

## EFFECT OF A SINGLE AMINO ACID CHANGE IN MHC CLASS I MOLECULES ON THE RATE OF PROGRESSION TO AIDS

XIAOJIANG GAO, PH.D., GEORGE W. NELSON, PH.D., PETER KARACKI, B.A., MAUREEN P. MARTIN, M.D., JOHN PHAIR, M.D., RICHARD KASLOW, M.D., JAMES J. GOEDERT, M.D., SUSAN BUCHBINDER, M.D., KEITH HOOTS, M.D., DAVID VLAHOV, PH.D., STEPHEN J. O'BRIEN, PH.D., AND MARY CARRINGTON, PH.D.

### ABSTRACT

**Background** From studies of genetic polymorphisms and the rate of progression from human immunodeficiency virus type 1 (HIV-1) infection to the acquired immunodeficiency syndrome (AIDS), it appears that the strongest susceptibility is conferred by the major-histocompatibility-complex (MHC) class I type HLA-B\*35,Cw\*04 allele. However, cytotoxic T-lymphocyte responses have been observed against HIV-1 epitopes presented by HLA-B\*3501, the most common HLA-B\*35 subtype. We examined subtypes of HLA-B\*35 in five cohorts and analyzed the relation of structural differences between HLA-B\*35 subtypes to the risk of progression to AIDS.

**Methods** Genotyping of HLA class I loci was performed for 850 patients who seroconverted and had known dates of HIV-1 infection. Survival analyses with respect to the rate of progression to AIDS were performed to identify the effects of closely related HLA-B\*35 subtypes with different peptide-binding specificities.

**Results** HLA-B\*35 subtypes were divided into two groups according to peptide-binding specificity: the HLA-B\*35-PY group, which consists primarily of HLA-B\*3501 and binds epitopes with proline in position 2 and tyrosine in position 9; and the more broadly reactive HLA-B\*35-Px group, which also binds epitopes with proline in position 2 but can bind several different amino acids (not including tyrosine) in position 9. The influence of HLA-B\*35 in accelerating progression to AIDS was completely attributable to HLA-B\*35-Px alleles, some of which differ from HLA-B\*35-PY alleles by only one amino acid residue.

**Conclusions** This analysis shows that, in patients with HIV-1 infection, a single amino acid change in HLA molecules has a substantial effect on the rate of progression to AIDS. The different consequences of HLA-B\*35-PY and HLA-B\*35-Px in terms of disease progression highlight the importance of the epitope specificities of closely related class I molecules in the immune defense against HIV-1. (N Engl J Med 2001; 344:1668-75.)

Copyright © 2001 Massachusetts Medical Society.

**H**LA class I molecules present antigenic epitopes to T lymphocytes, thereby initiating a specific immune response and the clearance of foreign material.<sup>1-3</sup> The genes encoding HLA class I molecules are highly polymorphic. The great diversity of these genes appears to have been selected over time so that mammals can

resist a wide variety of pathogens.<sup>4,5</sup> If this model accurately explains the diversity of class I molecules, alleles encoding functionally distinct molecules should provide a range of protection against a given pathogen. However, accurately assigning HLA alleles or loci to the defense against a particular infectious disease has been difficult for several reasons. Because of the extreme polymorphism and fairly even distribution of alleles that characterize the HLA loci, studies would require large cohorts to achieve sufficient statistical power. The effects of the genetic makeup of the host on infectious diseases are complex and may involve multiple loci, a fact that complicates analyses of the influence of HLA alleles on disease. The patterns of linkage disequilibrium (the nonrandom association between two linked loci) among the many functionally related loci in the major-histocompatibility-complex (MHC) genes also make it difficult to identify the causative disease locus.

HLA-B\*35, which almost always neighbors HLA-Cw\*04 on chromosome 6, has been the only allele consistently associated with rapid progression to the acquired immunodeficiency syndrome (AIDS) among a large number of conflicting or unconfirmed associations between HLA alleles and various outcomes in patients with human immunodeficiency virus type 1 (HIV-1).<sup>6-10</sup> Strong evidence for an effect of HLA-B\*35,Cw\*04 on progression to AIDS has been observed in white but not in black HIV-infected patients for whom the date of seroconversion is known, raising the question of whether HLA-B\*35 or HLA-Cw\*04 operates immunologically or, alternatively, is simply associated with another locus that accelerates the progression to AIDS.<sup>10</sup> Protection against HIV-1 and simian immunodeficiency virus (SIV) has been strongly correlated with cytotoxic-T-lymphocyte activity.<sup>11-14</sup> The molecule encoded by HLA-B\*3501, the most common allele among HLA-B\*35 subtypes,

From the Intramural Research Support Program, Science Applications International Corporation Frederick and the National Cancer Institute, Frederick, Md. (X.G., G.W.N., M.P.M., M.C.); Johns Hopkins School of Medicine, Baltimore (P.K.); Northwestern University Medical School Comprehensive AIDS Center, Chicago (J.P.); the Department of Epidemiology, University of Alabama at Birmingham School of Public Health, Birmingham (R.K.); the Viral Epidemiology Branch, National Cancer Institute, Bethesda, Md. (J.J.G.); the San Francisco Department of Public Health, San Francisco (S.B.); the Gulf States Hemophilia Center, University of Texas Health Science Center, Houston (K.H.); Johns Hopkins School of Hygiene and Public Health, Baltimore (D.V.); and the Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Md. (S.J.O.). Address reprint requests to Dr. Carrington at P.O. Box B, NCI-FCRDC, Frederick, MD 21702, or at carringt@mail.ncifcrf.gov.

is known to bind and present a variety of HIV-1 antigenic epitopes and to induce cytotoxic-T-lymphocyte reactivity to these epitopes.<sup>1-3</sup> Thus, susceptibility associated with HLA-B\*35 cannot be attributed to an inability of HLA-B\*3501 to elicit cytotoxic-T-lymphocyte reactivity.

Differences in the amino acid sequences of the HLA class I peptide-binding region have been shown to affect the binding of peptides, particularly in the B and F pockets of the HLA molecule, which interact with the second amino acid residue (P2) and the carboxy-terminal amino acid residues (P9, in most cases) of bound peptides, respectively.<sup>15</sup> In this study, we investigated the rate of progression to AIDS among patients with subtypes of HLA-B\*35 alleles that correlate with the peptide-binding properties of each subtype.

## METHODS

### Patients

HIV-1-infected patients for whom the dates of seroconversion were known were from five cohorts: the Multicenter AIDS Cohort Study (MACS),<sup>16</sup> the Multicenter Hemophilia Cohort Study (MHCS),<sup>17</sup> the Hemophilia Growth and Development Study (HGDS),<sup>18</sup> the San Francisco City Clinic Cohort (SFCC),<sup>19</sup> and the AIDS Linked to Intravenous Experience (ALIVE) Study.<sup>20</sup> A total of 592 white patients, 219 black patients, and 39 patients from other racial groups were included in our study. Patients from the MACS and ALIVE cohorts who seroconverted were representative of all HIV-infected patients, whereas patients from the SFCC and MHCS cohorts showed a moderate survival bias, because biologic samples were unavailable for patients with the most rapid rates of progression to AIDS.<sup>21</sup>

### HLA Typing

For HLA typing, genomic DNA was isolated from lymphoblastoid B-cell lines or from peripheral-blood lymphocytes and amplified with a panel of 96 specific primers by the polymerase chain reaction (PCR) for HLA-A, B, and C.<sup>22</sup> Each reaction included primers used as positive controls that amplified a 796-bp fragment from the third intron of HLA-DRB1. HLA PCR products underwent electrophoresis on 1.5 percent agarose gels containing ethidium bromide, and PCR products were visualized under ultraviolet light. More precise typing for HLA-B\*35-related subtypes was performed by direct sequencing of the PCR product.<sup>23</sup> The sequences were analyzed with MatchTools and MT Navigator Allele Identification software (Applied Biosystems Division, Perkin-Elmer, Foster City, Calif.).

### Statistical Analysis

Survival analyses were performed separately for white and black patients from the combined cohorts (MACS, MHCS, HGDS, SFCC, and ALIVE) who seroconverted.<sup>16-20</sup> Four AIDS-related outcomes were considered end points of survival analysis: a CD4 T-lymphocyte count of less than 200 per cubic millimeter, progression to AIDS according to the 1993 definitions of the Centers for Disease Control and Prevention (CDC),<sup>24</sup> progression to AIDS according to the CDC's more restrictive 1987 definition,<sup>25</sup> and death from an AIDS-related cause. We performed Kaplan-Meier and Cox model analyses using the LIFETEST and PHREG procedures of the SAS System (SAS Institute, Cary, N.C.). Genetic factors with a confirmed effect on progression to AIDS (variant chemokine receptor alleles CCR5Δ32 and CCR2-641) were included as confounding covariates in all Cox model analyses<sup>26,27</sup>; overall homozygosity for HLA class I alleles was included as a confounding covariate in some analyses, as noted.<sup>10</sup>

## RESULTS

### Association of HLA Class I Alleles with Progression to AIDS

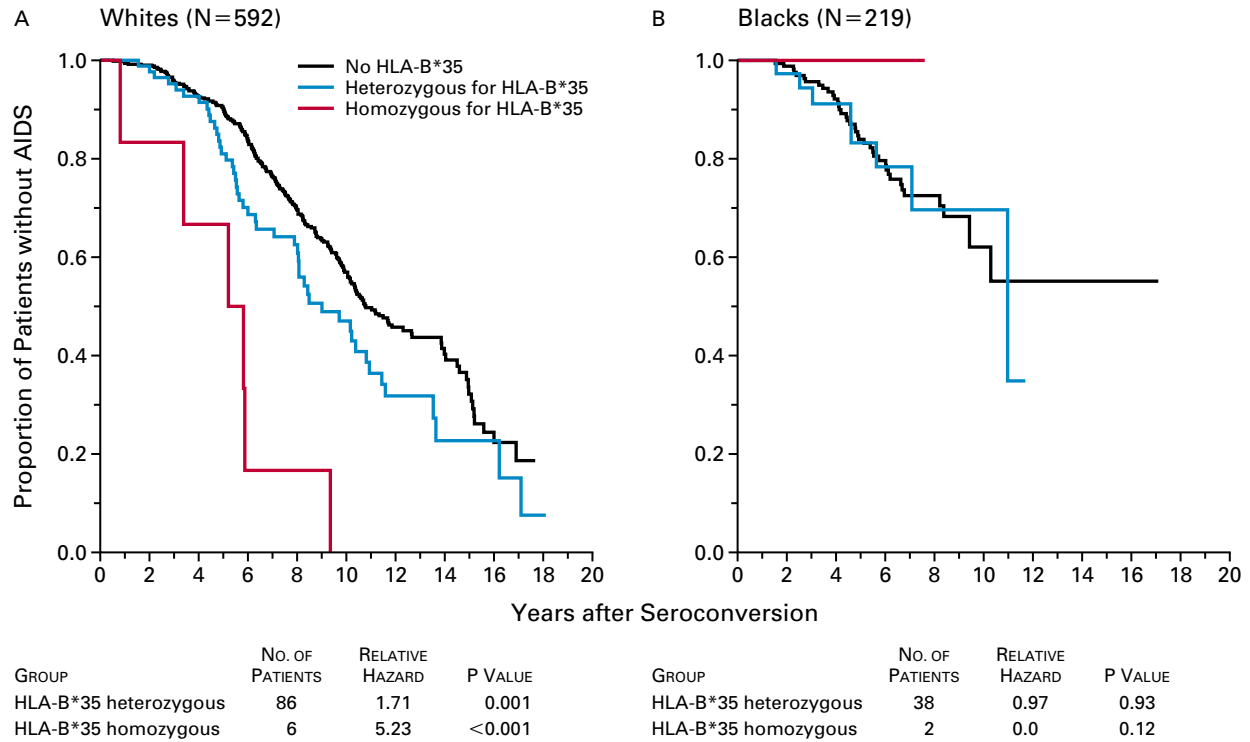
The influence of 61 individual HLA alleles (grouped according to serologic specificities) on the rate of progression to AIDS was determined in a sample of 592 white and 219 black patients who had seroconverted (for more information, see <http://rex.nci.nih.gov/lgd/pubs/2001.htm>). HLA-B\*27 showed a protective effect against progression to AIDS in whites (relative hazard of progression in those with the allele, 0.43; 95 percent confidence interval, 0.26 to 0.72;  $P=0.001$ ), as was previously predicted for this allele,<sup>28,29</sup> and HLA-B\*57 was also weakly protective in whites (relative hazard, 0.55; 95 percent confidence interval, 0.31 to 0.99;  $P=0.04$ ), also confirming previous reports.<sup>30,31</sup> The results also agree with the previous observation of a strong susceptibility to AIDS in whites with the HLA-B\*35 group of alleles and HLA-Cw\*04,<sup>6-10</sup> which are the only HLA class I genotypes significantly associated with progression to AIDS after statistical correction for multiple tests<sup>32-34</sup> (Fig. 1).

Survival analysis of the effect of all HLA-B\*35 subtypes combined indicated that the effects of HLA-B\*35 are codominant. The relative hazards of progression of 1.71 for patients with a single copy and 5.23 for those with two copies of any HLA-B\*35 allele were significant (Fig. 1). However, no effect of the HLA-B\*35 group of alleles was observed in blacks, although the lack of an effect of homozygosity for HLA-B\*35 in this racial group is inconclusive because the sample included only two patients who were homozygous for HLA-B\*35.

### Association of HLA-B\*35 Subtypes with Progression to AIDS

Only a single subtype of HLA-Cw\*04 (HLA-Cw\*0401) was present in both the white and the black cohorts. On the other hand, five HLA-B\*35 alleles were present in our patients; they encode products that vary from each other by no more than three amino acids throughout the entire HLA molecule (Table 1). The amino acid composition of the P2 pocket, which recognizes peptides that have proline (P) at the second position (P2), is identical in all of these HLA-B\*35 molecules. However, the amino acid composition of the P9 pocket varies in the different HLA-B\*35 subtypes, corresponding to variation in amino acid preference at the carboxyl terminal of the presented peptide.

We included the HLA-B\*53 allele in our analysis of HLA-B\*35 subtypes for several reasons. First, HLA-B\*5301 is phylogenetically closely related to the HLA-B\*35 group of alleles and is probably derived from a single gene conversion within the sequence encoding the P9 pocket of an HLA-B\*35 precursor.<sup>35,36</sup> Second, HLA-B\*5301 binds specifically



**Figure 1.** Survival Analysis of the Effect of HLA-B\*35 on AIDS-free Survival (According to the 1987 CDC Definition) in White Patients (Panel A) and Black Patients (Panel B) from Combined Cohorts.

Patients who were heterozygous (blue curve) or homozygous (red curve) for HLA-B\*35 are compared with patients who did not carry HLA-B\*35. Relative hazards and significance were calculated in Cox-model analyses, with heterozygosity and homozygosity for HLA-B\*35 considered as covariates, and with overall HLA class I homozygosity and the presence of protective genotypes of the chemokine receptors CCR2 or CCR5 considered as confounding covariates.<sup>10,26,27</sup>

**TABLE 1.** VARIATIONS IN THE PEPTIDE-BINDING SITES AND MOTIFS OF HLA-B\*35 SUBTYPES DETECTED IN PATIENTS WITH AIDS AND THEIR EFFECT ON PROGRESSION TO AIDS.\*

HLA ALLELE	PEPTIDE-BINDING SITE†						ANCHOR RESIDUE		WHITE PATIENTS (N=592)			BLACK PATIENTS (N=219)		
	77	80	81	114	116	156	P2	P9	FREQUENCY (%)	RELATIVE HAZARD (95% CI)	P VALUE	FREQUENCY (%)	RELATIVE HAZARD (95% CI)‡	P VALUE
B*35-Px														
B*3502	S	N	L	N	Y	L	P	L/V	1.3	2.90 (1.57–5.37)	<0.001	0.0	—	—
B*3503	—	—	—	D	F	—	P	M/L	2.2	2.70 (1.70–4.30)	<0.001	0.5	—	—
B*3504	—	—	—	—	—	—	P	L/V	0.1	0.0	0.87	0.0	—	—
B*5301	N	I	A	D	S	—	P	None	0.8	1.70 (0.68–4.23)	0.25	12.3	2.11 (1.13–3.95)	0.02
Total							P	X§	4.4	2.65 (1.84–3.82)	<0.001	12.8	2.06 (1.10–3.86)	0.02
B*35-PY														
B*3501	—	—	—	D	S	—	P	Y	4.3	1.22 (0.80–1.88)	0.36	8.9	0.94 (0.43–2.04)	0.87
B*3508	—	—	—	D	S	R	P	Y	0.4	2.22 (0.70–7.03)	0.17	0.0	—	—
Total							P	Y	4.7	1.30 (0.87–1.95)	0.20	8.9	0.94 (0.43–2.04)	0.87

\*A dominant model was used, in which the rate of progression to AIDS (according to the 1987 definition of the CDC) in patients carrying one or two copies of the HLA allele was compared with that in patients without that allele. CI denotes confidence interval. Relative hazards, significance, and confidence intervals were calculated with the use of the Cox model, with overall class I homozygosity and the presence of protective genotypes of the chemokine receptors CCR2 and CCR5 considered confounding covariates.<sup>10,26,27</sup>

†Numbers indicate amino acid positions. Dashes indicate identity with the sequence listed for B\*3502.

‡The relative hazard for B\*3503 was not calculated, but this allele was included in the total for P\*35-Px.

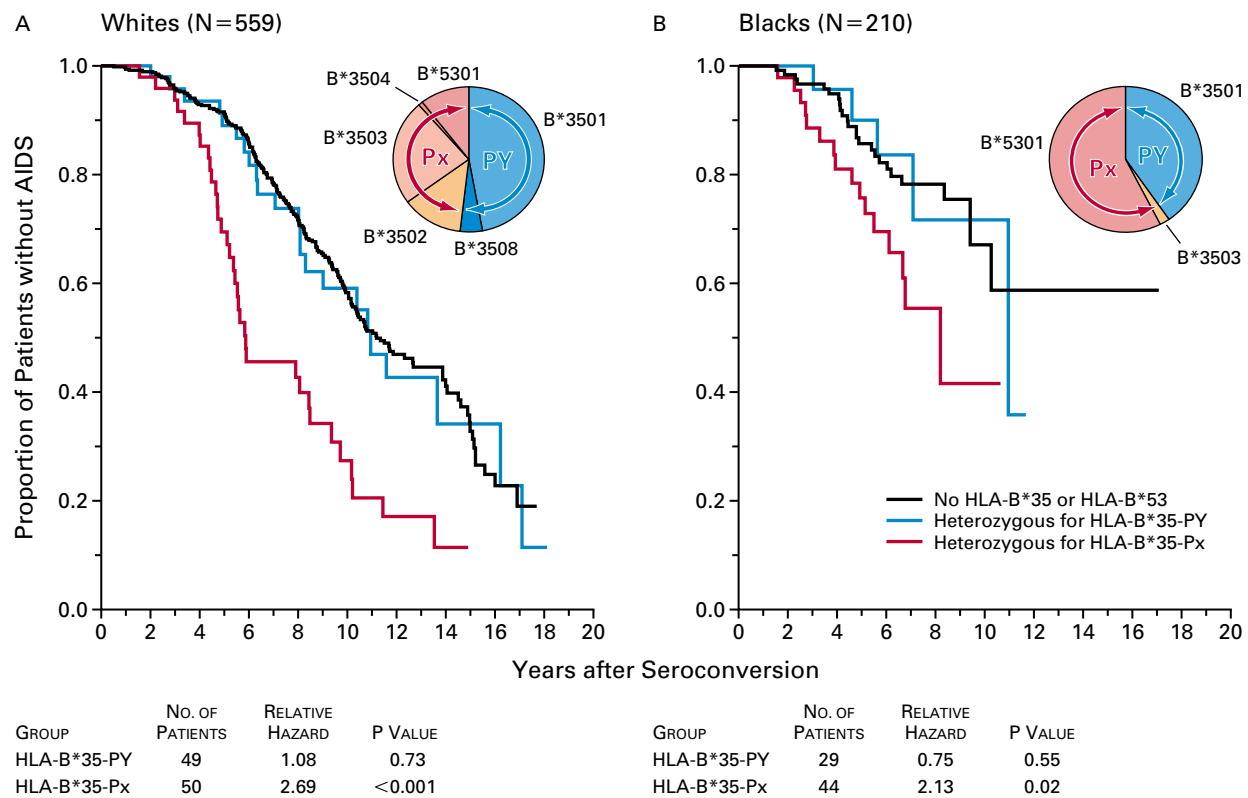
§X indicates a preference at the P9 pocket for a hydrophobic amino acid other than tyrosine or no clear preference.

to proline at P2 and nonspecifically at the carboxy-terminal (P9) site; these specificities are similar but not identical to those of other HLA-B\*35 subtypes (Table 1). Third, black participants carrying one or two copies of the HLA-B\*53 allele, in whom HLA-B\*5301 has a relatively high frequency (12.3 percent), showed a significant predisposition to rapid progression to AIDS (relative hazard, 2.11; 95 percent confidence interval, 1.13 to 3.95;  $P=0.02$ ).

The HLA-B\*35 and B\*53 subtypes fall into two general groups on the basis of peptide-binding preference: those that bind peptides containing proline at the P2 position but that show no preference for a specific amino acid at P9, which are termed the HLA-B\*35-Px group (P indicates proline and x indicates no single preference; the group includes HLA-B\*3502, B\*3503, B\*3504, and B\*5301); and those that bind peptides containing proline at P2 and tyrosine at P9, which are termed the HLA-B\*35-PY group (P indi-

cates proline and Y indicates tyrosine; the group includes HLA-B\*3501 and B\*3508) (Table 1). Survival analyses with respect to the progression to AIDS (according to the 1987 definition of the CDC)<sup>24</sup> were performed individually for all HLA-B\*35 subtypes (plus HLA-B\*5301) to test whether variability among subtypes might affect the rate of progression to AIDS (Table 1).

A striking observation was that HLA-B\*3501, the most common of the HLA-B\*35 subtypes, had no effect on disease progression in either racial group ( $P>0.3$ ) (Table 1). The effect of HLA-B\*35 on progression to AIDS in whites (Fig. 1A) can be attributed entirely to two subtypes, HLA-B\*3502 and B\*3503, both of which are significantly associated with rapid progression to AIDS (relative hazard for HLA-B\*3502, 2.90; relative hazard for HLA-B\*3503, 2.70;  $P<0.001$  for both) (Table 1), even though these two subtypes differ from HLA-B\*3501



**Figure 2.** Survival Analysis of the Effect of HLA-B\*35 Subtypes on AIDS-free Survival (According to the 1987 CDC Definition) in Patients with One Copy of an HLA-B\*35-PY Allele (B\*3501 or B\*3508) (Blue Curve) as Compared with Patients with One Copy of an HLA-B\*35-Px Allele (B\*3502, B\*3503, B\*3504, or B\*5301) (Red Curve) and Patients with No HLA-B\*35 or HLA-B\*53 Alleles (Black Curve). Only patients who were heterozygous for HLA-B were considered in the analysis, to exclude the strong, documented AIDS-accelerating effect of homozygosity at HLA-A, B, and C alleles.<sup>10</sup> The subjects were whites (Panel A) and blacks (Panel B) from combined cohorts who seroconverted. Relative hazards and significance are given for separate Cox-model analyses comparing each group with all other subjects in the analysis, with homozygosity at HLA-A or C and the presence of protective genotypes of the chemokine receptors CCR2 or CCR5 considered as confounding covariates.<sup>10,26,27</sup> The pie charts show the allelic frequencies for all patients who seroconverted in the two groups.

by only one or two residues in the peptide-binding region.<sup>37,38</sup> Furthermore, HLA-B\*5301 was significantly associated with progression to AIDS in blacks (relative hazard, 2.11;  $P=0.02$ ) and tends to be associated with susceptibility to progression to AIDS in whites (relative hazard, 1.70), although this trend falls short of significance ( $P=0.25$ ). The predominance of HLA-B\*3501 (which has no influence on progression to AIDS), as compared with all other HLA-B\*35 alleles in blacks (Fig. 2), probably accounts for our failure to detect an effect of HLA-B\*3501 on susceptibility in this racial group (blue vs. black curves in Fig. 1B).

Survival analysis involving four HLA-B\*35-Px alleles combined (HLA-B\*3502, B\*3503, B\*5301, and B\*3504), as compared with two B\*35-PY alleles (HLA-B\*3501 and B\*3508), was performed to assess the overall effect of shared attributes of peptide recognition on the progression to AIDS (Fig. 2). Only patients who were heterozygous for HLA-B were considered, to exclude the strong influence of homozygosity on progression to AIDS.<sup>10</sup> The results demonstrate a significant association with progression to AIDS in patients who had an HLA-B\*35-Px allele ( $P<0.001$  for whites,  $P=0.02$  for blacks). On the other hand, there was no apparent difference in the rate of progression between patients with an HLA-B\*35-PY allele and those without an HLA-B\*35 allele. Furthermore, among whites, the relative hazard of 2.69 that was determined for HLA-B\*35-Px (Fig. 2A) was greater than that for HLA-B\*35 as a whole (relative hazard, 1.71) (Fig. 1), since the over-

all HLA-B\*35 signal in Figure 1 is diminished by the inclusion of the patients with HLA-B\*35-PY.

We calculated relative hazards for the two HLA-B\*35 groupings, HLA-B\*35-Px and HLA-B\*35-PY, for the various AIDS-defining end points<sup>24,25</sup> (Table 2). Relative hazards were most significant with the use of a dominant model (including homozygotes and heterozygotes for a given allele in a single group), and  $P$  values were more significant for the later outcomes (particularly AIDS according to the 1987 CDC definition) in both white and black patients carrying HLA-B\*35-Px alleles, although an effect of these alleles was evident even in whites with CD4 cell counts of less than 200 per cubic millimeter, an early end point. The epidemiologic association of the HLA-B\*35-Px subtypes with progression to AIDS as compared with the HLA-B\*35-PY subtypes is consistent with the notion that the shared attributes of peptide recognition of the HLA-B\*35-Px alleles provide the functional explanation for rapid progression to AIDS in heterozygotes.

**Association of HLA-Cw\*04 with Progression to AIDS**

The differential association of the HLA-B\*35-PY and the HLA-B\*35-Px groups of alleles with progression to AIDS led us to reexamine the previously observed association of HLA-Cw\*04 with progression to AIDS.<sup>10</sup> Three of our observations point to the conclusion that most, if not all, of the association of HLA-Cw\*04 with rapid progression is due to its linkage disequilibrium with HLA-B\*35-Px alleles. First, all patients who were heterozygous for

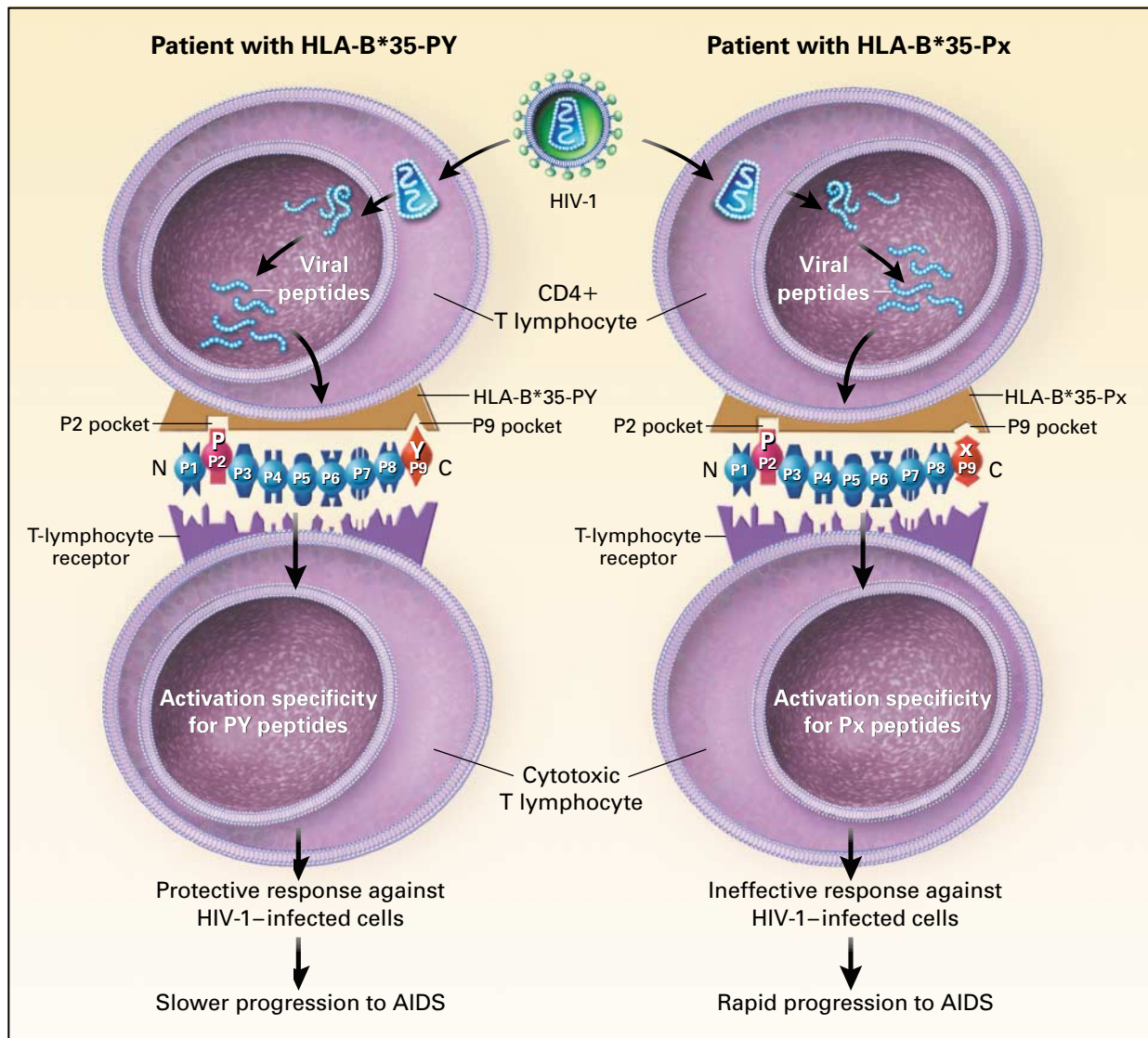
**TABLE 2. EFFECT OF GENOTYPES INCLUDING HLA-B\*35-Px AND HLA-B\*35-PY ON PROGRESSION TO FOUR AIDS-RELATED END POINTS.\***

AIDS-RELATED END POINT	No. OF PATIENTS	HLA-B*35-Px		HLA-B*35-PY	
		RELATIVE HAZARD (95% CI)	P VALUE	RELATIVE HAZARD (95% CI)	P VALUE
Whites	562				
CD4 cell count <200		1.77 (1.23–2.55)	0.002	0.73 (0.47–1.13)	0.16
AIDS (1993)†		1.94 (1.39–2.71)	<0.001	0.90 (0.62–1.31)	0.59
AIDS (1987)‡		2.69 (1.86–3.88)	<0.001	1.08 (0.69–1.69)	0.73
Death		2.51 (1.72–3.65)	<0.001	1.32 (0.86–2.02)	0.20
Blacks	211				
CD4 cell count <200		1.23 (0.72–2.09)	0.45	1.51 (0.81–2.28)	0.19
AIDS (1993)†		1.40 (0.87–2.23)	0.16	1.34 (0.75–2.38)	0.32
AIDS (1987)‡		2.13 (1.12–4.04)	0.02	0.75 (0.29–1.93)	0.55
Death		1.94 (0.85–4.40)	0.11	1.17 (0.39–3.45)	0.78
All racial groups	810				
CD4 cell count <200		1.59 (1.19–2.14)	0.002	0.92 (0.65–1.23)	0.63
AIDS (1993)†		1.77 (1.35–2.31)	<0.001	1.02 (0.75–1.38)	0.91
AIDS (1987)‡		2.37 (1.73–3.25)	<0.001	1.11 (0.76–1.63)	0.60
Death		2.25 (1.60–3.16)	<0.001	1.40 (0.96–2.05)	0.88

\*Relative hazards, significance, and confidence intervals (CI) were calculated for a dominant model as described in Figure 2.

†AIDS was defined according to the 1993 criteria of the CDC.

‡AIDS was defined according to the 1987 criteria of the CDC.



**Figure 3.** Model of the Difference in the Rate of Progression to AIDS between Patients with HLA-B\*35-Px and Those with HLA-B\*35-PY. HIV-1 replicates in the infected cells, and some viral protein is degraded into small peptides. The viral peptides, typically nine amino acids in length (P1 to P9), are bound by HLA class I molecules that have appropriate P2- and P9-anchoring pockets, and these complexes are expressed on the cell surface. Amino acids forming the P2-anchoring pockets of the HLA-B\*35-PY and HLA-B\*35-Px molecules are identical, and both pockets have a preference for proline (P) in the P2 position of the bound peptide. However, minor differences in the P9 pockets lead to distinct binding preferences for the carboxy-terminal amino acid of the peptides. The HLA-B\*35-PY molecules (HLA-B\*3501 and B\*3508) have a preference for tyrosine (Y) at the carboxy terminal of the peptide (P9), whereas the HLA-B\*35-Px molecules (HLA-B\*3502, B\*3503, B\*3504, and B\*5301) do not have a single clear preference at P9 (x) and do not bind peptides that have tyrosine at the carboxy terminal. T-cell receptors recognize distinct HIV-1 peptides bound by specific class I molecules expressed by the virally infected target cell. A poor or inappropriate cytotoxic-T-lymphocyte response occurs in patients with HLA-B\*35-Px, as is suggested by the rapid progression to AIDS in these patients, whereas a relatively protective response occurs in patients with HLA-B\*35-PY that corresponds with the slower progression to disease in these patients.

HLA-B\*3501 in our cohorts were also positive for HLA-Cw\*04, and rapid progression to AIDS is not associated with this haplotype in white or black heterozygotes (blue line in Fig. 2). Second, patients with HLA-Cw\*04 but without HLA-B\*35-Px subtype alleles are indistinguishable from those without HLA-

Cw\*04 in terms of the rate of progression to AIDS (further information is available at <http://rex.nci.nih.gov/lgd/pubs/2001.htm>). Third, in three of four whites who were heterozygous for HLA-B\*35-Px but who did not have HLA-Cw\*04, progression to AIDS occurred very rapidly (less than five years after

seroconversion). Thus, the differences in the rate of progression to AIDS between the HLA-B\*35-Px and HLA-B\*35-PY allele groups indicate that the previously observed HLA-Cw\*04 effect is predominantly, if not totally, due to linkage disequilibrium with HLA-B\*35-Px.

### DISCUSSION

The use of large, clinically well-defined cohorts in this study has allowed the identification of specific subtypes of HLA-B\*35 as responsible for the previously reported association between HLA-B\*35 and rapid progression to AIDS. The most common HLA-B\*35 subtype allele, HLA-B\*3501, has little or no effect on progression to AIDS in either white or black patients. The finding that specific HLA-B\*35-Px subtypes have similar effects in blacks and whites strongly supports the hypothesis that these HLA-B alleles exert an effect on the immune response to HIV-1 disease.

Peptide-binding assays have shown that amino acid substitution in the heavy chain at positions 114 (HLA-B\*3502) and 116 (HLA-B\*3502 and B\*3503) abolished the ability of the P9 pocket of HLA-B\*3501 to bind tyrosine at the carboxy-terminal anchor.<sup>39</sup> The relatively shallow P9 pockets of HLA-B\*3502 and B\*3503 do not bind tyrosine but preferentially accommodate smaller hydrophobic residues such as methionine, valine, or leucine.<sup>39</sup> The P9 pocket of HLA-B\*3501 is unable to accommodate tyrosine as well, and it appears to have no preference for a specific amino acid.<sup>40</sup> We suggest that the difference in affinity for tyrosine at the carboxy-terminal position of the peptide may be the critical distinction between HLA-B\*35-Px and HLA-B\*35-PY. This difference may influence the relative efficiency of HLA-B\*35-Px and HLA-B\*35-PY in presenting specific HIV-1 epitopes to cytotoxic T lymphocytes and may thereby account for the different effects on progression to AIDS (Fig. 3).

We have previously shown a strong effect of HLA class I homozygosity on susceptibility to progression to AIDS; this effect appeared to be additive, in that homozygosity at two or three loci had stronger effects than homozygosity at a single locus.<sup>10</sup> We could not assess whether homozygosity for HLA-B\*35-Px would cause even faster progression to AIDS than heterozygosity for this group, since only two patients homozygous for HLA-B\*35-Px alleles were identified among the white patients who seroconverted. However, the total group of HLA-B\*35 alleles had a codominant effect in whites (Fig. 1), in whom homozygosity for any combination of HLA-B\*35 alleles (in six study participants) was associated with significantly more rapid progression to AIDS than having a single copy of the HLA-B\*35-Px alleles. Because the effect of homozygosity for any combination of HLA-B\*35 alleles was so severe, it may be

possible that HLA-B\*35 alleles as a group, including HLA-B\*3501, have a recessive effect on susceptibility to progression to AIDS. However, five of the six patients who had any combination of two HLA-B\*35 subtypes were also homozygous at the HLA-C locus (HLA-Cw\*0401,Cw\*0401), and the single homozygote for HLA-B\*3501 in this group was homozygous at all three class I loci. Perhaps the most parsimonious explanation for the extremely rapid progression to AIDS in the HLA-B\*35 "homozygotes" is that all harbor at least two negative genotypes — namely, at least one copy of the HLA-B\*35-Px group of alleles plus homozygosity at the HLA-C locus.

Given the strength of the genetic effect described for HLA-B\*35-Px, an aggressive therapeutic regimen may be advisable for patients who are positive for these alleles (particularly for those newly infected with the virus). A test specifically designed to detect the presence or absence of HLA-B\*35-Px alleles could easily be developed and would be sufficient in terms of HLA typing, since only this set of alleles among all other HLA types has a strong influence on progression to AIDS. Functional studies designed to characterize cytotoxic-T-lymphocyte activity in HIV-1-positive patients carrying HLA-B\*35-Px may provide a deeper understanding of the mechanisms involved in susceptibility to HIV-1 disease and may enhance the efficacy of vaccines against HIV, drug treatment, or both in these patients.

Supported in part by a contract (NO-1-CO-56000) with the National Cancer Institute and by a research contract (R01-AI-41951) with the National Institutes of Health.

This article does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

This article is dedicated to the memory of Dr. Janis Giorgi, a longtime friend and colleague.

### REFERENCES

1. Tomiyama H, Miwa K, Shiga H, et al. Evidence of presentation of multiple HIV-1 cytotoxic T lymphocyte epitopes by HLA-B\*3501 molecules that are associated with the accelerated progression of AIDS. *J Immunol* 1997;158:5026-34.
2. Rowland-Jones SL, Dong T, Dorrell L, et al. Broadly cross-reactive HIV-specific cytotoxic T-lymphocytes in highly-exposed persistently seronegative donors. *Immunol Lett* 1999;66:9-14.
3. Shiga H, Shioda T, Tomiyama H, et al. Identification of multiple HIV-1 cytotoxic T-cell epitopes presented by human leukocyte antigen B35 molecules. *AIDS* 1996;10:1075-83.
4. Parham P, Ohta T. Population biology of antigen presentation by MHC class I molecules. *Science* 1996;272:67-74.
5. Hughes AL, Yeager M. Natural selection at major histocompatibility complex loci of vertebrates. *Annu Rev Genet* 1998;32:415-35.
6. Scorza Smeraldi R, Fabio G, Lazzarin A, Eisera NB, Moroni M, Zanussi C. HLA-associated susceptibility to acquired immunodeficiency syndrome in Italian patients with human-immunodeficiency-virus infection. *Lancet* 1986;2:1187-9.
7. Itescu S, Mathur-Wagh U, Skovron ML, et al. HLA-B35 is associated with accelerated progression to AIDS. *J Acquir Immune Defic Syndr* 1992; 5:37-45.
8. Sahnoud T, Laurian Y, Gazengel C, Sultan Y, Gautreau C, Costagliola D. Progression to AIDS in French haemophiliacs: association with HLA-B35. *AIDS* 1993;7:497-500.

9. Just JJ. Genetic predisposition to HIV-1 infection and acquired immune deficiency virus syndrome: a review of the literature examining associations with HLA. *Hum Immunol* 1995;44:156-69. [Erratum, *Hum Immunol* 1996;45:78.]
10. Carrington M, Nelson GW, Martin MP, et al. *HLA* and HIV-1: heterozygote advantage and B\*35-Cw\*04 disadvantage. *Science* 1999;283:1748-52.
11. Walker BD, Chakrabarti S, Moss B, et al. HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* 1987;328:345-8.
12. Yasutomi Y, Reimann KA, Lord CI, Miller MD, Letvin NL. Simian immunodeficiency virus-specific CD8+ lymphocyte response in acutely infected rhesus monkeys. *J Virol* 1993;67:1707-11.
13. Evans DT, O'Connor DH, Jing P, et al. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nat Med* 1999;5:1270-6.
14. Ogg GS, Jin X, Bonhoeffer S, et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 1998;279:2103-6.
15. Barber LD, Gillette-Castro B, Percival L, Li X, Clayberger C, Parham P. Overlap in the repertoires of peptides bound *in vivo* by a group of related class I HLA-B allotypes. *Curr Biol* 1995;5:179-90.
16. Phair J, Jacobson L, Detels R, et al. Acquired immune deficiency syndrome occurring within 5 years of infection with human immunodeficiency virus type-1: the Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr* 1992;5:490-6.
17. Goedert JJ, Kessler CM, Aledort LM, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. *N Engl J Med* 1989;321:1141-8.
18. Hilgartner MW, Donfield SM, Willoughby A, et al. Hemophilia Growth and Development Study: design, methods, and entry data. *Am J Pediatr Hematol Oncol* 1993;15:208-18.
19. Buchbinder SP, Katz MH, Hessel NA, O'Malley PM, Holmberg SD. Long-term HIV-1 infection without immunologic progression. *AIDS* 1994;8:1123-8.
20. Vlahov D, Anthony JC, Munoz A, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res Monogr* 1991;109:75-100.
21. O'Brien SJ, Nelson GW, Winkler CA, Smith MW. Polygenic and multifactorial disease gene association in man: lessons from AIDS. *Annu Rev Genet* 2000;34:563-91.
22. Bunce M, O'Neill CM, Barnardo MC, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens* 1995;46:355-67.
23. Cereb N, Maye P, Lee S, Kong Y, Yang SY. Locus-specific amplification of HLA class I genes from genomic DNA: locus-specific sequences in the first and third introns of HLA-A, -B, and -C alleles. *Tissue Antigens* 1995;45:1-11.
24. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep* 1992;41(RR-17):1-19.
25. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR Morb Mortal Wkly Rep* 1987;36:Suppl 1:3S-15S.
26. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CCR5* structural gene. *Science* 1996;273:1856-62. [Erratum, *Science* 1996;274:1069.]
27. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of *CCR2* and *CCR5* variants on HIV-1 infection and disease progression. *Science* 1997;277:959-65.
28. Phillips RE, Rowland-Jones S, Nixon DF, et al. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 1991;354:453-9.
29. Goulder PJ, Phillips RE, Colbert RA, et al. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* 1997;3:212-7.
30. Migueles SA, Sabbaghian MS, Shupert WL, et al. HLA B\*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci U S A* 2000;97:2709-14.
31. Kaslow RA, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med* 1996;2:405-11.
32. Holm SA. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1979;6:65-70.
33. Weir BS. Genetic data analysis II: methods for discrete population genetic data. 2nd ed. Sunderland, Mass.: Sinauer, 1996.
34. Schweder T, Spjøtvoll E. Plots of *P*-values to evaluate many tests simultaneously. *Biometrika* 1982;69:493-502.
35. McKenzie LM, Slattery JP, Carrington M, O'Brien SJ. Taxonomic hierarchy of HLA class I allele sequences. *Genes Immun* 1999;1:120.
36. Hayashi H, Ooba T, Nakayama S, Sekimata M, Kano K, Takiguchi M. Allospecificities between HLA-Bw53 and HLA-B35 are generated by substitution of the residues associated with HLA-Bw4/Bw6 public epitopes. *Immunogenetics* 1990;32:195-9.
37. Chertkoff LP, Herrera M, Fainboim L, Satz ML. Complete nucleotide sequence of a genomic clone encoding HLA-B35 isolated from a Caucasian individual of Hispanic origin: identification of a new variant of HLA-B35. *Hum Immunol* 1991;31:153-8.
38. Zemmour J, Little AM, Schendel DJ, Parham P. The HLA-A,B "negative" mutant cell line C1R expresses a novel HLA-B35 allele, which also has a point mutation in the translation initiation codon. *J Immunol* 1992;148:1941-8.
39. Steinle A, Falk K, Rotzschke O, et al. Motif of HLA-B\*3503 peptide ligands. *Immunogenetics* 1996;43:105-7.
40. Hill AV, Elvin J, Willis AC, et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature* 1992;360:434-9.

Copyright © 2001 Massachusetts Medical Society.