Class I HLA-A*7401 Is Associated with Protection from HIV-1 Acquisition and Disease Progression in Mbeya, Tanzania

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Here we explore associations between HLA variation and human immunodeficiency virus type 1 (HIV-1) acquisition and disease progression in a community cohort in Mbeya, Tanzania, a region that, despite harboring high rates of HIV-1 infection, remains understudied. African-specific allele HLA-A*74:01 was associated with decreased risk of infection (odds ratio [OR], 0.37; 95% confidence interval [CI], 0.14–0.80; P = .011) and with protection from CD4+ cell counts <200 cells/µL in women (OR, 0.31; 95% CI, 0.07–0.91; P = .032) and men (OR, 0.15; 95% CI, 0.01–0.78; P = .020). These associations remained significant after adjustment for linkage disequilibrium with HLA-B and HLA-C alleles. This observation calls for additional investigation of mechanisms by which HLA-A*74:01 may influence HIV-1 acquisition and control of the infection.

The class I HLA antigens are the most polymorphic loci in the human genome. They encode cell-surface molecules that present antigen peptides to cytotoxic T lymphocytes (CTLs) and serve as ligands for killer immunoglobulin-like receptors (KIRs) of natural killer cells. The class I HLA loci have been implicated in the course of many infectious diseases, including human immunodeficiency virus type 1 (HIV-1) [1, 2]. The consistent association of certain alleles (ie, B*57 and B*27) with HIV-1 disease progression and the association of HIV-1–specific CTL responses with reduced plasma viral load in early infection suggest an HIV-1–specific CTL control of HIV-1 replication once infection has been established [3]. However, the role played by class I HLA in HIV-1 infection susceptibility has been understudied, and the underlying mechanisms are not fully characterized.

Consistent with the out-of-Africa evolutionary model, modern East African populations exhibit extensive HLA diversity and contain African-specific alleles (eg, HLA-A*74:01, B*15:03, B*58:02) [4, 5]. Understanding protective or detrimental effects of class I HLA alleles in the region is critical, because this unique diversity poses challenges for designing vaccines against endemic pathogens and interpreting results from vaccine trials. The interpretation of immune responses against HIV-1 can be further complicated by the genetic diversity of the virus; the same HLA allele has shown opposing effects, depending on the subtype of the infecting virus [6, 7]. Subtype B dominates the HIV-1 epidemic in Western Europe and the Americas, and most large-scale studies of class I HLA variation and HIV-1 disease course have been carried out among white populations infected with this clade [1, 2]. On the other hand, East African HIV-1 infections are predominantly caused by subtypes A, C, and D and their recombinants [8], and the immunoepidemiological profile of this setting remains to be explored. One notable exception is the recent study that showed that carriage of A*36:01, on the one hand, and carriage of A*74, B*13, and B*57, on the other, were respectively associated with high and low viral load among seroconverters in Zambia [9]. Finally, another noteworthy confounding point is that the majority of the studies in East African cohorts involve high-risk groups [10], which may not fully reflect associations for lower risk populations.

Here we report an exploratory analysis on the effects of class
I HLA variation on HIV-1 acquisition and disease progression in the mixed HIV-1 subtype context of Mbeya, Tanzania.

Methods. The prospective community cohort study from Mbeya, Tanzania (CODE), is described in detail elsewhere [8, 11]. Briefly, at entry, 2581 people were HIV-1 seronegative, and 513 individuals were HIV-1 seropositive (modified slightly from Arroyo et al [8], due to updated information). Additionally, 2 volunteers, with an indeterminate serostatus, were lost to follow-up directly after baseline survey. Volunteers were followed up every 6 months, and 104 volunteers seroconverted during the 42-month study. Class I HLA genotyping was performed by sequence-specific priming real-time polymerase chain reaction with 4-digit resolution, as described elsewhere [5]. Samples available for HLA genotyping came from 508 seroprevalent, 98 seroincident, and 174 seronegative participants. HIV-1 subtype was determined by the multiregion hybridization assay [8] for seroprevalent samples, and by full-length sequencing for seroincident samples. Viral load set point was calculated as described elsewhere [11].

HLA allele and carrier frequencies were determined by direct counting. Haplotype frequencies were computed using PyPop software, distributed by the authors (version 0.6.0; http://www.pypop.org). Odds ratios were determined using logistic regression (SAS Institute). Time-to-event analysis was determined using Kaplan-Meier life-table methods with significance assessed using the log-rank tests. The study was conducted jointly by the Mbeya Regional AIDS Control Programme (Tanzanian Ministry of Health), the Department of Infectious Diseases and Tropical Medicine, Ludwigs-Maximilians University (Munich, Germany), and the US Military HIV Research Program (MHRP, Rockville, Maryland). Review boards of participating institutions approved the study protocols, and informed consent was obtained from all study participants.

Results. The class I HLA genetic composition of seronegative individuals from Mbeya has been described elsewhere [5]. These samples were used as a control group for comparison with seroincident cases to explore the effect of class I HLA on risk of HIV-1 acquisition. A statistically significant protective effect was observed for carriage of alleles A*02:05 (odds ratio [OR], 0.14; 95% confidence interval [CI], 0.01–0.78; \( P = .021 \)), A*74:01 (OR, 0.37; 95% CI, 0.14–0.80; \( P = .011 \)), and Cw*04:01 (OR, 0.52; 95% CI, 0.29–0.90; \( P = .020 \)), whereas a significant detrimental effect was observed for carriage of Cw*07:02 (OR, 2.88; 95% CI, 1.17–7.38; \( P = .021 \)) (Figure 1A). These effects were reiterated in time-to-event plots of carriers and noncarriers with respect to time to seroconversion (Figure 1B–1E). Demographic variables exhibiting an influence on HIV-1 acquisition in univariate analysis included age (OR, 0.96; 95% CI, 0.93–0.99; \( P = .003 \)), but neither sex nor religion (data not shown).

In multivariate analysis, carriage of A*74:01 remained statistically significantly protective in several models, including when age and sex (OR, 0.36; 95% CI, 0.15–0.80, \( P = .011 \)), HLA-B and HLA-C alleles in linkage disequilibrium with A*74:01 (OR, 0.37; 95% CI, 0.15–0.83; \( P = .015 \)), and class I alleles that had shown a \( P \) value <.1 in the univariate analysis (OR, 0.35; 95% CI, 0.14–0.79; \( P = .011 \)) were added as covariates. Multivariate analysis for alleles A*02:05, and Cw*04:01 and Cw*07:02 remained significant with most covariates (data not shown).

Class I HLA variation has been previously shown to influence viral load set point [6, 12]. The only alleles with significant effects on viral load set point were HLA-A*30:02 and Cw*18:01 (\( P = .03 \) and .006, respectively), each associated with lower viral load set point in women. The stratification based on sex [11] and low number of carriers of particular alleles limited the power to explore associations between class I HLA variation and viral load set point to a subset of alleles, excluding the protective alleles A*02:05, A*74:01, Cw*04:01. Among seroconverters, carriage of class I HLA alleles showed no association with infecting HIV-1 subtype (data not shown).

The association between class I HLA and HIV-1 disease progression at study entry was cross-sectionally explored through the proportion of seroprevalent individuals with CD4 cell counts <200 cells/\( \mu \)L. Women exhibited significantly higher CD4 cell counts (\( P = .007 \)) and thus were analyzed separately from men (Figure 2A). Among women, carriage of A*74:01 (OR, 0.31; 95% CI, 0.07–0.91; \( P = .032 \)), A*30:02 (OR, 0.44; 95% CI, 0.18–0.98; \( P = .044 \)), B*57:03 (OR, 0.19; 95% CI, 0.01–0.92; \( P = .036 \)), and Cw*18:01 (OR, 0.18; 95% CI, 0.03–0.62; \( P = .004 \)) were associated with significantly higher CD4 cell counts (Figure 2B). Carriage of A*02:05 (OR, 3.54; 95% CI, 1.24–2.30; \( P = .020 \)) and B*58:02 (OR, 2.54; 95% CI, 1.07–5.68; \( P = .035 \)) were associated with significantly lower CD4 cell counts. Among men, carriage of A*74:01 (OR, 0.15; 95% CI, 0.01–0.78; \( P = .020 \)) was associated with significantly higher CD4 cell counts, whereas carriage of B*58:01 (OR, 4.52; 95% CI, 1.14–18.33; \( P = .032 \)) and Cw*07:04 (OR, 9.19; 95% CI, 2.22–47.12; \( P = .002 \)) were associated with significantly lower CD4 cell counts (Figure 2C). HLA-A*74:01 maintained statistically significant association with CD4 cell counts in women in multivariate analysis accounting for age and alleles with \( P \) values <.1. Women exhibited significantly higher CD4 cell counts, whereas carriage of B*58:01 (OR, 4.52; 95% CI, 1.14–18.33; \( P = .032 \)) and Cw*07:04 (OR, 9.19; 95% CI, 2.22–47.12; \( P = .002 \)) were associated with significantly lower CD4 cell counts.

Discussion. In the current analysis, the African-specific allele HLA-A*74:01 displayed a protective effect for HIV-1 acqui-
Figure 1. A, Effect of class I HLA on human immunodeficiency virus type 1 (HIV-1) acquisition. Age-adjusted odds ratios and 95% confidence intervals are presented for class I HLA alleles found above a carrier frequency of 5% in cohort. P values are uncorrected for multiple comparisons. Kaplan-Meier (time-to-event) plot of months to HIV-1 seroconversion for carriers (blue line) and noncarriers (red line) of A*02:05 (B), A*74:01 (C), HLA-Cw*04:01 (D), and Cw*07:02 (E).
The protective role that A*74:01 played in disease progression is likely due to its restriction of CD8+ T cell responses, although the current repertoire of epitopes restricted by this allele is poorly characterized. Only one defined epitope restricted by A*74:01 is noted in the Los Alamos HIV database (http://www.HIV.lanl.gov/). Interestingly, A*32:01 and A*74:01 share common peptide-binding properties [13], and the former has been recently associated with lower viral load set point in a genome-wide association study in whites [14]. The mechanism of class I HLA protection for HIV-1 acquisition may be different from that proposed for disease progression and has yet to be elucidated. A*74:01 may be in strong linkage disequilibrium with another polymorphic gene, thus serving as a marker for a protective factor. Alternatively, the protective effect may be indirect, with A*74:01 restricting the immune response to an unknown endemic pathogen, thereby diminishing the level of immune activation and reducing the susceptibility toward HIV-1. This hypothesis is consistent with the observation that, in sub-Saharan Africa, elevated immune activation is associated with poor outcomes in HIV-1 pathogenesis. Finally, A*74:01 may mediate innate immune responses by providing early control of HIV-1 infection. A*74:01 is highly related to A*32:01, which serves as a ligand for KIR3DL1/S1 on natural killer cells. By serving as KIR ligands, HLA can potentially act before the mounting of adaptive immune responses without the need of priming. The repertoire of KIR ligands is of ongoing study, and it will be necessary to confirm A*74:01 as a KIR ligand.

In the current study, several other alleles demonstrated associations with HIV-1 acquisition and disease progression, consistent with previous reports. The protective role played by A*02:05 confirms previous observations among high-risk Kenyan cohorts, where it was associated with low transmission rates [10]. However, in our study, A*02:05 was detrimental for HIV-1 disease progression in seroprevalent cases among women, which suggests that separate mechanisms are involved in acquisition and disease progression. We also found that Cw*04:01 exhibited a protective effect on HIV-1 acquisition, consistent with clade C-infected South Africans, where it provided a protective effect on viral load [6] but in contrast with reports on the association of Cw*04 with rapid disease progression in clade B-infected whites [2], which reiterates the impact of viral genetic context on the HIV-1 immunoepidemiological profile. The only allele in the current study associated with increased acquisition risk was Cw*07:02, in concordance with a previous study of mother-to-child transmission among Kenyans [15]. The detrimental effect of B*58:02 carriage on CD4 cell counts among women was consistent with previous reports from South African clade C-infected women [6]. Furthermore, among women, B*57:03 was associated with protection from disease progression, consistent with one of the most reproducible class I HLA associations to date, irrespective of ethnicity, virus clade,

**Figure 2.** A, CD4 cell count distributions in seroprevalent men and women. Nonparametric significance testing for comparisons between groups (Mann-Whitney U test) was used. Effect of class I HLA allele carriage on human immunodeficiency virus type 1 (HIV-1) disease progression (total CD4 cell count, <200 cells/μL) in seroprevalent women (B) and men (C). Age-adjusted odds ratios and 95% confidence intervals are presented for class I HLA alleles found above a carrier frequency of 5% in cohort.
and risk group [1, 2]. In the current study, A*30:02 and Cw*18:01, found in linkage disequilibrium with B*57:03, were also protective among women, in agreement with results from Zambian clade C-infected cohorts [9]. Among men, B*58:01 and Cw*07:04 were associated with lower CD4 cell counts, effects not previously reported. However, when significant covariates (P < .05) were included in multivariate analysis, neither allele remained associated with disease progression.

The study of 104 seroincident cases and the focus on the genetically complex HLA loci imposed limitations to the statistical power to detect positive associations between HLA alleles and HIV-1 acquisition. Another limitation of the current study was the unavailability of viral load information for seroprevalent cases.

The current study was carried out within a mixed HIV-1 epidemic, where 3 subtypes and unique recombinants cocirculate, coupled with an extensive variation in HLA polymorphisms. In this setting, the African-specific A*74:01 demonstrated strong protection from both HIV-1 acquisition and disease progression. The current report on A*74:01, together with recent findings by others, warrant additional investigation on the mechanisms by which HLA variation affects acquisition of HIV-1. As an exploratory study, the current findings will benefit from verification in similar cohorts and may ultimately contribute to the development of effective HIV-1 vaccines.

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