD4	CD8
1996	7/90	5/92	7.01	1	1	1	720	1307
463	3/89	8/92	1.1	1	1	7	684	1367
231	9/87-4/89	5/90	4.5	1.9	3.2	4.5	409	1008
414	7/87-3/89	10/91	1	1.6	1	5	437	878
353	7/87-1/89	9/92	7	1	1	5	528	1548
292	6/87-8/88	3/93	1.5	2.2	2.6	3.5	640	454
2212	7/90	8/93	13	10.3	5.27	3	371	1074
Summary	N¶							
Mean	7	CMV-R	5.01	2.6	2.15	4.14	541	1091
Mean	142	Control**	27.24	8.48	5.86	3.66	527	1080
	t test‡‡	*P*=	<.001	.002	.0005	0.26	0.40	0.47

* Date of T cell proliferation assay. A range indicates multiple responses were averaged. ‡ Date of diagnosis of CMV-R. § Proliferation response, SI of 1.95 or more considered a positive response. Responses <1 were rounded to 1.0. ‖ (peptide count) is the number of HIV peptides which elicited a response of 1.95 or more (out of 31) ¶ N is number of individuals. ** Patients with >200 CD4 T-cells who have not developed CMV-R. Individual responses are not shown. ‡‡ *t* test 2 tailed, 2 sample unequal variance.

Table III. CMV-R Patients Have Increased Frequencies of HLA B44.51 and DR-7

Alleles	Exp.* (N = 150)	Obs.‡ (N = 16)	Obs.§ (N = 21)	P value‖ (N = 16)	P value¶ (N = 21)
	%	%	%		
DR7	21	40	45	.07	.02
B44	20	37	38	.1	.06
B51	10	6	20	.4	.14
DR7 or B44, or 51	34	69	76	.008	.0005

* Expected percentage of individuals with that allele in the sample group based on the frequency in the population of 150 HLA typed HIV infected individuals. ‡ Observed frequencies in 16 CMV-R patients HLA typed after initial predictions. § Observed frequencies of all HLA typed CMV-R patients. ‖ *P* values calculated using Fisher's Exact Test, for 2 stage analysis. ¶ *P* values for total CMV-R group.

Immunogenetic analysis. To investigate whether the low immune responses and susceptibility to CMV retinitis were associated with specific HLA types, allele frequencies for HLA-A, B, C, and DR were initially analyzed for the five patients with CMV-R who had previously been HLA typed. In this small sample, B44, B51, and DR7 were at increased frequency compared to HLA frequencies of 150 HIV infected HNRC participants. From this initial observation, it was hypothesized that if these alleles were consistently associated with CMV retinitis, they would be increased in a second, non overlapping sample. This two step analysis is a method to adjust for multiple comparisons (many HLA alleles) (15).

The expected and observed frequencies for HLA B44, B51, and DR7 are shown in Table III, both for the second group of 16 and the total of 21 typed CMV-R patients. Individually, the frequencies of DR7 and B44 are close to twice that expected and approach, or are significant, in the 16 and 21 retinitis patient groups. The combination (B44, 51 or DR7) is highly significant in both the non overlapping group of 16 and the total of 21. These results suggest that the HLA alleles DR7, B44, and possibly B51 may predispose AIDS patients to CMV-R. Although B51 was not significantly increased in the second group of retinitis patients, it was included in the analysis since (*a*) it was part of the initial hypothesis (in fact, the allele most significantly increased), (*b*) the ratio of observed to expected is similar to DR7 and B44, and (*c*) B51 is at low frequency in the general population and would also be rare in a small sample. HLA typing of larger populations of retinitis patients will be required to confirm and assess the risk of individual HLA alleles.

HLA B44, B51, and DR7 and low immune response to CMV. If HLA types DR7, B44 and B51 are associated with CMV-R, and CMV-R is associated with low immune response to CMV before severe immunosuppression, then selection of individuals with HLA alleles B44, B51, and DR7 should identify individuals with low immune responses. The immune responses to CMV (first assay, at entry to the study) were compared for those with, and without HLA B44, 51, or DR7. Patients known to have retinitis and those with fewer than 100 CD4 T cells at the time of assay were excluded from this analysis. As shown in Table IV, negative responses to CMV were twice as common (61 vs. 30%) in those with, than in those without, predisposing alleles ($P = .02$). Thus, HLA B44, DR7 and possibly B51, appear to be associated with comparatively low T cell proliferative responses to CMV in HIV infected individuals, prior to onset of retinitis.

Table IV. Individuals with B44,51 or DR7 Have an Increased Chance of Developing CMV Retinitis when CD4 Levels Drop Below 100

HLA alleles*	Retinitis	No retinitis	Totals
B44,51, or DR7	17 (68%)	8 (32%)	25
Not B44, 51 or DR7	5 (31%)	11 (69%)	16
$P = .018$‡	22	19	N = 41

* Individuals with fewer than 100 CD4 T-cells were grouped according to those with, versus those without HLA B44, 51, or DR7. ‡ Calculated using Fisher's Exact Test.

Risk of CMV-R in immunogenetically predisposed patients. In HIV infected individuals, most CMV-R develops when CD4 T cell counts fall below 100 (6). To examine the predictive value of these HLA alleles at this stage, the frequency of retinitis was calculated for the 41 patients with CD4 counts less than 100 (see Table V). Of 25 HIV infected individuals with HLA B44, B51, or DR7 and CD4 counts < 100, 17 (68%) have developed retinitis (relative risk is 4.7) compared with $^5/_{16}$ (28%) without the predisposing alleles. In a similar analysis of the 13 HLA typed patients who have died, all 6 (100%) with B44, 51 or DR7 had retinitis compared to $^2/_7$ (29%) of those without ($P = .02$, relative risk = 15).

In a recent study of CMV-R and CMV encephalitis (CMV-E), 42% ($^{16}/_{38}$) of patients with CMV-R had CMV-E at autopsy compared with only 2% ($^1/_{41}$) of those without existing CMV-R. CMV-E was defined post mortem, by the presence of microglial nodules with cytomegalic cells or frank ventriculitis (21, 22). The results suggest that the majority (between 50 and 100%) of AIDS patients with HLA B44, B51, or DR7 eventually develop CMV retinitis and 40% of those individuals also have CMV infection of brain.

Discussion

HIV infected individuals who have low immune reactivity to CMV antigens in vitro are at comparatively higher risk for developing CMV-R. Low T cell proliferative responses, both at early and later stages of disease, were associated with in-

Table V. Positive T Cell Proliferative Responses to CMV Are Less Frequent in Individuals with HLA B44, B51, or DR7

Prlf. CMV*†	B44,51 or DR7‡	NoB44, 51 or DR7	Totals
SI < 1.95	11 (61%)	17 (30%)	28
SI > 1.95	7 (39%)	38 (70%)	45
$P = .02$§	18	55	‖N = 73

* T cell proliferation to CMV antigens in vitro. Threshold for a positive response is a stimulation index (SI) of 1.95 or higher. ‡ HIV infected individuals with any of these alleles. § Calculated using Fisher's Exact Test. ‖ N = number of individuals, tested at entry to study who had 100 or more CD4 T-cells.

creased risk of CMV-R. Also, immunogenetic phenotype was associated with both low immune response to CMV and onset of CMV-R. Individually, HLA DR7 was most strongly associated with CMV-R, followed by B44. Weak associations were noted for B51. The association of CMV-R and low immune response with presence of one or more of three alleles was highly significant.

Neither low CMV specific immunity nor HLA phenotype have been previously identified as risk factors for CMV disease in AIDS. Immunosuppressed transplant recipients are also a risk group prone to CMV viremia, although there may be important differences from AIDS patients with respect to manifestations of CMV (retinitis is rare in transplant patients and pneumonia is rare in AIDS patients), sero-status for CMV (many transplant recipients are initially CMV antibody negative), specificity of host cells infected by HIV, versus more general immunosuppression with iatrogenic agents, and complication of allogeneic responses in transplant recipients. The kinetics of immunosuppression in AIDS is also gradual and progressive which may lead to lower levels of protective (T cell dependent) antibody to CMV late in disease. In contrast, acute immunosuppression prior to transplant might have less effect on existing CMV antibody production. Despite differences, in patients and disease states, some results do suggest that low T cell proliferative responses to CMV following iatrogenic immunosuppression are associated with clinical symptoms (23, 24). Interestingly, two groups have recently reported that transplant recipients who had, and were matched with the donor graft for HLA-DR7, were at increased risk for CMV disease, compared with all other patients (25, 26).

In this study, T cell proliferation of PBMC in vitro was used as a general indicator of immune response to CMV and low response predicted CMV-R better ($^3/_5$) than would CD4 count alone (30%, based on the average frequency of retinitis in all AIDS patients), but additional observation on assayed individuals, as they drop to below 100 CD4 T cells, will be necessary for accurate estimates of the predictive value. One HIV infected individual developed CMV-R 3 years after a high response to CMV (SI of 10). Such exceptions to the association between T cell proliferation and CMV disease could be due to a number of factors. Concurrent opportunistic infections can depress or amplify T cell proliferation non-specifically and CMV reactivation is known to alter the CMV response. Abrupt rises in proliferation responses are detected in certain individuals prior to severe immunosuppression (21 individuals had stimulation indices between 12 and 75). In general, high reactivity appeared to reflect successful responses to sub clinical reactivations since only one of these 21 has displayed symptoms of CMV-R (or any other manifestation of CMV). Interpretation of a low to moderate proliferation response is more difficult since it could represent either inability to respond to CMV and suppress viremia (which could cause further immunosuppression) (27) or that endogenous reactivations did not occur. Therefore, future studies of pathogenesis will include assay for the presence and levels of CMV in peripheral blood as well as proliferation response. Also, while proliferation is a simple method for measurement of T cell response, it can represent activation of a number of cell types. Examination of the specific T cell subpopulations responding (CD4, Th1, Th2, CD8: by CTL assay) may reveal which populations are most closely correlated with disease resistance or susceptibility in specific patients.

Investigation of HLA associations with disease requires adjustment for chance over-representation of one allele from a pool of numerous alleles, as well as careful choice of reference groups. In this study we used two stage analysis to adjust for multiple comparisons. Bonferoni correction (multiplying the p value by the number of alleles) is an alternate method, but is not hypothesis driven and was not used since it is considered statistically impractical since the number of alleles detectable has risen to between 40 and 60, depending on methodology (15). Thus, observed HLA types for five initial retinitis patients provided a hypothesis to be tested in a second set of 16 CMV-R patients. Our expected frequencies of HLA alleles were calculated from our HLA phenotyped participant population of 160 HIV infected individuals, rather than an external reference group. This insured that differences in composition between the retinitis and reference population would not confound the analysis.

Although HLA associations have been identified for a number of diseases, the mechanism by which the HLA alleles influence susceptibility or resistance to a given syndrome is only beginning to be investigated. Since it is known that primed, specific immune responses (i.e., HLA restricted) are an important component in resolution of CMV reactivation disease, it is not unlikely that predisposition to clinical infection could be directly related to specific T cell recognition. Therefore, it is possible that the observed low immune response to CMV in the majority of those with the identified HLA phenotypes could involve comparatively poor recognition or activation by CMV specific CD4 (DR7) or CD8 T cells (B44), resulting in low T cell proliferation and IL-2 production or low CTL activity, respectively. A direct immunologic explanation would be that few viral peptides have a strong binding affinity for the antigen pocket of these particular HLA proteins, compared to other HLA proteins. Since CMV-R usually develops when CD4 T cells drop below 100, progressive compromise of the immune system by HIV may serve to reveal which HLA alleles are relatively less efficient for immune recognition and control of CMV. It is also known that CMV contains sequences similar to HLA proteins, which could lead tolerance if there were sufficient identity between a host's specific HLA protein and the virus (28, 29).

In addition to our observations and those noted for transplant recipients for CMV, HLA DR7 associations have been observed for both recurrent HSV-2 disease and Burkitts Lymphoma (30, 31) two other herpes virus syndromes. In our study, average immune responses to HSV-1 were also low in patients who went on to develop CMV-R. While these herpes viruses do not all share any antigenic determinants, T cell immunity is important in control of infection in each case. T cell immunity is also now thought to be important in protection from malaria, and in a recent study of HLA, DR7 was at increased prevalence in the group of African children who developed severe, compared to those with mild *P.* falciparum disease (7). Given the absence of antigenic similarity among these pathogens, it is possible that the DR7 protein associates with relatively few epitopes in general, is sparsely expressed, or is relatively inefficient in interacting with the T cell receptor complex and that the resulting low CD4 T cell response is associated with susceptibility to infections for which CD4 immunity is important for protection. The hypothesis that DR7 might specify an overall low CD4 response is consistent with observations that very few autoimmune diseases are associated with DR7 (15).

In AIDS, HLA DR7 has been identified as protective for HIV disease progression in one study, and associated with increased frequency of opportunistic infections in another (32, 33). These two observations are not necessarily contradictory since individuals who survived for extended periods, might develop more opportunistic infections overall, including CMV retinitis.

Further investigation of the immune response to CMV and HIV in AIDS should increase our understanding of how HIV mediated immunosuppression affects the response to an endogenous opportunistic pathogen such as CMV. Examination of HLA types and response profiles (eventually to specific epitopes) should allow a better resolution as to how immunogenetics and theories of T cell recognition relate to practical immune control of disease in vivo. At the clinical level, our findings suggest that host immune response and HLA phenotype may serve as early markers for CMV-R, facilitating prophylaxis to prevent blindness.

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