Frequent Human Leukocyte Antigen Class I Alleles Are Associated With Higher Viral Load Among HIV Type 1 Seroconverters in Thailand

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Summary: The loss of viral control by the host may be due to the evolution of viruses with mutations that limit presentation by human leukocyte antigen (HLA) to cytotoxic T cells. The authors hypothesized that the consequence of such evolution might be that persons with common HLA class I alleles would be less able to control viremia, on average, than would those with rare alleles. HLA class I typing was completed for 128 injection drug users who seroconverted in a prospective cohort study in Bangkok, Thailand. Logistic regression was used to model viral load (greater than or equal to the median) at 9 and 12 months after seroconversion with an HLA score that profiled the relative prevalence of each individual's alleles. At 12 months after seroconversion, injection drug users with the most common HLA alleles (highest quartile HLA score) had an almost 4-fold increased risk for higher viral load (≥32,055 copies/mL) than injection drug users with less common HLA alleles (adjusted odds ratio, 3.92; 95% confidence interval, 1.3–11.8). These findings support the importance of frequency-dependent effects of host genes on HIV type 1 evolution in different populations and suggest that HLA-driven viral evolution critically influences control of viremia in early HIV type 1 infection.

Key Words: Thailand, Asia, HIV type 1 (HIV-1), human leukocyte antigen (HLA), host immune system, viral load

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The highly polymorphic major histocompatibility complex (MHC) genes that encode the human leukocyte antigen (HLA) class I molecules are located on human chromosome 6.[1] It is believed that the high degree of HLA variation in different human populations is the result of strong evolutionary selection pressures from contact with various infectious agents over thousands of years.[1,2] A mechanism proposed for this variation is frequency-dependent selection in which pressure exerted by various epidemics of infectious diseases selects for particular HLA alleles with distinct peptide binding properties.[2] As a result, pathogens evolve to escape immune responses mediated by common HLA alleles but remain susceptible to responses mediated by rarer alleles.

HLA polymorphism could influence the control of viremia in HIV type 1 (HIV-1) infection through a number of immune pathways. Allelic variants can bind and display various epitopes with differing specificities and affinities, thereby influencing the efficiency of the cytotoxic T lymphocyte (CTL) response to recognize HIV epitopes as well as affecting the specificity of the T-helper-cell response. The effect of HLA may be further modified by other genes on chromosome 6 involved in antigen processing, such as *TAP*[3] and *LMP*.[4] Epistatic interactions with HLA have been demonstrated with *KIR* genes that modulate natural killer cell responses to HLA on target cells.[5] Other linked genes in the MHC region, such as those encoding tumor necrosis factor and complement, may influence viremia as well.[6]

HIV-1 infection is characterized by an early acute phase of high viremia followed by strong host responses that result in suppression of viral loads[7] to lower steady-state levels, which predict the rate of subsequent disease progression.[8–10] Although the level of viral suppression appears to be influenced by various host immune factors,[11] the CTL response to HIV-1 infection appears to be particularly important.[7,12,13] Because HLA polymorphism influences CTL responses, there has been increasing interest in understanding the influence of HLA, and several studies have shown that specific HLA alleles and haplotypes are associated with different rates of HIV-1 disease progression.[14,15] Homozygosity at certain HLA class I loci has also been associated with more rapid disease progression.[14,16]

In contrast to the relatively slow changes in HLA allele frequencies in different human populations over time, the ex-

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tensive genetic variation of HIV-1 has resulted from very rapid viral evolution since the first human infections several decades ago[17] from cross-species transmissions of related simian counterparts.[18,19] A substantial viral burden and rapid turnover of virions during early HIV-1 infection result in great viral heterogeneity even within a single host.[20,21]

In a population with an established HIV-1 epidemic, such as that in Thailand,[22–24] selective pressures could be expected to generate more viruses with escape mutations from presentation by predominant HLA class I alleles than by less common ones. Indeed, HLA-driven HIV-1 variation possibly indicative of CTL escape on a population level was recently observed for subtype B by Moore et al.[25] In Thailand, the epidemic was characterized by at least 2 independent introductions of HIV-1 subtype B and CRF01_AE.[22–24] A prospective cohort of injection drug users in Bangkok[26,27] provided a unique opportunity to test the hypothesis that persons in this cohort with common HLA alleles would be less able to control HIV-1 viremia, on average, than individuals with rare HLA alleles. The objective of this study was to evaluate the association between HLA class I allele frequencies and HIV-1 levels during the first year after seroconversion.

METHODS

Study Population and Procedures

The Bangkok Metropolitan Administration manages a large municipal drug treatment program serving ~8000–10,000 drug users annually in Thailand. As described previously, 130 seroconverters were identified between 1995 and 1998.[26,27] With voluntary informed consent, blood samples were collected as soon as possible after the first positive HIV-1 enzyme immunoassay, 1-month later, and subsequently at 4-month intervals. The estimated date of seroconversion was defined as the midpoint between the dates of the last negative enzyme immunoassay and the first positive enzyme immunoassay. Study protocols were approved by the Ethical Review of Research Committee, Ministry of Public Health (Nonthaburi, Thailand), and the Institutional Review Board, Centers for Disease Control and Prevention (Atlanta, GA).

We previously reported that ~80% of seroconverters were infected with HIV-1 CRF01_AE and 20% were infected with subtype B.[28] HIV-1 RNA load in blood plasma was determined by the Amplicor HIV-1 Monitor Test version 1.5 (Roche Diagnostics, Branchburg, NJ). Genomic DNA for 128 (98.5%) of the 130 seroconverters was extracted from peripheral blood mononuclear cells using commercial kits, according to the manufacturer's protocol (Qiagen, Chatsworth, CA).

Typing of the HLA class I loci (HLA-A, -B, and -C) was performed using polymerase chain reaction analysis and sequence-specific oligonucleotide probes according to manufacturers' instructions (Orchid Diagnostics, Stamford, CT; and Dynal Biotech, Inc., Lafayette Hill, PA) by the Laboratories at Bonfils (Denver, CO) to obtain 2- to 4-digit resolution of the alleles. HLA typing was completed without knowledge of subjects' clinical status. Allele frequencies for 128 seroconverters were calculated as frequency (%) = (total count of a given allele/256) × 100. Homozygosity at a particular locus was defined as identity for 2 digits, regardless of whether results were resolved to the 2- or 4-digit level. Because high resolution typing and sequencing were not performed, our definitions may have overestimated the true frequency of homozygosity.

Statistical Analysis

On the basis of overall HLA allele frequencies among the 128 seroconverters, an individual HLA profile score variable was created that described the relative prevalence of each individual's alleles. This score was calculated as the sum of the population allele frequency percentages for each of the 6 HLA alleles present in each individual. For example, consider the hypothetical population HLA allele frequencies of 19.6% for A*02, 2.3% for B*05, 1.8% for B*57, 9.2% for Cw*04, and 15.2% for Cw*08. Thus, an individual with A*02/A*02, B*05/B*57, and Cw*04/Cw*08 would have an HLA profile score of (19.6 + 19.6) + (2.3 + 1.8) + (9.2 + 15.2) = 67.7.

The nonparametric rank sum test was used to test the null hypothesis that median viral loads between any 2 groups of seroconverters divided by HLA score (median, quartiles) were equal against the 1-sided alternative that the group with higher HLA scores would have significantly higher viral loads. To assess the effects of HLA and other factors on HIV-1 load at selected time points after seroconversion, logistic regression models were developed with the primary outcome variable as HIV-1 load greater than or equal to the median viral load at a particular time. We chose the median rather than specific categories (eg, clinically relevant criteria) of viral load because the data were distributed normally and this partitioning generated sample size adequate for analysis. Although no antiretroviral therapy was reported for any of the participants during the first 12 months after seroconversion, antiretroviral therapy use increased subsequently. To avoid any bias from the effect of antiretroviral use on viral loads, we focused our analysis on viral loads during the first year after the estimated date of seroconversion when all seroconverters were treatment naive. In addition, because the variance of our outcome variable (HIV-1 load) was extremely high during the first 6 months after seroconversion, we excluded time points before this and focused our model on 9 and 12 months after seroconversion. Both time points were used to assess the impact of previously identified subtype-specific differences in viral load present at 9 months and not at 12 months.[26] The model further controlled for the presence of presumed homozygosity at any of the 3 HLA class I loci as well as for factors that have previously been documented for this cohort to be associated with HIV-1 load, including viral subtype (B or CRF01_AE), CD4 lymphocyte count (<400 or ≥400/mL), sex, age at seroconversion, and cal-

endar time of seroconversion.[26,29] All data analysis and modeling were completed with SAS System for Windows, version 8.2 (SAS Institute, Cary, NC).

RESULTS

Class I HLA-A and HLA-B allele frequencies are reported in Tables 1 and 2, respectively. Of the 13 HLA-A types identified, the predominant alleles, occurring at a frequency of >10%, were A*11 (26.2%), followed by A*24 (21.9%), A*02 (20.3%), and A*33 (17.6%). At the HLA-B locus, 21 HLA-B types were identified, of which only B*15 (19.9%) had a frequency of >10%. A total of 9 HLA-C alleles were detected; these were Cw*07 (30.5%), Cw*03(14.8%), Cw*08 (14.8%), Cw*04 (12.1%), Cw*01 (11.7%), Cw*06 (5.9%), Cw*12 (5.5%), Cw*14 (2.7%), and Cw*15 (2.0%). The distribution of the most common class I alleles did not differ by infecting viral strain.

The median viral loads at 9 and 12 months after seroconversion were 42,269 copies/mL (log 4.63) and 32,055 copies/mL (log 4.51), respectively. The HLA score variable was normally distributed, with a median of 94.73 (range, 22.66–123.44). HIV-1 loads were significantly higher among individuals with HLA scores greater than or equal to the median than among persons with HLA scores less than the median at both 9 months (49,855 vs. 27,189 copies/mL, respectively; $P = 0.03$) and 12 months (47,387 vs. 18,665 copies/mL, respectively; $P = 0.02$) after seroconversion. For seroconverters with the most frequent HLA alleles (the upper quartile of HLA scores [ie, ≥105.08]) compared with the remaining individuals, viral loads were also higher at both 9 months (52,830 vs. 33,732 copies/mL, respectively; $P = 0.08$) and 12 months (51,374 vs. 22,149 copies/mL, respectively; $P = 0.045$) after seroconversion.

Table 3 reports results of modeling viral load at 9 and 12 months after seroconversion. At 9 months, predominant HLA alleles (the upper quartile of HLA scores [ie, ≥105.08]) were not significantly associated with higher viral load. Seroconversion during the period of high incidence, the presence of HIV-1 subtype CRF01_AE, and $CD4^+$ cell counts of <400/mL at 9 months after seroconversion were associated with 3- to 4-fold greater risks of higher viral load. However, at 12 months, subtype and $CD4^+$ cell count were no longer significantly associated with higher viral load. At this time, predominant HLA alleles were associated with an almost 4-fold increased risk for higher viral load (odds ratio, 3.92; 95% confidence interval,

TABLE 1. HLA-A Allele Frequencies Among HIV-1–Positive IDUs Versus Uninfected Thais

HLA Allele	No.	Frequency (%) IDUs n = 128	Frequency (%) Uninfected Thais* n = 140
A*01	9	3.52	4.00
A*02	52	20.31	19.60
A*03	5	1.95	2.90
A*11	67	26.17	28.90
A*24	56	21.88	20.30
A*26	6	2.34	0.70†
A*29	2	0.78	0.70
A*30	5	1.95	1.10
A*31	2	0.78	2.50†
A*33	45	17.58	14.60
A*34	3	1.17	0.30
A*68	1	0.39	1.10
A*74	3	1.17	0.70

Only P values of < 0.10 are reported.
*Data are from Chandanayingyong et al.[34] Due to exclusion of null alleles, referent frequencies do not sum to 100.
†$P = 0.09$.
IDUs, injection drug users.

TABLE 2. HLA-B Allele Frequencies Among HIV-1–Positive IDUs Versus Uninfected Thais

HLA Allele	No.	Frequency (%) IDUs n = 128	Frequency (%) Uninfected Thais* n = 140
B*05	2	0.78	1.40
B*07	17	6.64	3.30
B*08	3	1.17	1.10
B*13	17	6.64	5.50
B*15	51	19.92	26.80
B*18	15	5.86	4.70
B*27	9	3.52	4.40
B*35	16	6.25	4.70
B*37	4	1.56	1.40
B*38	10	3.91	2.20
B*39	5	1.95	2.20
B*40	21	8.20	10.00
B*44	20	7.81	7.90
B*46	25	9.77	9.40
B*51	10	3.91	2.50
B*52	2	0.78	0.70
B*54	2	0.78	1.10
B*55	2	0.78	1.10
B*56	2	0.78	1.80
B*57	5	1.95	1.80
B*58	18	7.03	4.60

Only P values of <0.10 are reported.
*Data are from Chandanayingyong et al.[34] Due to the exclusion of null alleles, referent frequencies do not sum to 100.
IDUs indicate injection drug users.

TABLE 3. Multivariate Analyses of HIV-1 Load and Associated Factors in IDUs

	9 mo				12 mo			
	Viral Load				Viral Load			
Risk Factor	≥Median* No. (%)	<Median No. (%)	Odds Ratio†	95% CI†	≥Median No. (%)	<Median No. (%)	Odds Ratio	95% CI
HLA profile score								
≥Upper quartile‡	17 (13.5)	8 (14.5)	1.98	(0.62–6.28)	20 (38.5)	8 (14.8)	3.92	(1.30–11.82)
<Upper quartile	37 (68.5)	47 (85.5)		Referent	32 (61.5)	46 (85.2)		Referent
HLA class I homozygosity								
At any locus	21 (38.9)	14 (25.5)	1.73	(0.63–4.77)	16 (30.8)	16 (29.6)	0.62	(0.22–1.74)
At no locus	33 (61.1)	41 (74.5)		Referent	36 (69.2)	38 (70.4)		Referent
HIV-1 subtype								
CRF01_AE	49 (90.7)	39 (70.9)	3.77	(1.10–12.91)	43 (82.7)	41 (75.9)	1.37	(0.46–4.08)
B	5 (9.3)	16 (29.1)		Referent	9 (17.3)	13 (24.1)		Referent
$CD4^+$ cell count								
<400/mL	21 (38.9)	10 (18.2)	3.21	(1.18–8.78)	22 (42.3)	16 (29.6)	1.84	(0.75–4.53)
≥400/mL	33 (61.1)	45 (81.8)		Referent	30 (57.7)	38 (70.4)		Referent

*Median viral load at 9 months, 42,269 copies/mL (log 4.63); median at 12 months, 32,055 copies/mL (log 4.51).
†Adjusted odds ratios and 95% CI, controlling for sex, age at seroconversion, and calendar time of seroconversion.
‡Upper quartile of HLA score, 105.08.
CI indicates confidence interval; IDUs, injection drug users.

1.30–11.82). Homozygosity at any HLA class I locus was not predictive of viral load at either time point during the first year after seroconversion.

DISCUSSION

The most significant new finding of our study was the demonstration of an increased risk of higher HIV-1 loads within the first year of infection among individuals with the most common HLA class I alleles. The risk of higher viral load was significant even when controlling for other factors associated with viremia.[26,29] Although this finding will need to be confirmed in other cohorts, our results are consistent with the notion that coevolution of viruses and host immune responses can lead to pathogen evasion of the immune response. Viruses such as HIV-1 can evade human host responses by generating viral variants that can escape CTL recognition, as has been demonstrated by the occurrence of mutations in immunodominant viral peptides.[30] Other mechanisms whereby HLA polymorphisms could provide the host with an advantage against viral variation could involve linked genes within the MHC, such as those involved in antigen processing or in regulation of tumor necrosis factor and complement levels. Such effects could be independent or combine with direct effects of MHC variation on CTL epitope recognition. Given that HLA heterogeneity far outweighs the polymorphism known to exist in linked genes, it seems most likely that mechanisms directly related to HLA polymorphism account for the greatest part of the effect on viral load observed in this study.

Heterogeneity in HLA alleles, especially rare ones, could benefit the host when HLA-driven viral adaptations acquired in 1 host may not be advantageous to the virus in subsequent hosts with different HLA. Conversely, viruses that have evolved in hosts with common HLA alleles will have already acquired successful mutations that will limit the effectiveness of CTL responses in subsequent hosts with the same or similar HLA alleles. Moore et al[25] provided evidence for this process in that circulating HIV-1 strains acquired polymorphisms in reverse transcriptase that were likely driven by certain HLA class I alleles. Predominant HLA alleles were also associated with a lack of evidence for HLA-driven viral variation, suggesting viral adaptation to CTL responses restricted by predominant HLA types in the host population. Another study, using an MHC supertype–based classification of HLA class I alleles, demonstrated an association between the frequency of HLA supertypes and HIV-1 load that provides an advantage to individuals expressing rare supertypes.[31] This study and ours, although based on different classification systems, directly support a model whereby MHC-driven frequency-dependent selection of HIV-1 variants generates a population of viruses that have an advantage in hosts with similar or frequent MHC types.

An interesting observation in this study was a difference in the timing of the associations of CD4 cell count, viral subtype, and HLA frequency with higher viral loads. CD4 cell count and viral subtype associations were observed at 9 months but not at 12 months, whereas the HLA frequency as-

sociation was observed only at 12 months. The effect of viral subtype on viral load earlier in infection is consistent with previous research showing higher viral loads in injection drug users infected with CRF01_AE compared with subtype B, but the magnitude of these differences decreased over time.[26] Furthermore, these subtype-specific effects were independent of the effects of CD4 cell count, inoculum size, or dynamics of the HIV-1 epidemic on injection drug users in Bangkok. The finding of a temporal association of HLA frequency with viral load only at 12 months could be due to masking of the HLA effects by viral subtype earlier in infection; once the subtype effect diminishes after 12 months, the HLA effect is detectable. The HLA effect could also persist at later time points, which were not examined in this study due to introduction of antiretroviral therapy. A delayed dominance of HLA-mediated effects could also be consistent with the development of mutations in HLA-restricted CTL epitopes after prolonged infection and immune pressure.

Our approach is useful in evaluating the potential impact of HLA allelic frequency in general rather than attempting to assess the effects of specific alleles, especially uncommon ones, with respect to HIV-1 disease progression. Instead of using a genetic profile based on the presence or absence of specific host factors,[32] our HLA score directly measured the relative frequency of alleles in an individual with respect to the population of interest without dependence on a priori knowledge from previous association studies that may be confounded by differences in allele frequencies among ethnic groups. In doing so, our data provide a broader analysis of HLA effects at the population level and warrant further validation in other populations. Methods of classifying HLA alleles, such as HLA supertypes,[33] have also been applied in studying associations with HIV-1.[31] Because supertypes are based upon peptide binding motifs, they are functional classifications of HLA and imply assumptions about CTL-driven mechanisms in explaining observed HLA effects. The use of a classification based on historically serologically defined HLA alleles allowed us to consider HLA effects on viral load that may be independent of CTL response. By using a classification based upon the 2-digit resolution of alleles, we may have incorrectly grouped alleles as common that at a 4-digit resolution would be rare alleles and vice versa. However, any bias would be toward the null, indicating that the effects of HLA observed in this study were likely underestimated.

The absence of any significant differences in HLA class I allele frequencies between our cohort and comparable uninfected populations[34] suggests that HLA frequencies among HIV-infected injection drug users are probably similar to those among the general population in Bangkok and that HIV-driven MHC evolution has not yet occurred. Given that the HIV epidemic in Thailand has been established relatively recently, this is not surprising.

If HLA rarity provides an advantage in terms of viral control in the host, one would expect HLA homozygosity to have a negative effect. We did not find such an association at either 9 or 12 months in this cohort. As stated earlier, our analysis may have overestimated the true frequency of homozygosity and hence limited our ability to assess the relative contribution of this factor. Another explanation could be that the rarity of an allele, whether heterozygous or homozygous, has a greater relative effect. Finally, it is possible that the effects of homozygosity may be maximal later in disease than during the period evaluated in this study. Support for this explanation is provided by previous studies that, in general, have shown an effect of homozygosity on time to AIDS rather than on early viral load.[14,16]

As is the case for all infectious diseases, the course of HIV-1 infection is a function of the genetic variability of both the pathogen and the host. The effects on HIV-1 load are multifactorial and may vary in magnitude for HLA and other factors such as CD4 cell count, viral subtype, and risk of infection. The consideration of genetic factors in a population-based manner, such as this one, in future studies will greatly improve our understanding of host–pathogen interactions as well as potential approaches to prevention and treatment.

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