

Homozygosity for *HLA-Bw4* is not Associated with Protection of HIV-1-Infected Persons of African Ancestry

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ABSTRACT

Background: In HIV-1+ Caucasians, homozygosity at the serogroup level for the Bw4 epitope has been associated with better control of HIV/AIDS (Flores-Villanueva et al, *PNAS* 2001;98:5140). The association appeared independent of a number of reported markers of progression. Interaction between Bw4 and its natural killer (NK) cell receptor was suggested as a possible mechanism. We searched for a protective Bw4 effect in HIV-1+ persons of African ethnicity.

Methods: Class I *HLA* molecular typing has been performed on 202 Rwandans, 160 African-American adolescents, and 259 Zambians. Associations of genetic determinants established elsewhere were observed here in cohort-specific analysis of HIV disease "control"—measured as earlier viral load (VL) in Zambians (clade C), as interval to appearance of clinical and hematologic abnormalities in Rwandans (clade A), and as categories of combined VL and CD4+ cell count in African-Americans (clade B). Proportions of participants who carried 0, 1 ("heterozygous"), or 2 ("homozygous") Bw4+ alleles were compared across disease control categories.

Results: Bw4+ alleles did not occur in successively higher proportions of participants with better disease control. In the presence or absence of B*57 (strongly protective in all three cohorts), homozygous Bw4+ Zambians did not have lower mean VL, homozygous Bw4+ Rwandans experienced no more benign course, and homozygous Bw4+ African-Americans showed no more favorable VL/CD4+ cell combinations. In B*57-negatives, homozygous Bw4+ was found, respectively, in 9, 5 and 12 percent of Zambians with high, intermediate and low VL; in 20, 18, 20 and 12 percent of Rwandans with increasing intervals to recognition of abnormalities; and in 8, 12 and 0 percent of African-Americans with progressively better virologic/immunologic control.

Conclusions: Homozygosity for Bw4 alleles did not protect against disease progression in three African groups infected with different viral subtypes. Confirmation that homozygosity for Bw4 protects Caucasians could imply important differences from ethnic Africans in control of HIV-1 infection through Bw4 modulation of CTL response or ligation with its specific NK cell receptor.

BACKGROUND

Homozygosity at any of the classical *HLA* class I loci is quite disadvantageous for HIV infection.¹⁻³ Individual class I alleles have also been associated with either favorable or unfavorable course of infection. Some have appeared consistently advantageous (B*27³⁻⁶ and B*57^{3,5-7}) or disadvantageous (B*35 and B*53^{1,3,8-9}). Reported associations of other alleles have been less consistent across populations (e.g. A24³, B8^{3-4,10}, B51¹¹, and B14⁵⁻⁶). Differences in populations, study designs, numbers and types of subjects, genetic typing methods, and statistical analytic procedures probably contribute to the more divergent findings.

HLA B-locus alleles display one of two forms of a "public epitope" – Bw4 or Bw6. These alternative forms are defined by amino acids at positions 77-83 near the peptide binding cleft of the heavy chain (Figure 1). The Bw4 motif is also found on a few A-locus alleles but may not function in the same way there. Among ethnic Caucasians and Africans about 40% of B alleles display Bw4 and 60% display the alternative Bw6 motif (Table 1). Bw4 may alter CTL responses by modulating peptide binding or by some other mechanism¹²⁻¹⁴ that could regulate CTL control of viral replication in the pathogenesis of AIDS. Alternatively, because Bw4 serves as principal ligand for certain inhibitory killer immunoglobulin-like receptors (KIRs) on NK cells, it may be central to the interaction between the innate (NK) system and the adaptive (*HLA* class I) systems,¹⁸ as investigated in HIV and other viral infections.¹⁵⁻¹⁷

The recent report¹⁹ that a small group of HIV-1-infected individuals carrying two Bw4+ alleles showed relatively greater suppression of early HIV-1 RNA levels and a larger group experienced longer AIDS-free time has posed a readily testable hypothesis. Here, in three groups of infected subjects of African ethnic background, we describe our test of the hypothesis that Bw4 homozygosity confers virologic and clinical protection.

Fig. 1. Heterogeneity in the Bw4 and Bw6 epitope sequences. Number of HLA alleles in each subgroup is shown on the left, proportion and common HLA-B alleles on the right.

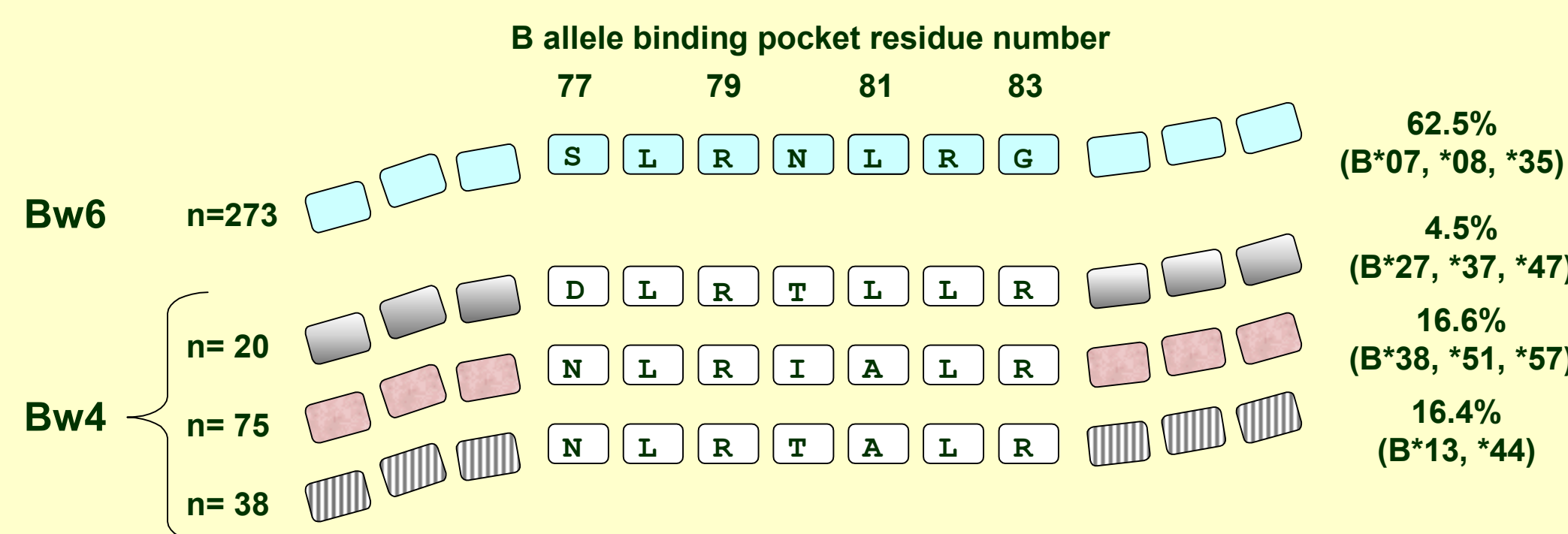


Table 1. Frequency of common alleles displaying Bw4 motif or Bw6 motif in three cohorts of African ancestry

	Bw4			Bw6		
	Rwanda	REACH	Zambia	Rwanda	REACH	Zambia
(TOTAL)	42.8	43.1	35.6	57.2	56.9	64.4
B*15	2.9	3.2	2.6	B*07	5.9	7.8
B*44	3.2	7.8	4.8	B*14	3.7	7.7
B*49	10.9	3.5	1.2	B*15	15.8	10.6
B*51	2.5	0.9	2.1	B*18	3.0	2.2
B*53	5.7	14.7	10.6	B*35	3.0	7.2
B*57	6.9	4.1	3.9	B*42	5.0	6.9
B*58	11.6	6.9	9.1	B*45	11.1	5.9
				B*81	2.0	3.1

SUBJECTS AND METHODS

Subjects: The analysis was performed in three populations of African or African-American ethnic background: 202 Rwandan women infected primarily with HIV-1A, 12 years of follow-up, validated clinical and hematologic staging²; 160 African-American adolescents of both genders from multiple US centers infected with HIV-1B, categorized according to mutually exclusive combinations of HIV-1 RNA levels and CD4+ cell counts²¹; and 259 men and women in the Zambia-UAB HIV Research Project (ZUHRP) infected largely with HIV-1C, categorized according to HIV-1 RNA measurements in the early and middle stages of infection²².

***HLA* class I typing:** *HLA* class I alleles were initially typed by PCR with sequence-specific primers (SSP) (Pel-Freez Clinical Systems, Brown Deer, Wisconsin, USA), which defined alleles largely as two-digit specificities. Individuals with apparent homozygosity at any class I locus were either sequenced using the ALFexpress automated sequencer (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) or analyzed by reference strand conformation analyses (RSCA)²³ (Pel-Freez Clinical Systems, Brown Deer, Wisconsin, USA) for confirmation. Bw4/Bw6 assignment was made according to the sequence data available from the ImMunoGeneTics (IMGT) Database (<http://www.ebi.ac.uk/imgt/hla>).

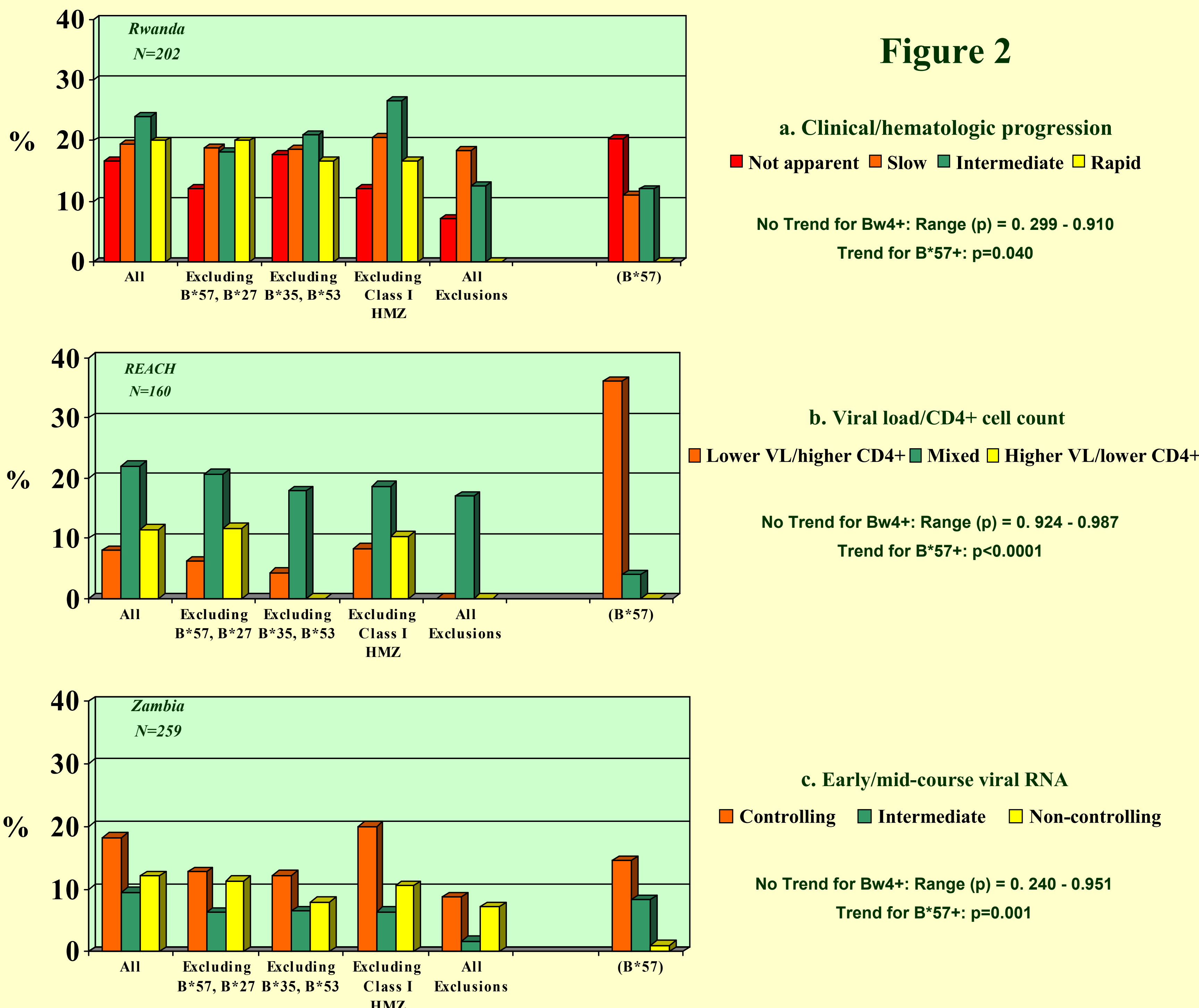
Analysis: In ordered categorical analysis, we examined: 4 groups at successively advanced disease stage, by clinical and hematologic criteria (Rwanda; n = 54, 108, 25, 15), 3 groups at successively advanced disease stage, by combinations of HIV-1 RNA level and CD4+ cell count (REACH; n = 25, 100, 35), and 3 groups with successively higher viral loads (Zambia; n = 99, 105, 55). Proportions of participants who carried 0 or 1 vs 2 ("homozygous") Bw4+ alleles were compared across disease control categories using the Cochran-Mantel-Haenszel test for differences in row mean scores.

Stratification/adjustment: Because the two most favorable alleles (B*27 and B*57) display Bw4 and the two most unfavorable B*08 and B*35 display Bw6, the relative contributions of each must be considered. Homozygosity at class I loci has been associated with high early viral load [Kaslow, unpublished] and rapid progression.¹⁻³

Sensitivity: A high degree of discrimination across above-defined categories has been established by demonstrating a strong B*57 effect in each cohort, consistent with that reported in Caucasians as well.^{3,5,6,7,11}

RESULTS

In each cohort, there was minimal if any evidence for a protective effect of Bw4 homozygosity or of a dose-response relationship in heterozygotes and homozygotes (Figure 2 a, b, c). Exclusion of other factors that might have obscured a Bw4 effect did not materially alter the overall relationships. In contrast, the recognized protective effect (from B*57) was apparent in all three cohorts, indicating sufficient power and sensitivity to detect a protective effect of moderate magnitude when it was present.



DISCUSSION

We observed no protective effect of Bw4 homozygosity in three populations of African ancestry. Several possible explanations should be considered for that observation, in strong contrast to findings reported in two groups of North American Caucasians.¹⁹ To begin with, the ethnic Africans resembled the Caucasians broadly in their distributions by age, gender and duration of infection; those factors could not have accounted for such a striking difference. Nor was the difference due to viral subtype: both the REACH African-Americans and the Caucasians in the published report were infected with subtype B.

Study size, subject selection and analytic approach in the two published groups may have partially explained the differences. The study of HIV-1 viremia included only 39 subjects in controller and non-controller groups, which appeared to be ethnically/genetically heterogeneous.²⁴ Together, B*27 and B*57 occurred quite frequently in controllers with well suppressed viremia, and the Bw4/Bw4 protective effect depended heavily on that unequal distribution. In the second published group, survival analysis of clinical/immunologic outcome in 103 men with adjustments for effects of several known factors like B*57 revealed a similarly favorable Bw4 effect. Adjustment for *HLA A, B, and C* homozygosity, somewhat more likely among individuals with Bw6 alleles (e.g. B*08 and B*35), was not described; however, Bw6-related homozygosity probably could have only partly explained the association.

Is it possible that the Bw4 protects in ethnic Caucasians but not in Africans? As yet unknown modifiers could operate differentially in the two racial groups. However, data from other sources suggest that Caucasians do not uniformly exhibit the favorable Bw4 effect. First, preliminary analyses in other cohorts of Caucasians indicate only slight a trend toward protection, which is dampened when analyses account for additional known genetic effects (Kaslow, unpublished data). Second, a study of CTL response to canarypox HIV vaccine²⁰ offers another indirect indication; it confirmed the strong influence of B*27 and B*57 but not an independent effect of Bw4 homozygosity.

Of course, it is possible that the observed Bw4 protection is unrelated to the classical class I-restricted CTL response. As the ligand for an inhibitory NK receptor, Bw4 may act via the NK pathway instead. In that case, numerous further questions would arise. Which structural or regulatory elements encoded by the HIV genome interact with the NK system through Bw4? Because Bw4 alleles show slight sequence polymorphism (Figure 1), do they all mediate NK inhibition equally well, or do those Bw4 sequence variations produce functional differences in NK response? How does the small Bw4 piece fit into the highly complex puzzle of interactions between *HLA-B* and *-C* as ligands for multiple NK cell receptors?

In the absence of any evidence for a protective Bw4 effect in HIV-1-infected individuals of African ancestry, extensive effort at elucidating the mechanism by which Bw4 may reduce viral burden and retard disease progression should probably await results of analysis in additional Caucasian populations.

CONCLUSIONS

* Homozygosity for the Bw4 public specificity on *HLA-B* alleles was not associated with more favorable virologic, immunologic or clinical response to HIV-1 in infected subjects of African ethnic background.

* Failure to observe any protective association was not due to lack of power or discriminatory capacity within the comparison groups because the protection known to be conferred by B*57 was readily detected in each cohort.

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