MEDICAL SCIENCE

A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection

A report from the Multicenter AIDS Cohort Study

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108 seropositive homosexual men were examined for associations between HLA phenotype and progression of human immunodeficiency virus type 1 (HIV-1) infection. Among men of predominantly European ethnic origin, 49 with very rapid 2-year declines in $CD4^+$ lymphocyte counts showed significant differences in antigen frequencies from 59 men matched for ethnic background, study centre, and initial $CD4^+$ cell count but with little or no decline in $CD4^+$ cells. Relations of varying strength (odds ratios 6·1–10·3) were seen with several HLA antigens often linked in the A1-Cw7-B8-DR3 haplotype. The strongest relation was with the A1, Cw7, B8 combination (odds ratio 10·3). Associations between these antigen combinations and development of AIDS were weaker. The frequency of HLA A24 was also significantly higher in rapid than in slow decliners (odds ratio 4·3). These findings strengthen the suggested link between the product of a gene in the A1-Cw7-B8-DR3 haplotype and HIV-1-related disease.

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Introduction

There is substantial interest in the possibility that products of genes in the major histocompatibility complex (MHC), either HLA antigens or related immunogenetic factors, affect the acquisition or progression of infection with human immunodeficiency virus type 1 (HIV-1).[1–10] The location and function of HLA antigens on the surface of cells are characteristic of proteins that could determine viral attachment, penetration, replication, or some other process in HIV-1 infection. In mice, class I antigens, in conjunction with products of other non-MHC genes, seem to confer varying degrees of susceptibility to progressive murine retroviral infection.[11]

Although epidemiological studies have suggested HLA associations, particularly with Kaposi's sarcoma,[1,2] relations with acquired immunodeficiency syndrome (AIDS) or with specific late manifestations of HIV-1 infection are not consistent.[3–9,12] Few studies have addressed the possibility

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that HLA antigens are associated more with the rate of immunological deterioration than with the individual clinical manifestations. In a cohort of homosexual men with infection of unknown duration, during an average 43 months of follow-up, AIDS appeared significantly more rapidly in men with the DR1 allele than in those without it; however, with further follow-up the difference diminished.[8,12]

Steel and colleagues[9] have reported that one or more alleles in the A1-B8-DR3 haplotype seem to increase the risk of HIV infection and early deterioration. The genes coding for this triplet of antigens show tight linkage disequilibrium in Caucasian populations—ie, they and other genes occur together on the same haplotype more often than would be expected from their individual frequencies.[13] The implication of such a relation is that a genetic marker linked to that haplotype promotes more immediate or intense expression of HIV-1 infection.

We sought relations between HLA antigens and progession of HIV-1-induced immunodeficiency by comparing groups of participants in the Multicenter AIDS Cohort Study (MACS) with different rates of decline in peripheral-blood T-helper lymphocytes.

Methods

The recruitment and regular evaluations of MACS participants have been described previously.[14,15] Homosexual men initially free of AIDS were seen twice a year for a history, a brief physical examination, and laboratory testing. Serum was tested by DuPont or Genetic Systems enzyme immunoassay at each visit and by immunoblot assay at one or more visits depending on the previous serological status.[16,17] T-lymphocyte phenotyping[16] was carried out on seropositive men at every visit. We chose for the HLA study men who were seropositive on entry to the study, rather than men who seroconverted subsequently, because more serial specimens were available for counting of $CD4^+$ cells and more AIDS-defining outcomes were available for analysis.

The inclusion criteria for HLA study participants were to be Caucasian, to have sufficient serological and haematological data for classification, to be seropositive at entry, and to be without AIDS for 12 months thereafter. For each eligible individual a separate straight line was fitted to serial measurements of the number, and the percentage, of $CD4^+$ lymphocytes over time by the least squares method.[18] To fit this straight line and calculate its slope, we required that $CD4^+$ cell data were available from at least the entry visit and one other visit 1–2 years later, but we incorporated all available data from visits during the first 2 years of follow-up. We defined rapid decliners as men whose rates of decline in $CD4^+$ cells were in the fastest 9% (based on the most rapid 15% by both count and percentage of $CD4^+$ cells) and slow decliners as those whose $CD4^+$ cell declines were among the slowest 23% (based on the slowest 35% by both count and percentage). The range had to be wider for slow decliners to allow matching of rapid and slow decliners by study centre, ethnic group, category of initial T-helper-cell count (5·00–6·49, 6·50–7·99, and 8·00–9·99 $\times 10^8$/l), and T-helper-cell percentage (less than 20%, 20–29%, 30–39%, and 40% or above).

We also compared men grouped according to clinical manifestations including AIDS (by Centers for Disease Control criteria),[19] other clinically significant findings as measured by an index similar to one previously described,[16,20] and persistent generalised lymphadenopathy.[16]

Lymphocytes cryopreserved by routine methods were thawed and prepared. Standard National Institutes of Health microcytotoxicity techniques were used to type peripheral-blood mononuclear cells for sixteen A, seven Cw, twenty-two B, and two Bw supertypic antigens and to type B cells for ten DR, three DQ, and the MC1 antigens.[21,22] Typing was done without knowledge of the clinical or immunological data.

TABLE I—RELATION BETWEEN $CD4^+$ CELL DECLINE AND COMBINATIONS OF HLA ANTIGENS

—	No (%) with antigen: Rapid decliners (n=44–49)	No (%) with antigen: Slow decliners (n=54–59)	Odds ratio (logit)	p*
A1, Cw7, B8	9 (18·4%)	0	10·3	0·0005
A1, B8	9 (18·4%)	1 (1·7%)	6·1	0·003
A1, Cw7	10 (20·8%)	2 (3·4%)	6·1	0·004
A1, Cw7, B8, DR3	5 (10·6%)	0	7·6	0·01
Cw7, B8	10 (20·8%)	5 (8·5%)	2·7	0·0600
A1, B8, DR3	5 (10·6%)	1 (1·9%)	3·8	0·05
A1, Cw7, DR3	5 (10·6%)	2 (3·4%)	3·1	0·13
Cw7, B8, DR3	6 (12·8%)	3 (5·3%)	2·7	0·15
B8, DR3	7 (14·9%)	4 (7·0%)	2·1	0·19
A1, DR3	7 (14·9%)	5 (8·6%)	1·8	0·34
Cw7, DR3	8 (17·4%)	7 (12·7%)	1·4	0·49

*Not corrected for multiple comparisons.

For relations with changes in $CD4^+$ cells and with clinical manifestations, we screened single antigens, the six combinations of two antigens that can be made from A, B, Cw, and DR, four combinations of three antigens, and the combination of all four antigens (table I). When there was a substantial difference between rapid and slow decliners in the frequency of specific antigens in a combination, we did analyses similar to those for single antigens. Findings of previous studies directed our attention to certain single antigens and combinations (eg, DR1 and A1, Cw7, B8, DR3).

For comparisons between two proportions, the chi-square test or Fisher's exact test was used for unmatched and the Mantel-Haenszel procedure for matched groups; the overall odds ratios for matched groups were calculated by the logit method of Woolf.[23] No correction for multiple comparisons was applied; a correction in the statistics for certain previously observed relations would have been inappropriately conservative. A t test was applied to differences in means of cell counts and means of slopes. Survival analysis techniques[24] were used to examine differences in time to AIDS.

Results

HLA typing was done for 108 of the 1227 seropositive men eligible for the study—49 rapid decliners and 59 slow decliners. 75% of the pairs matched completely by ethnic history (60% entirely western European, 10% partly western European, and 5% entirely Mediterranean or eastern European). In the remaining 25%, men in the matched pairs differed only partially in ethnic origin. The mean age at entry was 33 years in both rapid and slow groups.

The mean differences at entry between rapid and slow decliners in number of $CD4^+$ cells ($0{\cdot}12 \times 10^8$/l) and percentage $CD4^+$ cells (0·9%) were not significant, and 82% of the men had $CD4^+$ cell data for the full 2-year follow-up period. The 6-month rates of change in the number of $CD4^+$ cells among rapid decliners ranged from $-0{\cdot}46$ to $-2{\cdot}56 \times 10^8$/l (mean $-1{\cdot}35$ [SEM 0·07], median $-1{\cdot}30 \times 10^8$/l). The range of rates of change among slow decliners was $-0{\cdot}23$ to $+2{\cdot}21 \times 10^8$/l (mean $+0{\cdot}57$ [0·06], median $+0{\cdot}54 \times 10^8$/l). There was no significant difference between the two groups in the nature and frequency of the early clinical manifestations of HIV-1 infection they experienced at entry.

The differences between rapid and slow decliners in combinations of any two, three, or four antigens commonly seen as part of the A1-Cw7-B8-DR3 haplotype are shown in descending order of statistical significance in table I. All 9

TABLE II—ASSOCIATIONS OF SINGLE AND COMBINED HLA ANTIGENS WITH OCCURRENCE OF AIDS

—	No (%) with antigen		Odds ratio	p*
	AIDS (n=22–29)	No AIDS (n=72–79)		
A1	8 (27·6%)	19 (24·1%)	1·2	0·71
Cw7	15 (51·7%)	34 (43·0%)	1·4	0·42
B8	6 (20·7%)	12 (15·2%)	1·5	0·78
DR3	6 (23·1%)	17 (23·6%)	0·9	0·96
A24	8 (27·6%)	11 (13·9%)	2·4	0·25
A1, Cw7, B8	5 (17·2%)	4 (5·1%)	3·9	0·04
A1, Cw7	6 (20·7%)	6 (7·6%)	3·2	0·05
A1, B8	5 (17·2%)	5 (6·3%)	3·1	0·08
Cw7, B8	6 (20·7%)	9 (11·4%)	2·0	0·22
Cw7, B8, DR3	3 (10·7%)	6 (7·9%)	1·4	0·45
A1, Cw7, B8, DR3	2 (7·1%)	3 (3·8%)	1·9	0·48
A1, B8, DR3	2 (7·1%)	4 (5·1%)	1·4	0·50
A1, Cw7, DR3	2 (7·1%)	5 (6·4%)	1·1	0·60
A1, DR3	3 (10·7%)	9 (11·7%)	0·9	0·60
B8, DR3	3 (10·7%)	8 (10·5%)	1·0	0·61
Cw7, DR3	4 (14·8%)	11 (14·9%)	1·0	0·61

*Not corrected for multiple comparisons.

men who carried A1, Cw7, B8 were rapid decliners. In the comparison between pairs of rapid and slow decliners, the relative odds that the rapid decliner carried that combination were 10·3 ($p = 0·0005$). Ethnic diversity did not account for this finding; 75% of the pairs including an A1, Cw7, B8-positive rapid decliner were fully matched and the remainder were partly matched. Other related combinations showed weaker associations with rapid $CD4^+$ declines. Although the numbers were small, there was a suggestion that the strength of the relation with immunological deterioration diminished when DR3 was included in the antigen combinations. The mean 6-month $CD4^+$ cell declines were no greater in rapid decliners who carried any of the antigen combinations than in those who did not.

There were differences between the groups with and without AIDS in the frequencies of three of the antigen combinations associated with rapid $CD4^+$ decline (table II), but the odds ratios ranged between 3·1 and 3·9, and the significance was borderline.

We found no significant difference between the rapid and slow decliners (table III) or between those with and without AIDS (table II) in the proportions with any of the single antigens from the A1-Cw7-B8-DR3 haplotype. The A24 antigen was the only individual HLA antigen that showed a significant difference in frequency between rapid and slow decliners (table III). Among the rapid decliners, men with A24 showed a greater mean 6-month decline in the number of $CD4^+$ cells than men without the antigen ($-1·56$ *vs* $-1·24 \times 10^8/l$; $p < 0·05$). A24 was no more frequent among the 29 men with AIDS than among the 79 men without AIDS (table II).

TABLE III—ASSOCIATIONS OF SINGLE HLA ANTIGENS WITH DECLINE IN $CD4^+$ CELLS

—	No (%) with antigen		Odds ratio	p*
	Rapid decliners (n=44–49)	Slow decliners (n=54–59)		
A24	14 (28·6%)	5 (8·5%)	4·3	0·006
A1	15 (30·6%)	12 (20·3%)	1·7	0·22
B8	11 (22·4%)	7 (11·9%)	2·1	0·142
Cw7	24 (49·0%)	25 (42·4%)	1·3	0·492
DR3	12 (27·3%)	11 (20·4%)	1·5	0·423

*Not corrected for multiple comparisons.

Rapid decline in $CD4^+$ cell count was not associated with the presence of HLA DR1. Fewer rapid than slow decliners carried the DR1 antigen, and it was no more frequent among rapid decliners with AIDS than among those without AIDS (data not shown).

No single antigen was substantially more frequent among the 7 men in whom Kaposi's sarcoma developed or among the 41 men with persistent generalised lymphadenopathy than among the groups of men without those disorders. No combination of antigens showed more than borderline significance in its association with Kaposi's sarcoma, although there were too few cases for meaningful analysis. The combination Cw7, DQ1 showed a weak association with more extensive and persistent lymphadenopathy ($p < 0·02$).

Discussion

In this study seropositive MACS participants who carried the A1, Cw7, B8 combination were about ten times more likely to suffer an accelerated course of HIV infection than men who did not carry that combination. Only one individual antigen, A24, occurred in significantly different frequency among the rapid and slow decliners; however, there has been no previous suggestion of its association with rapid progression or late manifestations of HIV-1 infection, so this finding may reflect chance distribution.

Our findings are consistent with those of Steel et al[9] on 18 haemophiliacs in Edinburgh who acquired HIV-1 infection from contaminated factor VIII concentrate. In that cohort the closely related combination of HLA A1, B8, DR3 was seen in 8 of 18 (44%) men who seroconverted and 3 of 14 (21%) who did not. More importantly, during the 4-year follow-up AIDS developed in 7 of 9 seroconverters with that antigen combination but in only 1 without it. The single antigens DR3[4] and Cw7[8] have been found in high proportions of AIDS patients with opportunistic infections and in another study[10] HIV-1-seropositive subjects with Kaposi's sarcoma and lymphoma were distinguished by the virtual absence of B8 and DR3. The authors suggested that these clinical manifestations may occur after a more gradual decline in $CD4^+$ cells, whereas people with B8 or DR3 may experience more rapid progression to fatal opportunistic infection and thus have less opportunity to manifest a malignant disorder.

Our study was similar to that of Steel et al[9] in that all the participants had similar ethnic backgrounds. The analysis was confined to men whose entry $CD4^+$ cell counts were between 5·00 and $10·0 \times 10^8/l$. We intentionally excluded men with the highest entry counts to avoid mixing those whose decline may have represented regression to the mean with men whose rapid T-cell loss was probably real. Furthermore, the important comparisons were made between groups showing the greatest difference in rate of progression (decline in $CD4^+$ cells) after matching for entry cell counts. Our selection of men with uncertain durations of infection instead of seroconverters is unlikely to have biased the study in favour of detecting an HLA association. The MACS seropositives who were typed showed a slower mean decline and less overall dispersion of slopes than did MACS seroconverters with known duration of infection who were eligible for the study but were not typed (data not shown). This greater similarity between the two groups of seropositives would tend to reduce the likelihood of detecting HLA associations.

We found weaker associations between HLA and development of AIDS than between HLA and $CD4^+$ decline. However, because the study was not designed to include men representative of all seropositive men, the search for associations with AIDS may have been less sensitive than that addressing immunological change. Nevertheless, our results suggest that less carefully controlled studies of AIDS in patients with infection of unknown duration may obscure the type of relation found here.

As population studies, the Edinburgh[9] and MACS investigations could not identify haplotypes as directly as could family studies, but the strong linkage disequilibrium characteristic of the A1, Cw7, B8, DR3 combination suggests strongly that most, if not all, people expressing at least three of the antigens do carry the controlling genes in a haplotype relation. There is little support for a direct biological role of any single established antigenic specificity so far associated with expression of HIV-1 infection, but perhaps the product of an as yet unknown gene in the HLA region determines how rapidly HIV-1 infection progresses. Although the numbers of men with the A1, Cw7, B8, DR3 antigen combinations are small, the relative strengths of the associations with various portions of the haplotype suggest that the putative gene might be located nearer to the loci at the A, Cw, B or class I end of the HLA region. Progression of HIV-1 infection is accompanied by rising levels of beta-2-microglobulin, which occurs on cells in physical conjunction with class I antigens. Both types of proteins may be involved in the response to HIV-1.

There is much evidence that genes in the A1-Cw7-B8-DR3 haplotype are associated with disorders characterised by a loss of suppressor cell activity and by excessive autoantibody production.[13,25] If a gene within this HLA haplotype did control progression of HIV-1 infection, it might be related through autoimmune or immune activation or other indirect regulatory phenomena, rather than through direct physical interaction between virus and cell.

All A1, Cw7, B8-positive subjects experienced rapid decline in $CD4^+$ cells during this study. However, more than half of the rapid decliners lacked any of the antigens in this haplotype and there was no difference in mean slope between those with and without the haplotype. Thus, other factors must help to determine the rate of progression. We were unable to detect an association between HLA and Kaposi's sarcoma. In the absence of other suggestive findings, the validity of the association between the combination Cw7, DQ1 and lymphadenopathy must be doubtful. Further effort to define the immunogenetic determinants of progression should take into account the possible effects of ethnic diversity, duration of infection, haplotype relations within the HLA region, and interactions between HLA and other genetic markers.

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