HLA-B Signal Peptide Polymorphism Influences the Rate of HIV-1 Acquisition but Not Viral Load

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Human leukocyte antigen alleles influence the immune response to HIV-1. Signal peptides cleaved from those alleles bind to HLA-E and mediate natural killer cell function. Signal peptides of HLA-A and HLA-C proteins carry methionine (Met) at anchor position 2 (P2); those of HLA-B carry Met or threonine (Thr). Different P2 residues alter HLA-E binding to its cognate receptors and may impact HIV-1 acquisition. Among Zambian couples (N = 566) serodiscordant for HIV-1, P2-Met accelerated acquisition in the HIV-1-negative partner (relative hazard [RH], 1.79). Among seroconverting Zambian (n = 240) and Rwandan (n = 64) partners, P2-Met also accelerated acquisition (RH, 1.47 and RH, 1.83 respectively). HLA-B alleles displaying the reportedly protective Bw4 epitope carry P2-Thr. Bw4/P2-Thr and Bw6/P2-Thr showed similar protective effects compared with Bw6/P2-Met. Neither motif was associated with viral load. The influence of HLA-B alleles on HIV/AIDS may derive from multiple motifs in and beyond the mature proteins.

Human leukocyte antigen (HLA) molecules influence immune responses through interactions with cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells [1, 2]. Classical HLA class I (HLA-I) molecules interact with CD8-bearing CTLs for host discrimination between self and foreign molecules, and there is definitive evidence for differential influence of the allelic forms of the HLA-I binding pocket on the course of human immunodeficiency virus type 1 (HIV-1) infection [3].

The nonclassical HLA-E molecule binds with NK cells expressing C-type lectinlike heterodimeric CD94/NKG2 receptors [4]. The inhibitory NKG2A has a higher affinity for HLA-E than the activating NKG2C receptor [5, 6]. HLA-E binds peptides from the amino acid residues 3–11 of the signal peptide of HLA-A, -B, -C, and -G molecules and peptides derived from other cellular and viral sources [7]. HLA-E expression levels on the cell surface serve as a marker of overall HLA expression. Although far less polymorphic than classical class 1 genes, HLA-E is expressed differentially according to allelic differences at position 107, with E*01:01 having arginine and E*01:03 having glycine. HLA-E*01:03 exhibits increased cell surface expression [8]. A previous report described an association of HLA-E*01:03 with a 4-fold decreased risk of HIV-1 infection in Zimbabwean women [9].

HLA-I molecules display other polymorphisms that alter the occurrence and control of HIV-1 infection. Variation in the binding residue at the second position (P2) of the HLA-B signal peptide can affect CD94-NKG2 receptor recognition of that peptide when it is bound to HLA-E [10, 11]. Amino acid substitutions at P2 alter the stability of the HLA-E/peptide complex

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and impact cell surface expression [11–13]. Alleles of *HLA-A* and *HLA-C* encode methionine at P2 (P2-Met) [12]. In contrast *HLA-B* alleles encode either Met or Thr (P2-Thr) at P2, with Thr causing aberrant folding and presentation of the HLA-E molecule [14].

The products of the *HLA-B* gene can also be divided into Bw4 and Bw6 epitope-bearing groups, based on variation in residues at positions 77–83 of the α1 domain on the peptide-binding pocket [15]. Alternative forms of Bw4 molecules, bearing isoleucine (Ile) or threonine (Thr) at position 80, contribute to alterations in receptor specificity on T and NK cells [16, 17]. HLA-Bw4 homozygosity has been associated with control of HIV-1 load [18] and decreased risk of HIV-1 transmission [19]. HLA-Bw4 with Ile at position 80 (HLA-Bw4 80Ile) with certain forms of its cognate NK receptor, KIR3DS1/KIR3DL1, is associated with delayed progression to AIDS [20]. All HLA-B molecules exhibiting the Bw4 epitope variant except HLA-B*38 also carry P2-Thr, whereas Bw6 molecules carry either P2-Thr or P2-Met [21].

No study to date has determined the separate contributions of the signal peptide polymorphism and the Bw4/Bw6 epitope variant on HIV-1 acquisition and viral load (VL) control. In a single report, homozygosity for the P2-Thr variant of the signal peptide was associated with lower frequency of infection and long-term nonprogression to AIDS [22]. Although the authors recognized the potential simultaneous influences of the Bw4 motif, they did not describe analyses aimed at teasing apart the several likely associations with HLA-I alleles (eg, HLA-B*57), which not only display both P2-Thr and Bw4 but also exert highly favorable effects in the $CD8^+$ CTL response pathway. To resolve those ambiguities, we examined data from our cohort of serodiscordant Zambian couples, one of the largest investigations of prospectively documented intracouple transmission. In this cohort, we previously described the influences of polymorphisms in HLA and killer immunoglobulinlike receptor (KIR) genes on 3 outcomes of HIV-1 infection: transmission by index partners, acquisition by their seronegative partners, and control of viremia [23–27]. We have now elucidated the impact of the signal peptide P2 polymorphism on HIV-1 seroconversion, while accounting for effects potentially attributable to other HLA polymorphisms (ie, allelic variation in the peptide binding pocket, the Bw4/Bw6 epitope variant, and the single-residue variation in the molecule encoded by *HLA-E*). In addition, our data on the comparable effect of the signal peptide polymorphism in Rwandan couples with prospectively observed transmission corroborated the findings in Zambians.

SUBJECTS AND METHODS

Study Population

Between 1995 and 2006, HIV-1 discordant couples from Lusaka, Zambia, were followed as part of the Zambia-Emory HIV-1 Research Project. Participants in this study were antiretroviral treatment naive and provided consent. We included all transmitting couples whose initially HIV-1–exposed seronegative partner acquired virologically linked HIV-1 from the index partner during follow-up. For closer comparability to these transmitters, nontransmitting couples with ≥9 months of follow-up were selected from the cohort of eligible couples through the application of an algorithm incorporating self-reported behavioral or clinical measures of their risk; their risk of transmission was higher than the average for all couples in the cohort [28, 29] (See Supplementary Material). Additionally we included transmitting couples enrolled in Kigali, Rwanda (Rwanda-Zambia HIV Research Group), in whom HIV-1 was acquired during follow-up of a large cohort of discordant couples. For both Zambian and Rwandan couples, HIV-1 transmission status was determined at the quarterly examination by dipstick HIV-1/HIV-2 Ab assay with Capillus latex aggregation for confirmation and Uni-Gold Recombigen HIV test (Trinity Biotech). The viral subtype and intracouple linkage of virus was established by sequencing and phylogenetic analysis for both the index and seroconverting partners [30]. Data on couples were censored at the time of seroconversion, withdrawal from the study, or on 31 December 2006.

For the 566 couples in this study, biologic specimens adequate for HLA and KIR genotyping were available, sequencing confirmed epidemiologic linkage in HIV-1 transmission pairs, and nontransmission pairs had ≥9 months of follow-up. Depending on whether transmission had occurred by the censoring date, index partners were classified as transmission pair index partners or nontransmission index partners, and partners who were HIV-1 seronegative at enrollment were classified as exposed seronegatives or seroconverters.

HIV-1 VL Measurement and Analysis

HIV-1 RNA copies in patient plasma were quantified by Roche Amplicor 1.0 assay (Roche Diagnostics Systems) in laboratories certified by the Virology Quality Assurance Program of the AIDS Clinical Trials Group. The lower detection limit was 400 copies/mL of plasma.

Genotyping of HLA Class I Genes

Genomic DNA was extracted from whole blood or buffy coat using QIAamp blood kits (Qiagen). HLA genotyping was performed by polymerase chain reaction as previously described [31]. Bw4 and Bw6 epitopes were assigned using the ImMunoGeneTics/Human Leukocyte Antigen database[32].

SNP Typing of HLA-E

Discrimination of the alleles of HLA-E*01:01 and *01:03 by Taqman assay was performed as described [33].

Statistical Analysis

Statistical routines in SAS version 9.2 were used for analyses. We compared patient characteristics in exposed seronegatives and seroconverters by χ^2 analysis for categorical variables or *t* test for continuous variables. Hardy–Weinberg equilibrium of *HLA-B* and *HLA-E* alleles was tested by global log likelihood χ^2 test. Using Cox proportional hazards models we tested the relationships of *HLA-B* and *HLA-E* polymorphisms to HIV-1 acquisition events within the study period. Because Bw4/Bw6 epitope and P2 polymorphism are nonindependent variables, we analyzed these factors as a 3-level variable; Bw4 (always P2-Thr), Bw6/P2-Thr, and Bw6/P2-Met. In logistic regression and Cox proportional hazards models, we analyzed the following covariates: HLA-A*6802, which we previously associated with HIV-1 acquisition in the Zambian population [34], and nongenetic factors including genital ulcers and/or inflammation in either partner in the 3 months prior to the estimated date of transmission/censoring and $\log_{10}$ transformed VL measured in the index partner during the 3 months prior to transmission/censoring. We used transformed VL values in the proportional hazards model because their goodness of fit was better than for nontransformed values.

Kaplan-Meier plots are shown to compare the relative differences in time-to-acquisition according to the P2 polymorphism. Because of the deliberate inclusion of couples at relatively higher risk of infection, these plots do not reflect overall transmission rates in the entire discordant couple population. Uncertainty about when during follow-up the genetic factors of interest might be expected to exert their influence led us to apply both the Wilcoxon and log-rank significance tests, which tend to weight results from the earlier and later follow-up periods, respectively.

In a similar way we tested combinations of HLA-E/P2 polymorphism and of Bw4/KIR3DS1 in nonindex partners for relationships to HIV-1 acquisition. Lastly we examined the impact of *HLA-B* and *HLA-E* variants on HIV-1 viral load in index partners and seroconverters by proportional odds logistic regression with VL as a categorical variable and by linear regression with $\log_{10}$ VL as a continuous variable. Proportional odds logistic regression was used for data in which the outcome variable of VL was divided into more than 2 categories, and the differences in the effect across successive categories were relatively constant. The regression generated a proportional odds ratio (pOR) with no units of measurement.

RESULTS

Characteristics of Zambian Couples Included in This Study

Details of the Zambian study cohort have been described previously [31]. In brief, of 566 couples, 240 were transmission pairs with nearly all subtype C viral linkage and 326 were nontransmission pairs. Male-to-female transmission accounted for 61.2% of transmission events (n = 147). Mean follow-up times in the nontransmission pairs were nearly double the mean time to seroconversion in the transmission pairs ($P < .001$). Genital ulcers and/or inflammation in the 3 months preceding seroconversion or the censoring date was more frequent in seroconverters compared with exposed seronegatives (45.7% vs 10.6%; $P < .001$). Results were similar after stratification for female-to-male and male-to-female transmission.

In the Rwandan HIV-1 subtype A–infected transmission couples, female-to-male transmission was slightly more common (n = 34) than male-to-female transmission (n = 30) (Table 1). Genital ulcers and/or inflammation was more common in couples with female-to-male transmission (53.1%) than in male-to-female transmission (36.0%).

Distribution of HLA-B and HLA-E Polymorphism in Zambians

Of the 566 Zambians who were HIV-1 negative at enrollment, 337 (59.5%) carried the Bw4 epitope and 15.7% were homozygous for Bw4 (Table 2). All *HLA-B* alleles encoding the Bw4 epitope carried P2-Thr. In addition, 477 (84.3%) Zambians carried an *HLA-B* allele encoding ≥1 copy of the Bw6 epitope; of those, 322 (56.9%) carried ≥1 Bw6/P2-Thr. Bw6/P2-Met homozygotes made up 9.4% of the study participants. HLA-

Table 1. Characteristics of Rwandan and Zambian Transmitting Serodiscordant Couples

Cohort and Key Factors	Individuals		Couples	
	TPI	SC	FTM	MTF
Rwandans				
No.	64	64	34	30
Men age in years	38.8 (7.8)	39.4 (10.2)	39.4 (10.2)	38.8 (7.8)
Women age in years	34.0 (7.1)	31.8 (7.4)	34.0 (7.1)	31.8 (7.4)
GUI	36.8%	24.6%	53.1%	36.0%
VL, $\log_{10}$ copies/mL	4.2 (1.0)	4.2 (1.1)	3.7 (1.2)	4.7 (0.6)
Zambians				
No.	240	240	93	147
Men age in years	33.5 (7.5)	32.3 (7.6)	32.3 (7.6)	33.5 (7.5)
Women age in years	26.1 (5.9)	26.2 (6.2)	26.2 (5.9)	26.3 (6.2)
GUI	50.5%	45.7%	66.2%	74.1%
VL, $\log_{10}$ copies/mL	5.0 (0.7)	4.5 (0.8)	4.8 (0.7)	5.1 (0.6)

Age is expressed as the mean years and standard deviation. Viral load (VL) is expressed as the mean and standard deviation of $\log_{10}$ copies/mL. VL in couples is the index partner VL. Genital ulcers and/or inflammation (GUI) in couples is GUIs in either partner within 3 months of transmission.

Abbreviations: FTM, female-to-male transmission; MTF, male-to-female transmission; SC, seroconverter; TPI, transmission positive index partner.

Table 2. Frequencies of HLA-B Bw4/Bw6 and HLA-E*01:01/*01:03 Alleles in Zambian Nonindex Partners, Stratified by HLA-B Signal Peptide Position 2 (P2) Variants

Genotypes	N = 566	P2-Met/Met n = 53 (9.4%)	P2-Met/Thr n = 209 (36.9%)	P2-Thr/Thr n = 304 (53.7%)
HLA-B Bw4/Bw4	89 (15.7)	0	0	89 (15.7)
HLA-B Bw4/Bw6	248 (43.8)	0	102 (18.0)	146 (25.8)
HLA-B Bw6/Bw6	229 (40.5)	53 (9.4)	107 (18.9)	69 (12.2)
HLA-E*0101/*0101	138 (24.4)	13 (2.3)	49 (8.7)	76 (13.4)
HLA-E*0101/*0103	260 (45.9)	18 (3.2)	100 (17.7)	142 (25.1)
HLA-E*0103/*0103	168 (29.7)	22 (3.9)	60 (10.6)	86 (15.2)

All data are no. (%).

Abbreviations: Met,methionine; Thr, threonine.

E*01:03 was present in 428 (75.6%) of the participants, of whom 29.7% were homozygous. All *HLA-B* alleles with carrier frequencies ≥1% were in Hardy–Weinberg equilibrium.

Univariate Analyses of HLA Polymorphisms With HIV-1 Acquisition in All Zambian Nonindex Partners

Either 1 or 2 copies of P2-Met were present in 138 (42.3%) of the HIV-exposed seronegatives and 124 (51.7%) of the seroconverters (Table 3). During follow-up, seroconverters who were homozygous for P2-Met acquired HIV-1 more rapidly (median time to acquisition, 36 months; 95% confidence interval [CI], 26–51), compared with those heterozygous for P2-Met (median time to acquisition, 45 months; 95% CI, 36–69) or homozygous for P2-Thr (median time to acquisition, 69 months; 95% CI, 51–102) (Figure 1). Estimates of acquisition-free time for seroconverters with the 3 distinctive genotypes differed significantly by log-rank ($P = .01$) and Wilcoxon ($P = .004$) tests. By Cox proportional hazards regression, P2-Met was associated with accelerated acquisition of HIV-1 (RH, 1.79; 95% CI, 1.35–2.36; $P < .0001$). The effects of P2-Met were similar for 1 copy (RH = 1.77; 95% = CI 1.31–2.38, $P < .001$) and two (RH = 1.86, 95% CI = 1.18–2.91, $P = .007$).

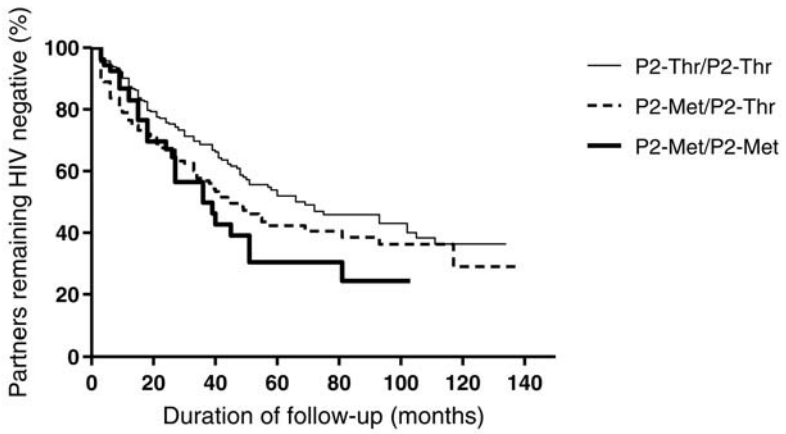
Either one or two copies of HLA-B Bw4 were present in 204 (62.6%) of HIV-1 exposed seronegatives and 135 (56.3%) of seroconverters (Table 3). HLA-B Bw4 was associated with delayed time to HIV-1 acquisition (RH = 0.70, 95% CI = .55–.97, $P = .041$). The effects of Bw4 were similar for 1 copy (RH, 0.77; 95% CI, .59–1.02; $P = .06$) and 2 (RH, 0.66; 95% CI, .45–.92; $P = .03$). Further stratification of Bw4 by the amino acid at position 80 did not demonstrate an obvious difference between the associations of HIV-1 acquisition with Bw4–80 Thr (RH, 0.65; 95% CI, .43–.98; $P = .04$) and Bw4–80 Ile (RH, 0.84; 95% CI, .65–1.08; $P = .17$). The *HLA-A* alleles encoding Bw4 epitopes (A*23, A*24, and A*32) were present in 26.2% of nonindex partners but were not associated with HIV-1 acquisition in a proportional hazards analysis ($P = .94$). Neither *KIR3DS1* nor HLA-E*01:03 was associated with HIV-1 acquisition.

Table 3. Univariate Associations of HLA Class I Polymorphisms, *KIR3DS1*, and Nongenetic Factors with HIV-1 Acquisition Among Zambian Serodiscordant Couples

		Total (N = 566), no. (%)			Cox Proportional Hazards		
	Co-factors in Model	Total	ESN (n = 326)	SC (n = 240)	RH	95% CI	*P* Value
Genetic factors	HLA-B signal peptide P2-Met	262 (46.3)	138 (42.3)	124 (51.7)	1.79	1.35–2.36	<.0001
	HLA-E*0103	428 (75.6)	252 (77.3)	176 (73.3)	0.94	.67–1.29	.74
	HLA-A Bw4	64 (26.2)	87 (26.7)	67 (27.9)	1.01	.75–1.37	.94
	HLA-B Bw4	339 (59.9)	204 (62.6)	135 (56.3)	0.70	.55–.97	.04
	HLA-B Bw4–80Ile	282 (49.8)	165 (50.6)	117 (48.8)	0.84	.65–1.08	.17
	HLA-B Bw4–80Thr	80 (14.1)	56 (17.2)	24 (10.0)	0.65	.43–.98	.04
	KIR3DS1	60 (10.6)	37 (11.3)	23 (9.6)	0.88	.57–1.35	.56
Nongenetic factors	GUI	220 (46.4)	74 (28.1)	146 (69.2)	3.58	2.66–4.83	<.0001
	Female sex	296 (52.3)	149 (45.7)	147 (61.2)	1.11	.81–1.51	.52
	Age	NA	NA	NA	0.96	.75–1.68	.12
	Donor VL	NA	NA	NA	1.83	1.51–2.22	<.0001

Among the study participants, 92 couples have no genital ulcers and/or inflammation (GUI) data with 29 of those SC and 63 ESN; 44 couples do not have donor viral load (VL) data, with 30 ESN and 14 SC. The frequency of genetic markers analyzed in Table 2 did not differ significantly ($P > .05$) in individuals with VL data compared with those without.

Abbreviations: CI, confidence interval; ESN, exposed seronegative; NA, not applicable; P2, position 2; RH, relative hazard; SC, seroconverter.



		Acquisition-free individuals remaining at various intervals (months)				
	Acquisition Events: n (%)	0	16	32	64	128
P2-Thr /P2-Thr	116 (38.2)	304	220	138	54	6
P2-Thr / P2-Met	96 (45.9)	209	129	79	27	3
P2-Met /P2-Met	28 (52.8)	53	35	20	6	1

Figure 1. Kaplan–Meier plot showing human immunodeficiency virus type 1 (HIV-1) acquisition-free time in nonindex partners with position 2 (P2)-threonine (Thr)/P2-Thr, P2- methionine (Met)/P2-Thr, and P2-Met/P2-Met genotypes. For the Wilcoxon test, $P = .004$, and for the log-rank test, $P = .01$. The summary table shows numbers of acquisition-free couples at various follow-up intervals. Plots shown represent relative occurrence among those with the factors of interest, not overall incidence in the population. Rates of acquisition depicted here in discordant couples selected for risk characteristics are higher than rates in the entire discordant couple cohort.

Analysis of P2-Met After Exclusion of Bw4 Epitopes in All Zambian Nonindex Partners

To assess the effect of P2-Met independent of any Bw4 influence, we analyzed the 229 individuals who carried 2 Bw6 alleles (Table 2). Carriage of P2-Met in a Bw6 allele was associated with accelerated HIV-1 acquisition compared with carriage of a Bw6 allele with P2-Thr. (RH, 1.68; 95% CI, .98–2.44, $P = .08$). Although the statistical power was lower in this smaller group of homozygous Bw6 individuals, carriage of 1 or 2 copies of P2-Met was associated with accelerated HIV-1 acquisition in Bw6 homozygotes (RH, 1.24 and 1.71, respectively).

Multivariable Modeling of HIV-1 Acquisition in All Zambian Nonindex Partners

By multivariable analysis, we tested the association of selected factors on HIV-1 acquisition (Table 4). We compared Bw4, Bw6/P2-Thr, and Bw6/P2-Met as a 3-level variable with Bw6/P2-Thr as the reference group. Of all factors, only P2-Met, HLA-A*6802, genital ulcers and/or inflammation, and index partner VL showed significant associations in the multivariable analysis, and these were included in the reduced model. P2-Met was strongly associated with more rapid HIV-1 acquisition (RH, 1.68; 95% CI, 1.28–2.11; $P = .002$); HLA-A*6802 ($P = .003$), higher VL in the index partner ($P = .005$), and genital ulcers and/or inflammation in either partner showed independent associations ($P < .0001$).

HLA-B Signal Peptide Polymorphisms and HIV-1 Acquisition in Zambian and Rwandan Seroconverters

We examined the impact of P2 polymorphism on rate of HIV-1 acquisition in an analysis confined to the seroconverters (Table 5). In the Zambians, where 51.7% of seroconverters had at $\geq$1 copy of P2-Met, this genetic factor was associated with relatively more rapid acquisition of HIV-1 (RH, 1.47; 95% CI, 1.10–1.96; $P = .009$). Zambian seroconverters with $\geq$1 copy of P2-Met acquired HIV-1 more rapidly (median time to acquisition, 15 months; 95% CI, 10–19) then those homozygous for P2-Thr (median time to acquisition, 19 months; 95% CI, 16–27). Estimates of acquisition-free time for seroconverters with or without P2-Met differed significantly by Wilcoxon ($P = .01$) and log-rank ($P = .02$) tests. The association was similar in the Rwandan seroconverter group (RH, 1.83; 95% CI, 1.05–3.35; $P = .048$), where 37.5% had $\geq$1 copy of P2-Met. Rwandan seroconverters with P2-Met acquired HIV-1 more rapidly (median time to acquisition, 8 months; 95% CI, 4–10) than Rwandan seroconverters without P2-Met (median time to acquisition, 10 months; 95% CI, 7–13). Estimated acquisition-free time for seroconverters with or without P2-Met differed significantly by log-rank ($P = .05$) but less so by Wilcoxon tests ($P = .13$).

Table 4. Multivariable Models of Associations of HLA Class I Polymorphisms, *KIR3DS1*, and Nongenetic Factors With HIV-1 Acquisition Among Zambian Serodiscordant Couples

Full Model	Genetic or Nongenetic Factor HLA-B epitope/ signal peptide	Cox Proportional Hazard model (time to acquisition) RH	95% CI	Adjusted *P* Value
	Bw6/P2-Thr[a]	REF	REF	REF
	Bw4/P2-Thr	0.82	.65–1.04	.12
	Bw6/P2-Met	1.66	1.17–1.99	.02
	HLA-E*0103	1.07	.67–1.53	.74
	KIR3DS1	1.22	.85–1.76	.27
	HLA-A*6802	1.69	1.32–2.19	.009
	Index VL	1.41	1.22–1.62	<.0001
	GUI	3.50	2.80–4.38	<.0001
Reduced model	P2-Met[b]	1.68	1.28–2.11	.002
	HLA-A*6802	1.59	1.19–2.40	.003
	Index VL	1.42	1.16–1.73	.0005
	GUI	3.51	2.57–4.78	<.0001

Abbreviatons: CI, confidence interval; GUI, genital ulcers and/or inflammation; Met,methionine; P2, position 2; REF, reference; RH, relative hazard; Thr, threonine; VL, viral load.

[a] In the full model, Bw4/Bw6 and P2-Met/P2-Thr were analyzed as a 3-level variable with Bw6/P2-Thr as the reference group.

[b] In the reduced model P2-Met (presence or absence) vs P2-Thr were analyzed.

HLA Class I Polymorphisms and VL in Zambians

In contrast with the findings for HIV-1 acquisition, no association of P2-Met with VL was detected in either the seroconverters or the index partners (Table 6). HLA-B Bw4 was significantly associated with lower VL as a continuous variable in seroconverters analyzed by a generalized linear model ($\beta = -0.25 \pm 0.11$ $\log_{10}$; $P = .03$), but when B*57 alleles were excluded from the analysis, the Bw4 epitope was no longer associated with VL ($\beta = -0.07 \pm 0.08$ $\log_{10}$; $P = .42$).

Table 5. Multivariable Models of Association of HLA-B Signal Peptide Polymorphism with the Time to HIV-1 Acquisition Among Zambian and Rwandan Seroconverters

Cohort	Genetic or Nongenetic Factor	Cox Proportional Hazards Model RH	95% CI	Adjusted *P* Value
Zambians (N = 240)	P2-Met	1.47	1.10–1.96	.009
	Index VL	1.01	.85–1.18	.98
	GUI	1.75	1.31–2.35	<.001
Rwandans (N = 64)	P2-Met	1.83	1.05–3.35	.048
	Index VL	1.05	.79–1.39	.73
	GUI	1.15	.78–2.03	.26

Abbreviations: CI, confidence interval; GUI, genital ulcers and/or inflammation; Met, methionine; P2, position 2; RH, relative hazard; Thr, threonine; VL, viral load.

DISCUSSION

HLA-B molecules possess multiple elements that mediate several immunopathogenetic pathways. In one of the largest cohorts of HIV-1 discordant heterosexual couples under study, we tested the hypothesis that HLA-B alleles might affect HIV-1 pathogenesis through their polymorphism at P2 of the signal peptide, and we verified the critical result in a second cohort of serodiscordant couples. As noted earlier, different residues of the HLA-B signal peptide can differentially modulate recognition of the peptide-bound HLA-E molecule by CD94-NKG2 receptors [10, 11]. HLA-E molecules bind P2-Met nonamers selectively; when presented with P2-Thr peptides, HLA-E surface expression is decreased [35]. In vitro binding assays suggest that signal peptides with P2-Thr have decreased binding affinity for HLA-E [11]. HLA-E-bound P2-Thr peptides do not interact with CD94/NKG2A receptors and do not inhibit NK cell-mediated lysis [7].

Because HLA-B has a higher level of expression than HLA-A and is more inducible by interferon-α and -γ [21], both forms of signal peptide derived from HLA-B alleles probably contribute more than A or C alleles to HLA-E peptide presentation and to its variability in HIV-1 infection. HLA-E molecules present peptides to CD94/NKG2A and CD94/NKG2C receptors on NK cells, with higher affinity for the former (inhibitory) receptor than for the latter (activating) one [6]. Binding of P2-Met–bearing signal peptide with subsequent expression of HLA-E and its presentation to CD94/NKG2A would inhibit NK cell-mediated lysis of the presenting cell [7], whereas the P2-Thr signal peptide would enhance effective killing of infected cells and retard initiation of productive HIV-1 infection [35]. Interestingly, human cytomegalovirus (CMV) encodes a ligand for HLA-E, UL40, that confers resistance from NK cell lysis of the infected cell [36]. UL40 contains P2-Met and is identical to the signal peptide derived from HLA-C molecules [37]. It has been suggested that HIV-1 may also encode a ligand with isoleucine at P2 capable of stabilizing HLA-E expression [38]. If that finding is confirmed, then P2-Thr–containing signal peptides might compete with the virally derived peptide. The observation that CMV and possibly HIV-1 have evolved to encode HLA-E binding peptides that inhibit NK cell-mediated lysis of virally infected cells reinforces the likely importance of this pathway in viral infections.

We also tested the association of the common allelic variant of HLA-E with HIV-1 acquisition. HLA-E is expressed on the surface of T cells, B cells, and placental and trophoblastic tissue [39, 40]. Although HLA-A and HLA-B surface

Table 6. HLA-B Motifs and HIV-1 Viral Load (VL) Among Subgroups of Zambians With HIV-1 Infections

	Distribution Across VL Categories			Test for Trend			Generalized Linear Model	
Subgroups and Genotypes	Low ($<10^4$)	Medium (10^4–10^5)	High ($>10^5$)	pOR	95% CI	*P* Value	β (mean ± SE)	*P* Value
Seroconverters[a]								
HLA-A Bw4	12 (18.9)	30 (46.9)	22 (34.4)	1.11	.67–2.21	.59	0.15 ± 0.13	.25
HLA-B Bw4	47 (32.4)	55 (37.9)	43 (29.7)	0.67	.36–1.24	.10	−0.25 ± 0.11	.03
HLA-B Bw4 excluding B*57	28 (24.8)	44 (38.9)	41 (36.3)	0.95	.75–1.22	.67	−0.07 ± 0.08	.42
HLA-B Bw4-Ile80	38 (30.2)	51 (40.5)	37 (29.4)	0.88	.22–2.54	.45	−0.16 ± 0.10	.15
HLA-B Bw4-Thr80	11 (42.3)	6 (23.2)	9 (34.6)	0.71	.35–1.78	.11	−0.22 ± 0.18	.22
P2-Met	35 (31.0)	43 (38.0)	35 (31.0)	1.38	.71–2.45	.52	−0.16 ± 0.11	.14
HLA-E*01:03	44 (24.7)	68 (38.2)	66 (37.1)	1.21	.87–2.56	.16	0.22 ± 0.13	.07
Index partners[b]								
HLA-A Bw4	24 (17.1)	54 (38.6)	62 (44.3)	1.14	.56–1.72	.45	0.14 ± 0.08	.09
HLA-B Bw4	64 (20.5)	119 (38.1)	129 (41.4)	0.87	.23–2.11	.44	−0.08 ± 0.07	.29
HLA-B Bw4 excluding B*57	43 (17.4)	96 (38.9)	108 (43.7)	0.97	.59–1.91	.70	0.01 ± 0.07	.56
HLA-B Bw4-Ile80	52 (20.7)	94 (37.5)	105 (41.8)	1.01	.54–2.01	.48	−0.04 ± 0.06	.60
HLA-B Bw4-Thr80	18 (22.5)	30 (37.5)	32 (40.0)	0.76	.46–1.99	.72	−0.12 ± 0.10	.22
P2-Met	55 (21.0)	102 (38.9)	105 (40.1)	1.45	.54–2.91	.63	−0.05 ± 0.07	.44
HLA-E*01:03	77 (18.8)	167 (40.8)	165 (40.3)	1.28	.82–2.82	.75	−0.01 ± 0.08	.94

Distribution across VL categories are expressed as number (%). Beta estimates from generalized linear models are expressed as $\log_{10}$ VL (copies/mL). Forty-four index partners and 12 seroconverters were missing VL data.

Abbreviations: CI, confidence interval; Met, methionine; P2, position 2; pOR, proportional odds ratio; SE, standard error; Thr, threonine.

[a] n = 228.

[b] n = 522 (226 transmission pair index partners and 296 nontransmission pair index partners).

expression is down regulated by *nef*, a product of HIV-1 [41], HLA-E surface expression increases in infected cells [42]. Although HLA-E preferentially binds nonameric peptides derived from HLA-A -B, -C, and -G molecules [43], it can also present peptides derived from heat shock proteins with subsequent loss of binding to CD94/NKG2A [44]. The two most common alleles, HLA-E*01:01 and HLA-E*01:03, are differentially expressed at the cell surface [45]. In an initial report on 397 retrospectively analyzed infected and uninfected Zimbabwean women, HLA-E*01:03 was associated with a 4-fold lower frequency of HIV-1 infection [9]. HLA-E is a versatile molecule, but if the dominant effect of this more highly expressed allele is greater inhibition of NK cell activity through NKG2A, the opposite relationship (ie, higher frequency of HIV-1 infection) would seem more plausible. Although 76% of our 566 Zambian nonindex partners carried HLA-E*01:03, providing ample power to detect a clear association of this variant with prospectively ascertained HIV-1 acquisition, no such association was seen.

In addition to signal peptide polymorphisms, HLA-B molecules also display either a Bw4 or a Bw6 epitope defined by sequence variation at residues 77–83 of the α1 domain on the peptide-binding cleft [15]. The HLA-B Bw4 epitope has been associated with decreased VL in HIV-1 infection [46], slower progression to AIDS [20, 47], and decreased likelihood of HIV-1 transmission [19]. HLA-B*57 and B*27, both Bw4 epitope bearing, have consistently been associated with lower VL and long-term nonprogression to AIDS [25, 48]. Alleles displaying Bw4 have been presumed to alter HIV-1 pathogenesis by presenting viral peptide fragments to $CD8^+$ T cells and/or by interacting with KIR3DL1/KIR3DS1 receptors on NK cells [46, 49].

To tease apart the effects of the signal peptide polymorphism and the Bw4/Bw6 epitopes, we analyzed HLA-B alleles as Bw4 (always P2-Thr), Bw6/P2-Thr, or Bw6/P2-Met. Alleles displaying Bw4/P2-Thr and Bw6/P2-Thr had similar protective effects, and in multivariable analysis, time to acquisition did not differ significantly between the HIV-1–exposed seronegatives with Bw4/P2-Thr and Bw6/P2-Thr. More rapid seroconversion was consistently associated with Bw6/P2-Met. The association of HLA-B Bw4 with delayed acquisition observed in Zambians was not altered by the presence or absence of *KIR3DS1*. Further support for the primacy of the P2 polymorphism in virus acquisition is the lack of an effect in Zambian carriers of Bw4-bearing HLA-A alleles (all P2-Met) despite evidence that Bw4 A and B alleles bind to the same NK cell receptors [50].

To replicate the highly significant P2-Met effect on acquisition, we confined the analysis to HIV-1 subtype C–infected Zambians, with whom we could compare a much smaller number of HIV-1 subtype A–infected Rwandans. In these 2

populations, the similar degree to which P2-Met was associated with accelerated HIV-1 acquisition suggests that the effect of this polymorphism may be generalizable to other HIV-1–susceptible populations.

We found a striking disparity between the effect of the signal peptide P2 polymorphism on acquisition of infection by HIV-1–exposed seronegative partners and absence of any effect on the level of viremia in infected partners. The lack of effect of P2-Met on viremic control in subtype C–infected Zambians contrasts with the protective association described in 2 smaller populations of primarily European HIV-1 subtype B–infected men who have sex with men [13]. There were obvious differences in race and HIV-1 subtype between our population and the others. Another important difference is that, compared with the largely unselected HIV-1-positives in Zambia, the deliberately selected, long-term nonprogressors would likely have included a disproportional number of subjects with highly protective HLA-B*27 and B*57 alleles, which happen to display both P2-Thr and Bw4. These differences may well have contributed to the inconsistent findings in the infected populations, and it should be possible to reconcile the inconsistencies with a combination of population and experimental studies.

Our study has demonstrated that the overall contribution of HLA-I alleles to the occurrence or control of HIV-1 infection, and by inference to the outcomes of studies of vaccines, will need to account for several simultaneous effects mediated by HLA-B: presentation of virus-specific peptides in the $CD8^+$ CTL pathway, interaction of the Bw4/Bw6 epitope in the KIR3DL1/3DS1 pathway, and involvement of the HLA-E/signal peptide complex in the NKG2A/C pathway.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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