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Possession of human leucocyte antigen DQ6 alleles and the rate of CD4 T-cell decline in human immunodeficiency virus-1 infection

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SUMMARY

Polymorphism amongst the human leucocyte antigen (HLA) class II genes could influence antigen presentation and the ability to control human immunodeficiency virus (HIV)-1 by modulating the virus specific CD4 immune response. To examine the effect of such polymorphisms on disease progression, we studied a cohort of 46 HIV-1 infected long-term non-progressors (LTNPs), 87 intermediate progressors (IPs) and 26 rapid progressors. Kaplan–Meier survival analysis of all patients in the cohort on time to a CD4 count less than 350 cells/μl, showed a trend for a slower rate of CD4 decline in patients with, compared to those without, the *DRB1*15-DQB1*06* haplotype (hazard ratio (HR) 0·69, 95% CI 0·46–1·01, $P = 0{\cdot}06$). A similar effect was not observed with the *DRB1*13-DQB1*06* haplotype (HR 1·18, 95% CI 0·75–1·88, $P = 0{\cdot}46$), but was observed when DQB1*06 alleles were considered irrespective of their DR association (HR 0·74, 95% CI 0·52–1·05, $P = 0{\cdot}06$). Major HLA-DQ6 alleles encode aspartate (Asp) at position 57 on the DQβ chain, a phenotype associated with protection from other immune disorders. We therefore examined the frequency of all DQβ57 Asp$^+$ alleles, but could not detect a significant effect on the rate of CD4 decline. To examine whether the genotype associated with slower CD4 decline was over-represented in patients with a slow rate of disease progression, we conducted a categorical analysis of a subset of patients with an extended follow-up of 14+ years. We found a higher proportion of LTNPs at 14+ years possessed the *DRB1*15-DQB1*06* haplotype compared to IPs at 14+ years (38·46 versus 18·18%), though this difference did not reach statistical significance. When *DQB1*06* alleles irrespective of their DR association were considered, the protective effect was greater (76·9% LTNPs versus 18·18% IPs, $P = 0{\cdot}04$). Our results highlight the potential protective effect of HLA DQB1*06 alleles on the course of HIV disease.

Keywords HIV-1: disease progression; HLA Class II: genes, haplotypes, polymorphisms; T cells: CD4$^+$

INTRODUCTION

Control of human immunodeficiency virus (HIV)-1 infection involves the induction of virus-specific CD8 cytotoxic T cells (CTL), that, upon recognition of HIV-derived peptides presented by human leucocyte antigen (HLA) class I molecules, promote virus clearance via cytolysis and/or secretion of antiviral chemokines (see 1 and 2 for review). This model of infection control is emphasized by the absolute requirement for specific CD8 cells for virus clearance in the simian immunodeficiency virus/Macaque model.[3,4] In addition, immunity to HIV-1 is dependent on CD4 T cells that recognize specific viral antigens in the context of HLA class II molecules.[5,6] HIV-specific CD4 T cells orchestrate the virus-specific CD8 response, governing both its magnitude[7–9] and quality in terms of proliferation and perforin expression.[10] In addition to their helper role, virus specific CD4 cells may directly curtail HIV spread by the secretion of antiviral factors.[11]

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In view of the known effects of HLA gene products on the immune response, there have been several attempts to correlate HLA gene polymorphisms with progression to disease following HIV infection[12–14] in particular using cohorts representing the extremes of rapid progression and non-progression.[15,16] In relation to class I HLA alleles, *HLA-B14, -B27, -B57*, and *-B44* have been found to be associated with slower progression[17–22] while the *HLA-A29*, *-B22* and *B-35* alleles were associated with rapid disease progression.[17,23–25] In relation to class II HLA alleles, *HLA-DR11 has* been associated with rapid progression[17] whilst three studies have highlighted the importance of the *HLA-DRB1*13-DQB1*06* haplotype in slower progression.[17,25,26] Indeed, in a large longitudinal study of 375 seroconverters aggregated from three cohorts, this was the only class II haplotype associated with acquired immune deficiency syndrome (AIDS)-free time.[25] Inheritance of *HLA-DRB1*13* alleles has also been linked with long-term survival among children with vertically transmitted HIV-1 infection.[26] In addition, Malhotra *et al.*[27] found that the *DRB1*13-DQB1*06* haplotype was associated with an improved ability to suppress viral replication over time in response to early treatment in the acute phase of infection. Patients with this haplotype mounted a more vigorous HIV-specific CD4 response than those without, indicating that CD4 T-helper cells influenced by gene products associated with *DRB1*13-DQB1*06* alleles may be important in immunity to HIV infection.[27]

In general, however, the reported effects of the *HLA-DRB1*13-DQB1*06* haplotype have been weak (see 25). One possible explanation is that specific structural features that are shared by different HLA molecules and influence peptide-binding sites confer a protective effect. Under these circumstances there may be several HLA molecules with similar risk-associated structural features, but because they are encoded on different haplotypes, the association with risk remains obscure using haplotype analysis alone. A second possible explanation is that the effects of HLA class II gene structure and the ensuing CD4 helper cell response exert a protective effect only in subjects with a relatively benign disease course and are overcome by other viral, host or genetic factors in those with rapid disease progression. Such effects are therefore unlikely to be evident in studies that focus only on the extremes of rapid versus non-progression.

These issues were addressed by examining earlier reports of an association between HIV non-progression and the *HLA-DRB1*13-DQB1*06* haplotype[17,25–27] in our cohort of HIV^+ patients with different rates of progression. As *HLA-DQ6* alleles typically occur in linkage disequilibrium with *HLA-DR15* and *-13*, the impact of the *HLA-DRB1*15-DQB1*06* haplotype on disease progression was also assessed. We also examined the impact of one of the most studied structural features of HLA-DQ6, namely the presence of an aspartate (Asp) residue at position 57 on the DQβ chain, a region of the molecule known to influence the affinity and repertoire of peptide binding.[28,29] Our results highlight the potential protective effect of *HLA-DQB1*06* alleles and we were unable to detect an independent protective effect of β57Asp on different haplotypes on the course of HIV disease.

MATERIALS AND METHODS

Clinical features of cohort

Samples were taken from participants in a previously well-characterized HIV-infected cohort, established in 1995, of 165 long-term HIV-1-infected patients attending clinics in London, UK, who had been enrolled into a nested case–control study of the biological and behavioural correlates of non-progression in HIV-1 infection.[30] The majority of subjects (95%) were white homosexual or bisexual men of European origin.[30] Gentoype data were available on 159 patients: including 46 HIV-1 infected long-term non-progressors (LTNPs) defined as a CD4 count >500 cells/μl, at >10 years of infection, 87 intermediate progressors (IPs) defined as a CD4 count <500 cells/μl at >10 years of infection and 26 rapid progressors defined as development of AIDS within 5 years of infection. In order to study the potential impact of genotype on disease progression in patients with an extended follow-up, we identified 33 subjects (17 LTNPs and 16 IPs) who remained antiretroviral treatment naïve despite HIV infection of 14 or more years. Functional studies on these 33 subjects have shown that the HIV Gag-specific interleukin-2^+ interferon-γ^+ CD4 T-cell response, an important correlate of non-progression and low virus load was preserved in this LTNP group and conversely impaired in this group of IPs.[7] Thirty of these 33 subjects were similar in race (Caucasian), gender (male) and behavioural characteristics (homosexual). Genotype analysis was known for 24 out of these 30 subjects (13 LTNPs and 11 IPs). The study was approved by the local Ethics Committee and written informed consent was obtained from all subjects.

DRB1 Gene amplification by polymerase chain reaction (PCR)

Primers employed for group-specific amplification and sequencing of DRB1 exon 2 were as detailed by Voorter *et al.*[31] A multiplex mix of 5′ primers specific for all allelic groups was employed. In ambiguous cases, allele specific amplification was subsequently performed. The PCR reaction was set up in a volume of 50 μl containing 10 mM Tris (pH 8·3), 50 mm KCl, 1·5 mm $MgCl_2$, 5% glycerol, 4 μg cresol red, 200 μm each dinuclotidetriphosphate (dNTP), 13·3 pmol of biotinylated primer, 26·6 pmol unlabelled primer, 200 ng of DNA and 1·4 U AmpliTaq DNA polymerase (Roche Ltd, East Sussex, UK). Cycle parameters used in a Perkin-Elmer 9600 thermal cycler were an initial denaturation step at 94° for 2 min prior to cycling, followed by 35 cycles at 94° for 30 s, 55° for 30 s, 72° for 45 s, followed by a final 5-min extension at 72°.

DQB1 amplification by PCR

Primers employed for amplification and sequencing of DQB1 exon 2 were as detailed by Voorter *et al.*[31] DQB1 alleles were amplified employing the 5′ primer DQB96912

in combination with DQB96008b (DQB1*02/03/04) or DQB95011b (DQB1*05/06). PCR reactions were performed in a final volume of 50 μl. The PCR reaction was set up in a volume of 60 μl containing 10 mm Tris (pH 8·3), 50 mm KCl, 1·5 mm $MgCl_2$, 5% glycerol, 1% dimethylsulphoxide, 6 μg cresol red, 200 μm each dNTP, 20 pmol of biotinylated primer, 40 pmol unlabelled primer, 500 ng of DNA and 2·0 U AmpliTaq DNA polymerase. Cycle parameters used in a Perkin-Elmer 9600 thermal cycler were an initial denaturation step at 94° for 2 min prior to cycling, followed by 10 cycles at 94° for 10 s, 65° for 60 s, and finally 20 cycles of 94° for 10 s, 61° for 50 s, and 72° for 30 s followed by a final 5-min extension at 72°.

Sequencing reactions and analysis

Ten μl of PCR reaction mixture was screened by agarose gel electrophoresis for the presence of PCR product of the correct size (bp). Positive PCR products were used as a template for sequencing. For DRB1 50 μl of each PCR product was subject to Solid phase sequencing reactions using the Pharmacia Autoload Solid Phase Sequencing Kit (Amersham Pharmacia Biotech UK Ltd, Amersham, UK) according to the manufacturer's protocol. Reaction products were separated on an ALFexpress automated sequencer. Generated sequencing data was processed automatically and evaluated manually, prior to HLA typing using the Pharmacia Sequityper software package. Sequencing ambiguities were resolved by allele specific amplification and sequencing or by DRB1/DQB1 SSP (Dynal Biotech UK, Wirral, UK) where appropriate.

Statistical analysis

Kaplan-Meier analysis was used to analyse the time to a CD4 count <350 cells/μl in individuals who had either one (heterozygous) or two alleles (homozygous) compared to neither; and Cox's proportional hazard regression models were used to estimate hazard ratios. Proportional hazards assumptions were tested and found to be not significant. Categorical analysis of LTNPs versus IPs at 14+ years was conducted. Association between possession of 1 (heterozygous) or 2 (homozygous) of *HLA-DRB1*13-DQB1*06* or *HLA- DRB1*15-DQB1*06* alleles or *HLA-DQB1*06* alleles compared to none, was examined using Fisher's exact test. Since there had been previous reports of an association between the *DRB1*13-DQB1*06* haplotype and non-progressors[25–27] the association of this haplotype with non-progression was an *a priori* hypothesis, and accordingly, no correction was made for multiple comparisons.

RESULTS

Possession of *DRB1*15-DQB1*06* haplotype retards the rate of CD4 decline in HIV-1 infection

We first determined the effect of the *DRB1*13-DQB1*06* haplotype on the rate of decline of CD4 T cells to below 350 cells/μl blood in all 159 patients in the cohort during the period from the first HIV test to more than 14 years of infection. Thirty-two patients at the time of their first HIV test had the the *DRB1*13-DQB1*06* haplotype. No association between this haplotype and rate of CD4 decline was noted (Fig. 1a; hazard ratio (HR) = 1·19, 95% CI = 0·75–1·88, $P = 0{\cdot}46$), for those with none versus one or two HLA-DR13/DQ6 haplotypes. We reasoned that the effect of the *DRB1*13-DQB1*06* haplotype observed in other studies[17,25,26] might be related to the presence of genes encoding the *HLA-DQ6* molecule, rather than HLA-DR alleles, and since *HLA-DQ6* genes are typically in linkage disequilibrium with either *HLA-DR13* or *HLA–DR15* alleles, we next examined the frequency of the *DRB1*15-DQB1*06* haplotype. Forty-eight subjects in the cohort presented with the *DRB1*15-DQB1*06* haplotype. A strong trend for an association between the rate of CD4 T-cell decline and the *DRB1*15-DQB1*06* haplotype in patients possessing one or more copies was observed (Fig. 1b, HR = 0·69, 95% CI 0·46–1·02, $P = 0{\cdot}06$) and this did not change statistically when both the HLA-DQ6 encoding haplotypes were considered together (Fig. 1c, HR = 0·74, 95% CI 0·52–1·05, $P = 0{\cdot}09$). Seventy-five subjects possessed the 13/6 or the 15/6 haplotypes. An additional five subjects had both the 13/6 and the 15/6 alleles, making the combined number of subjects with these alleles, 80. When we analysed the possession of HLA-DQ6 encoding alleles irrespective of the HLA-DR alleles (75 subjects in the cohort had DQ6 with DR13 and/or DR15, only one subject had DQ6 on a different DR allele), we found a similar level of protection as that noted with the *DRB1*15-DQB1*06* haplotype (Fig. 1d, HR = 0·72, 95% CI 0·50–1·02, $P = 0{\cdot}07$). Taken together these data indicate the potential importance of the DR15/DQ6 haplotype in delaying clinical progression in our cohort.

DQ6-encoding alleles associate with a subset of non-progressors

We further hypothesized that the protective effect of *HLA-DQ6*-encoding-alleles may be more apparent by studying a subgroup of the 17 'best' survivors, clinically stable and free of antiretroviral therapy despite a median duration of infection of 14·8 years (range 13–16·2 years). We therefore tested whether markers identified by the Kaplan–Meier analysis to be associated with a slower rate of CD4 decline were over-represented in this group of patients compared to a matched group of IPs who also had a median duration of infection of 14+ years (range 12·7–15·7 years) but whose CD4 count had declined below 350 cells/μl. Functional data on these IPs had previously revealed that their HIV-specific CD4 T-cell response was impaired compared to these LTNPs.[7] We noted a higher prevalence of the *DRB1*15-DQB1*06* and the *DRB1*13-DQB1*06* haplotypes in this subset of LTNPs compared to the subset of IPs though group differences for either marker did not reach statistical significance (Table 1). However, in the combined analysis of *DQB1*06* alleles linked to either *DRB1*-15* or *-13*

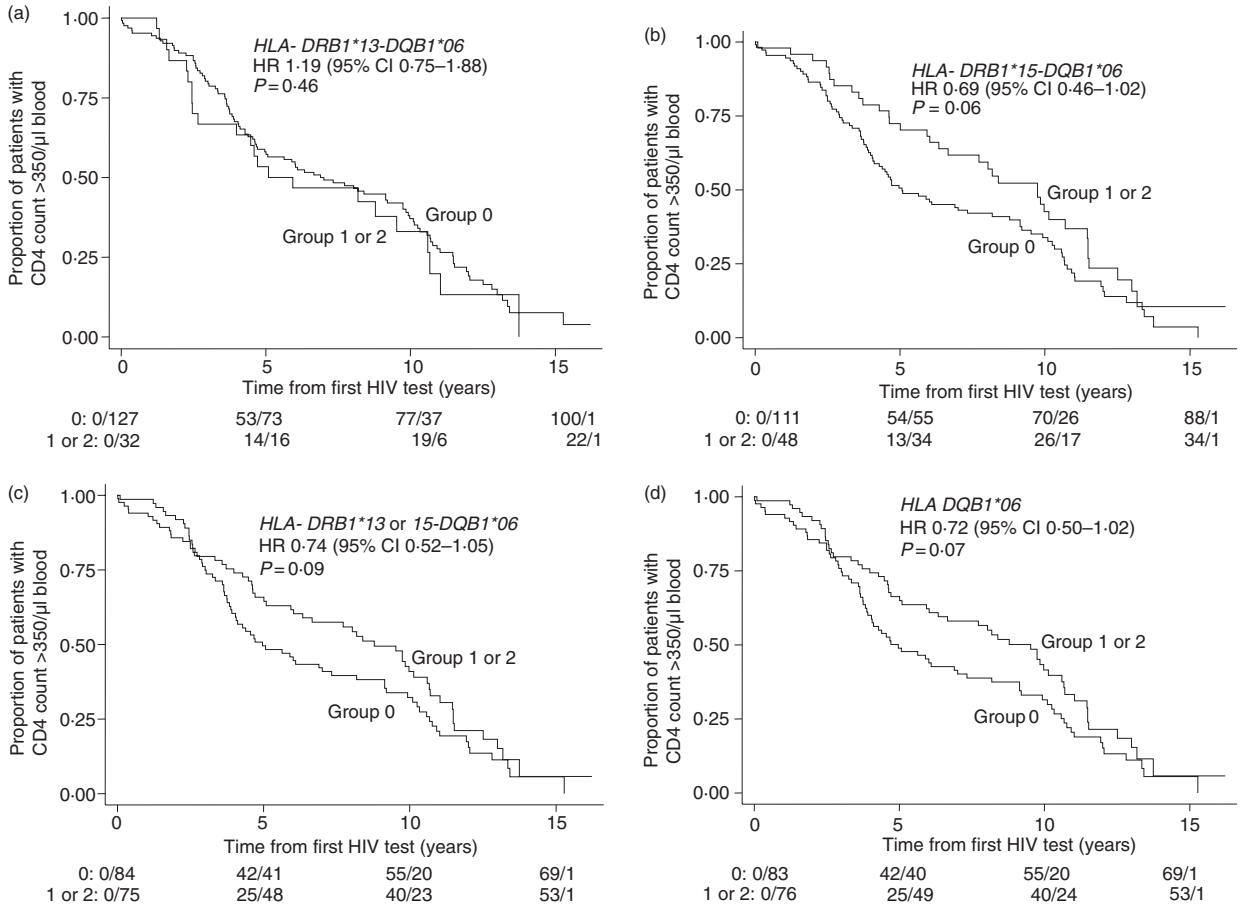

Figure 1. Effect of HLA DQ6 alleles on rate of CD4 decline. Kaplan–Meier analysis of the rate of CD4 T-cell decline to < 350 cells/μl from the time of the first HIV test to greater than 14 years of infection of 159 patients in the cohort is shown. Patients who possess one or two alleles of DQ6 (groups 1 or 2, respectively, d), or DQ6 linked to either *HLA DRB1*13* only (a), *HLA DRB1*15* only (c), or possess either the *HLA DRB1*13-/-15-DQB1*06* haplotype (c), were compared to those who lacked these alleles (Group 0). Cox's proportional hazard regression models were used to estimate hazard ratios and two-tailed *P*-values represented. The rows below each survival curve show the number of events/number of individuals still at risk at 0, 5, 10, 15 years after the first HIV test.

Table 1. Analysis of DQB1*06 alleles in long-term HIV-infected individuals at 14 + years

Parameters	LTNP $n = 17$	IP $n = 16$	*P*-value LTNP versus IP
Median duration of infection (years)	14.8 (13–16.2)	14.9 (12.7–15.7)	
Median virus load (copies/ml) (IQ range)	13 510 (50–104 000)	69 433 (1002–263 000)	0.007
Median CD4 count ($\times 10^6$/l) (IQ range)	656.50 (497–1993)	243 (85–530)	< 0.0001
Frequency of patients with *HLA DRB1*15DQB1*06* haplotype	5/13 (38.46%)	2/11 (18.18%)	0.3864
Frequency of patients with *HLA DRB1*13-DQB1*06* haplotype	5/13 (38.46%)	1/11 (9.09%)	0.1660
Frequency of patients with 1 or 2 *HLA DQB1*06* alleles	10/13 (76.9%)	3/11 (27.27%)	0.04

The genotype of a total of 13 LTNPs and 11 IPs at 14 + years was compared. The number and percentage of subjects who possessed one or two alleles of HLA *DRB1*15-* or *DRB1*13-DQB1*06* or *DQB1*06* alleles on either *DRB1*15-* or *DRB1*13-* alleles is summarized. Comparison of whether the frequency of one or two HLA alleles in the LTNP group was higher than that of the IP group was examined using Fischer's exact test and two-tailed *P*-values represented.

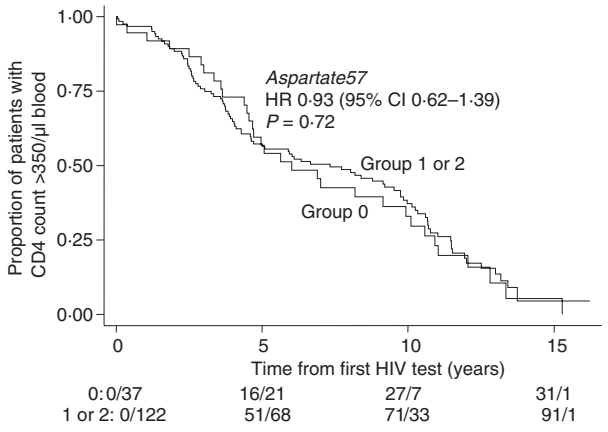

Figure 2. Effect of DQβ57Asp alleles on rate of CD4 decline. Kaplan–Meier analysis of the rate of CD4 T-cell decline to <350 cells/μl from the time of the first HIV test to greater than 14 years of infection of 159 patients in the cohort is shown. Patients who possess one or two alleles of DQβ57Asp (groups 1 or 2, respectively) were compared to those who lacked these alleles (Group 0). Cox's proportional hazard regression models were used to estimate hazard ratios and two-tailed *P*-values represented. The rows below the survival curve show the number of events/number of individuals still at risk at 0, 5, 10, 15 years after the first HIV test.

alleles, the protective effect was stronger (76·9% LTNPs versus 27·27% IPs, $P = 0{\cdot}04$).

Aspartate at position 57 on the HLA-DQβ chain does not alter the rate of CD4 decline

In structural terms, one of the most studied features of the protein products of the major alleles at the *DQB1*06* locus is the presence of an Asp residue at position 57 on the DQβ chain (DQβ57Asp)[28,29] which is, for example, associated with dominant protection from the autoimmune disease type 1 diabetes mellitus[32] irrespective of haplotype or racial origin. We therefore examined associations of DQβ57Asp-encoding alleles with clinical groups and effects on the rate of CD4 decline. DQβ57Asp-encoding alleles were assigned as described.[33] The majority of subjects in the cohort had one or two alleles encoding DQβ57 aspartate (122/159 = 76·7%) (Fig. 2). Kaplan–Meier survival and Cox-proportional hazards analysis showed no association with delay in CD4 decline and possession of 1 or more DQβ57Asp-encoding alleles (HR = 0·93, 95% CI 0·62–1·39, $P = 0{\cdot}72$; Fig. 2). Similarly, we were unable to detect evidence of a strong protective effect of DQβ5Asp57 in our analysis of the subgroup of the 'best' survivors at 14+ years. The percentage of alleles with this residue did not differ statistically between LTNPs and IPs (84·6% LTNPs had one or two alleles encoding DQβ57 aspartate compared to 54·54% Ips, $P = 0{\cdot}18$).

DISCUSSION

This study highlights the potential importance of the *HLA-DRB1*15-DQB1*06* haplotype in the control of HIV infection and raises the possibility that previous observations reporting a similar protective effect of the *HLA-DRB1*13-DQB1*06* haplotype[17,25,26] may reflect inheritance of DQ6 rather than DR13 alleles. The protective effect afforded by the *HLA-DRB1*13-DQB1*06* haplotype was noted to be weaker when cohorts that included subjects representing the extremes of HIV progression and non-progression were studied;[25] as emphasized by a recent study that looked at major histocompatibility complex (MHC) ancestral haplotypes and disease progression using a pooled cohort from several sites.[34] Our data support and extend these earlier studies.

The strength of the protective effect exerted by MHC class II molecules and the ensuing specific CD4 response is likely to be governed by additional host factors. Thus the potential protective effect of *DQB1*06* alleles was more apparent in the categorical analysis of the subgroup who included the extreme of non-progression at 14+ years, than in the survival analysis of all patients in the cohort. This is consistent with data from Malhotra *et al.*[27] who showed that although patients with and without the *DRB1*13-DQB1*06* haplotype had similar virus set-point after acquisition of HIV infection, patients with the haplotype had an improved ability to suppress viral replication over time in response to early treatment in the acute phase of infection. Taken together with our data, these results lead us to speculate that sustained levels of HIV-specific CD4 T-cell helper responses in patients inheriting the *DRB1*15-DQB1*06* alleles may control small bursts of HIV-1 replication, and this may occur either through release of antiviral cytokines, and/or maintenance of help for cytotoxic responses. This is consistent with the general paradigm that CD4 T-cell help is critical in controlling virus infections, including HIV-1.[5–9]

It was important to examine whether the protective effect of the DR15/DQ6 haplotype could be mediated by the presence of an aspartate residue at position 57 on the DQβ chain. Although HLA-DQ6 appears to confer the strongest protection from autoimmune diabetes, other DQβ57Asp-encoding alleles also exert varying degrees of protection.[32] Our survival analysis suggests that this particular structural feature may not, *per se*, confer protection. Rather, our data suggest that *DQB1*06* alleles are protective. The protective effect of *DQB1*06* alleles, when considered irrespective of their HLA-DR haplotype, was most evident in our categorical analysis of patients at 14+ years. Larger cohorts of patients with similar characteristics will be required to establish with confidence whether HLA-DQ6 haplotypes alone confer protection, or whether this effect requires particular structural features or extended haplotypes. The identification of HIV-encoded peptide epitopes that are selectively presented by HLA-DQ, but not by -DR molecules[35] will facilitate functional studies that will help resolve this issue.

HLA molecule-associated delay in HIV-1 disease progression might operate through selective presentation of epitopes found more frequently in diverse HIV-1 proteins. This has been noted for the two most prominent protective HLA class I alleles, *B*27* and *B*57*, that have been found

to promote immunodominant CTL responses to conserved HIV-1 epitopes.[36–38] The same HLA alleles were over-represented in canary pox HIV vaccine recipients with repeatedly detected CTL responses to certain viral proteins.[39] Whether the same phenomenon would apply to MHC class II restricted responses is presently not known. It is known, however, that subjects with *DRB1*13* alleles have a better prognosis in the context of other infections,[40,41] including viruses.[41,42] The pursuit of further comparable population studies combined with the demonstration of the functional significance of the putative markers will help refine and clarify the findings described in this report.

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