

Association of HLA-B22 with Increased Plasma HIV-1 RNA Concentration and Accelerated Progression to AIDS in Caucasian Cohorts

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ABSTRACT

Background: An association of alleles in the HLA-B22 group (*B*54*, **55* and **56*) with faster HIV disease progression has been suggested. B22 group alleles belong to the B7 supertype, as do *B*35* and the closely related *B*53*, also associated with faster progression. B7 supertype alleles preferentially bind CTL peptides containing proline at anchor residue position 2 but have shown different affinities for position 9 residues. We examined the B22 influence on early HIV RNA level and subsequent disease progression in infected Caucasian men from three cohorts.

Subjects and Methods: MACS, DCG, and Amsterdam Cohort Study (ACS) subjects (n=716) with closely estimated onset of infection were HLA typed at A, B and C loci by standard molecular methods. Log₁₀ mean plasma HIV RNA concentration (viral load=VL in copies/ml) was measured (Roche Amplicor/Monitor) for 380 participants in successive intervals during 42 months after seroconversion and analyzed by general linear model techniques. We compared time to AIDS (CDC 1987) in B22-positive (n=32) and -negative (n=684) men by Kaplan-Meier and Cox regression methods. Adjustments were made for other established genetic determinants.

Results: During each post-conversion interval, higher mean VLs (4.45-4.73) were observed for men with than men without a B22 subtype (4.26-4.34; $P=0.002-0.14$). B22-positive subjects progressed significantly faster than B22-negative subjects in each separate cohort and in aggregate (adjusted hazard ratio=1.98, $P=0.001$). The effects were independent of age and presence of *B*35/B*53* and other accepted genetic markers of progression in Caucasians. Other alleles of the B7 supertype have not shown such relationships.

Conclusion: Associations of B22 alleles with higher VL and faster disease progression resemble those reported for *B*35* and *B*53* alleles and contrast with effects of other members of the B7 supertype. Systematic dissection of the similarities and differences in HIV epitope binding among members of the B7 supertype at the molecular level may reveal important mechanisms of viral evasion of or escape from cell-mediated immunity.

INTRODUCTION

Host genetic factors influence the outcome of HIV-1 infection. Among the better characterized markers are the human leukocyte antigen (HLA), C-C chemokine receptor 5 (CCR5) and cytokine genes. In most cases, genotype-phenotype correlations are known and meaning of the association is clear. For example, the immune response against HIV-1 is regulated by the HLA genes, which may encode molecules that generate more effective responses (e.g. against conserved HIV-1 epitopes) or molecules that do not bind or present peptides as well or generate responses only against highly mutable epitopes.

In a case-control investigation of 200 slow and 75 rapid progressors to AIDS in the French cohort GRIV (Hendel, 1999), rapid progression was strongly associated with HLA-B22 serogroup. This was one of several previously unreported HLA associations found, along with certain others already known. With data on different endpoints available in several cohorts, we explored the influence of the HLA-B22 on both early control of infection as measured by HIV-1 RNA level and late outcome as measured by progression to AIDS in infected Caucasian men.

We were particularly interested in the B22 cluster (*B*54*, *B*55*, *B*56* consisting of 18 alleles) because it belongs to the HLA-B7 supertypic group, which also contains the HLA-*B*35* and -*B*53* alleles known for their negative impact on HIV-1 disease progression. The B22 and *B*35* groups have partially similar peptide binding motifs in that they share the affinity for proline at position 2 (P2). However, there are differences in affinities of certain *B*35* alleles for residues at P9, and they have been proposed as the basis for differential association of those *B*35* alleles with progression to AIDS (Gao, 2001). In contrast, the B22 group has shown no preference for any particular residue at position 9, and they might be expected to be more flexible than the *B*35* alleles in their peptide-presenting ability (Barber, 1995).

RESULTS

Descriptive Results: Overall age, mean post-seroconversion HIV-1 RNA levels (in MACS and DCG), and median AIDS-free times were comparable for the SCs from the three cohorts. Frequencies of contributing HLA class I alleles, including those of the B22 serogroup, and contributing CCR haplotype frequencies did not differ substantially among the cohorts. All loci analyzed were in Hardy-Weinberg equilibrium.

Viral Load: Crude mean viral RNA levels calculated jointly and separately for the MACS and DCG participants were consistently higher among B22 positive subjects than among the remaining SCs in all four intervals (Fig 1). The P values presented are adjusted.

Time-to-AIDS: Kaplan-Meier plots jointly and separately for each cohort demonstrated significantly more rapid progression among B22-positive than other SCs (Table 1 and Fig 2).

Linkage Disequilibrium: The observed effect was not due to LD between B22 cluster alleles and any specific A or Cw allele. The Cw allele most commonly found in B22 haplotypes is *Cw*0303*; it was in LD with *B*55* ($P=0.001$; $\Delta=0.0049$), the most frequent Caucasian B22 serogroup allele. However, the association with that haplotype was not nearly as strong as for *B*55* itself. Conversely, *Cw*0303* occurs principally in LD with *B*1501* ($P<0.0001$; $\Delta=0.0128$), but this haplotype was not associated with high viral RNA or rapid disease progression. No important LD between B22 group and alleles at DRB1 was observed.

Figure 1. Mean VL in four intervals

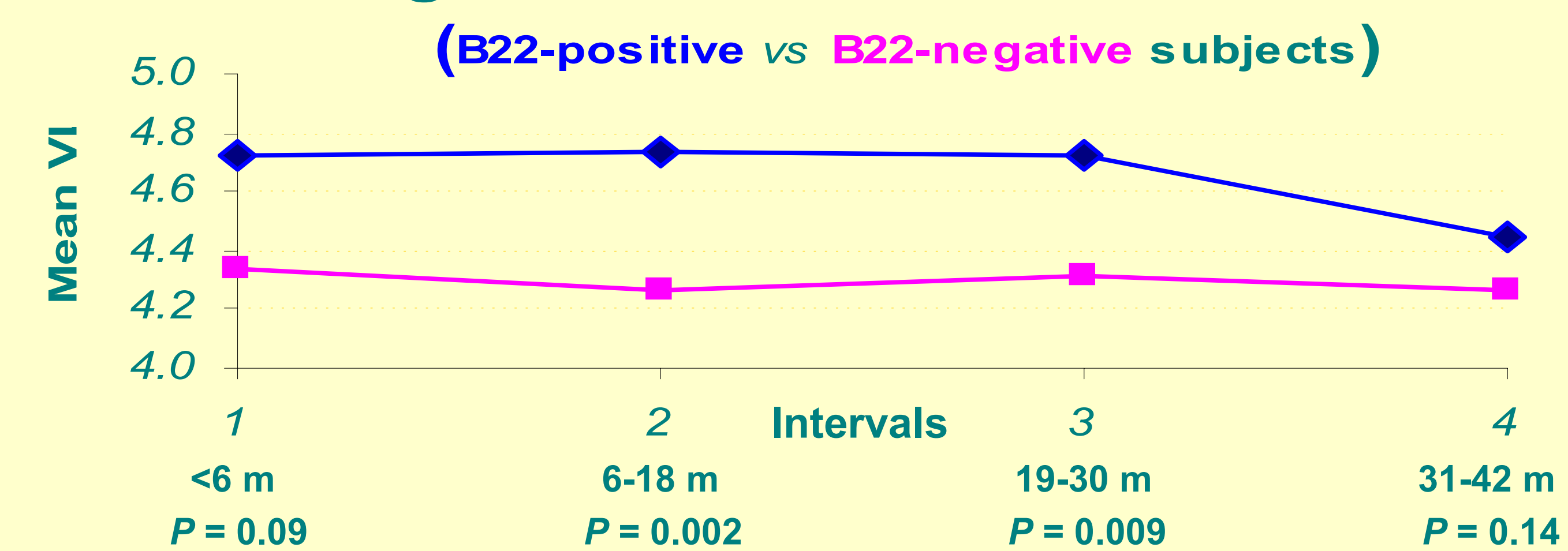


Table 1. Ratio of the hazards of AIDS for B22, adjusted for other associated markers, in the combined group of MACS, ACS and DCG (n=716)

Marker	n	Adjusted HR (95% CI)	P
B22 Serogroup	32	1.98 (1.30 - 3.00)	0.001
Class I homozygosity	219	1.41 (1.12 - 1.78)	0.004
<i>B*27</i> or <i>B*5701</i>	113	0.48 (0.33 - 0.70)	0.0001
<i>B*35</i> or <i>B*5301</i>	153	1.45 (1.12 - 1.88)	0.004
<i>CCR5 E/E</i>	79	1.38 (0.98 - 1.93)	0.063
<i>CCR5 F*2</i> or <i>G*2</i>	227	0.89 (0.70 - 1.14)	0.356

P value for the model <0.0001 . Unadjusted P value for B22 effect is 0.001; HR = 1.99, 95% CI = 1.31 - 3.01

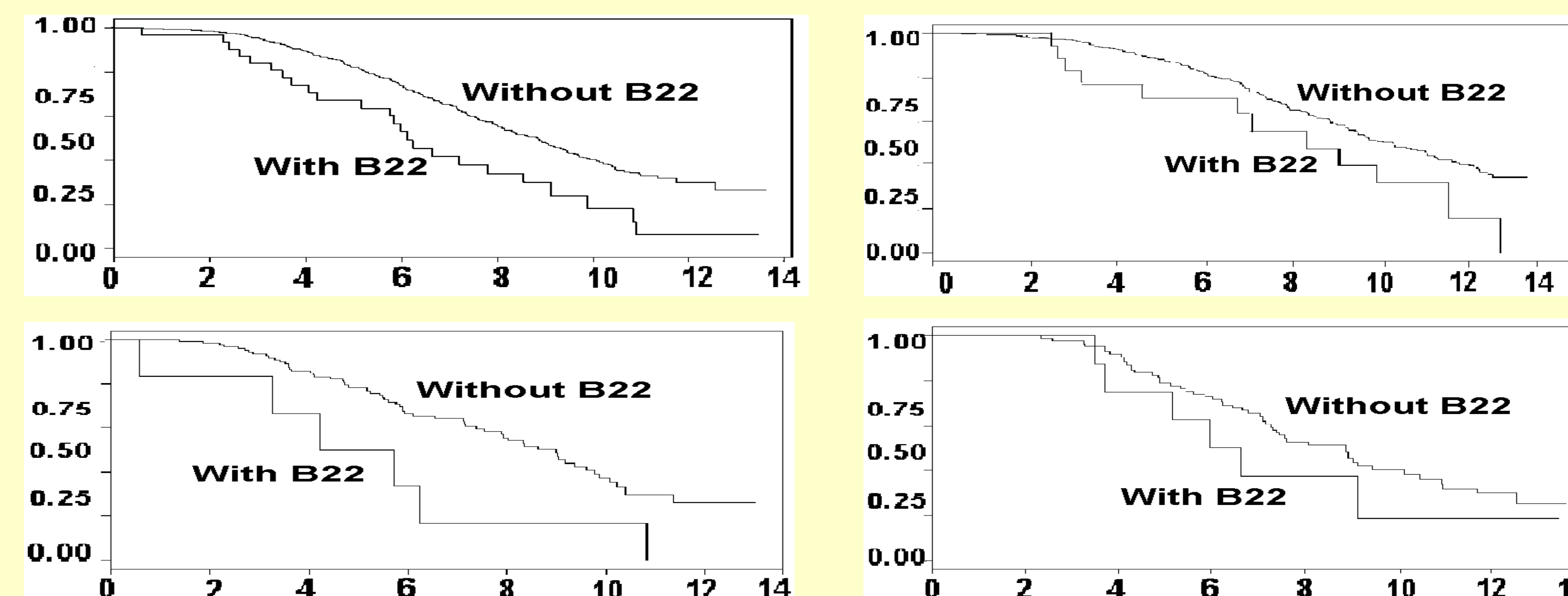


Figure 2. Kaplan-Meier plots of time-to-AIDS in subjects with and without B22 in combined cohorts (top left) and clockwise, MACS, ACS and DCG cohorts.

DISCUSSION

The HLA B22 serogroup was strongly associated with relatively high VL and unfavorable disease course in three cohorts of Caucasian men infected with HIV-1 subtype B. These findings corroborate a similar previous report in Caucasians (Hendel, 1999). Alternatives to a causal explanation for the B22 association seem unlikely. Typical selection bias must have been minimal because the relationship was consistent in three Caucasian cohorts. Ethnic admixture as an explanation is also improbable; it would have required that, in each of three rather diverse cohorts, the B22 lineage marked an ethnic subset with a coincident genetic trait in the cases but not the internal controls. LD with alleles at nearby loci (HLA A or C, or *DRB1*) did not reveal a relationship that could explain these results.

Epidemiologic confirmation that carriage of a B22 allele is disadvantageous may help verify or refute the recently proposed hypothesis about differential effects of *B*35* and *B*53* alleles (Gao, 2001). These alleles are all members of the B7 supertype, characterized by their affinity for peptide binding motifs containing a proline at P2 and any of several alternative residues P9. The unfavorable *B*35* effect appeared to be associated with particular *B*35* alleles (primarily *B*3502/B*3503* in Caucasians) and the closely related *B*53* (in persons of African ancestry) that cannot bind to a peptide with tyrosine (Y) at P9. Because B22 alleles have no such preference for P9 residues (Barber, 1995), they should be capable of presenting a greater variety of HIV-1 peptides and thus more effective at controlling the infection. With multiple cohorts displaying unambiguously increased risk conferred by HLA-B22, on the other hand, alternative mechanistic explanation for these effects may be needed.

An obvious possibility is that prediction based on linear binding motifs instead of conformational ones is not invariably informative. The physical shape and dimensions of the peptide binding grooves of *B*35/B*53* and B22 alleles may still resemble each other enough to make them similarly unfavorable for presentation of HIV-1 peptides, regardless of binding motifs. Alternatively, products of other genes may modulate the peptide-presenting capacity of B22 alleles. Although we excluded obvious candidates for LD with B22 alleles, those at other loci (e.g. nearby MHC class I-like or class III genes) could still account for the B22 effect.

The combined prevalence of B22 (mostly *B*54*) in Asians is about 6%, triple the frequency in Caucasians and Africans. If *B*54* confers the same risk as other B22 alleles, investigators studying persons of Asian ancestry should be alert to the potential clinical and epidemiologic ramifications of that allele along with those of other HLA and CCR determinants of HIV disease progression.

SUBJECTS AND METHODS

Subject selection and outcome measurement: We studied 716 Caucasian men (MACS= 483, DCG = 93 and ACS = 140) who had a relatively closely dated HIV-1 seroconversion (≤ 12 months in $> 90\%$), and follow-up for up to 13 years before the censoring date (January 1, 1996) prior to widespread use of antiretroviral therapy. At least one measurement of serum/plasma HIV-1 RNA concentration was taken within the first 42 months after seroconversion in 341 MACS and 39 DCG participants. For all three groups of SCs we used AIDS (CDC 1987) as the clinical endpoint.

Genotyping: HLA class I genes were typed by a combination of PCR-SSP (Pel-Freez Clinical Systems, USA), sequencing using the ALFexpress automated sequencer (Amersham Pharmacia Biotech Inc., USA) and reference strand conformation analyses (RSCA) (Pel-Freez Clinical Systems, USA). *CCR2* and *CCR5* haplotyping was performed as described by Tang et al (2002).

Statistical methods: Men carrying any allele in the B22 serogroup were compared with those who were not. With general linear model statistics applied to HIV-1 RNA levels, we compared B22 positive subjects to others, adjusted for the effects of class I homozygosity, *B*27*, *B*35*, *B*53*, *B*57*, and *CCR2-CCR5* haplotypes. We used repeated measures analysis for multiple post-seroconversion HIV-1 RNA measurements. For survival analysis, times from seroconversion to AIDS in Kaplan-Meier plots were compared with log-rank tests. By Cox proportional hazards models we estimated the hazard ratio (HR) of AIDS for B22, with adjustment for effects of established markers of progression. Viral RNA measurements (transformed to log₁₀) and survival analyses were censored at January 1, 1996. All analyses were performed in SAS (Statistical Analysis Software, NC).

Linkage Disequilibrium (LD) Analysis: Patterns of LD at HLA class I loci were determined for 313 subjects in the MACS cohort typed at HLA-A, -B, -Cw and -DRB1 loci, using the software PopGene v1.32 (Francis Yeh: <http://www.ualberta.ca/~fyeh>). Hardy-Weinberg equilibrium at the HLA loci was also examined using the same software.

REFERENCES

- Barber LD et al. Overlap in the repertoires of peptides bound in vivo by a group of related class I HLA-B allotypes. *Current Biology* 5:179-90, 1995
- Gao X et al. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med* 344:1668-75, 2001
- Hendel H et al. New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. *J Immunol* 162:6942-6, 1999
- Tang J et al. Distribution of chemokine receptor *CCR2* and *CCR5* genotypes and their relative contribution to HIV-1 seroconversion, early HIV-1 RNA concentration in plasma, and later disease progression. *J Virol* 76:662-72, 2002

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