Natural Killer Cell–Mediated Innate Sieve Effect on HIV-1: The Impact of KIR/HLA Polymorphism on HIV-1 Subtype-Specific Acquisition in East Africa

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Here we explore the association between killer cell immunoglobulin-like receptor (KIR)/HLA and human immunodeficiency virus type 1 (HIV-1) acquisition with different viral subtypes circulating in East Africa. In the prospective Cohort Development (CODE) cohort (Mbeya, Tanzania), carriers of KIR3DS1 and its putative ligand (HLA-A or HLA-B Bw4-80Ile alleles) showed increased HIV-1 acquisition risk (odds ratio [OR] = 3.46; 95% confidence interval [CI], 1.12–10.63; $P = .04$) and a trend for enrichment for subtype A and A-containing recombinants (78% vs 46%; OR = 4.05; 95% CI, .91–28.30; $P = .09$) at the expense of subtype C (11% vs 43%; OR = 0.17; 95% CI, .01–.97; $P = .08$). In vitro, only natural killer cells from KIR3DS1(+)/HLA-Bw4-80Ile (+) healthy donors showed a 2-fold increased capacity to inhibit replication of subtype C vs subtype A viruses ($P = .01$). These findings suggest the presence of an innate sieve effect and may inform HIV-1 vaccine development.

Keywords. HIV-1; innate immunity; KIR; HLA; sieve effect; subtypes; East Africa.

A major obstacle in the development of an effective human immunodeficiency virus type 1 (HIV-1) vaccine with global

application is the high level of worldwide viral genetic diversity. Group M HIV-1, which accounts for the vast majority of infections in the pandemic, is classified into 9 subtypes. Additionally, numerous circulating and unique intersubtype recombinants have been reported [1]. HIV-1 subtypes have a defined geographic distribution. Subtype B constitutes the most common strain in the Americas and Western Europe, while subtypes A, C, D, and their recombinants cocirculate in East Africa [2]. Founder effects have likely defined the initial introduction of particular strains in each geographic area, where they rapidly spread through established social networks, and local epidemics have further evolved by recombination and by the influx of new strains [3]. The question of whether genetic variation in host defense factors influence local HIV-1 subtype ecology remains unanswered.

HIV-1 subtypes differ by approximately 15% in their full-genome nucleotide sequences and by approximately 35% in the sequences of their gp120 envelope protein and may be associated with biological and clinical differences. Similarly, human genes involved in host restriction and innate [4] and adaptive immunity [5] present extensive genetic diversity, reflecting the demographic history of human populations along with past and present interactions with numerous pathogens. Natural killer (NK) cells are increasingly being recognized as major effectors of innate antiviral immunity. Their ability to recognize and lyse target cells is determined by signals from inhibitory and activating receptors expressed on their cell surface, including the killer cell immunoglobulin-like receptors (KIRs). Among them, KIR3DL1 and KIR3DS1 segregate as alleles of the same locus [6]. Based on receptor-binding and lysis-inhibition data, the strongest ligands for KIR3DL1 are HLA-A and -B molecules with the Bw4 public epitope and an isoleucine at position 80 [7] (ie, HLA-A Bw4(+) and HLA-B Bw4-80Ile, respectively, and jointly denoted here as “HLA-Bw4-80Ile”); these same HLA molecules are putatively the ligands for KIR3DS1 [8]. Along with HLA, KIR3DL1/S1 allele distribution varies considerably among world populations [4, 5]. In the context of HIV-1 subtype B, both KIR3DS1 and particular allotypes of KIR3DL1, when coexpressed with HLA-Bw4-80Ile, have been associated with lower viral load set point and slower HIV disease progression (reviewed in [6]). In addition, in vitro studies have demonstrated that the KIR3DS1/HLA-Bw4-80Ile genotype is associated with increased NK-cell-mediated HIV-1 inhibition

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[8]. Furthermore, recent results also demonstrate that sequence variation in HIV-1–derived peptides presented by HLA ligands can play a crucial role in the recognition by KIR [9].

Thus, a possible explanation for the global segregation of HIV-1 subtypes is that viral variants found in a given region present some level of selective advantage for their circulation in local human populations based on the host genetic background. While it is recognized that genetic variation in host defense mechanisms can translate into differences in susceptibility to HIV or control of viral replication, to date there is no conclusive evidence of differential activity on HIV-1 subtypes. Here we explore the hypothesis that the strong innate anti-HIV defense imposed by NK cells, mediated by polymorphic KIR recognizing HIV-1 in the context of HLA, plays a selective role in the global distribution of HIV-1 subtypes. This is done by imposing stronger antiviral barriers against the acquisition of particular viral strains compared to others.

METHODS

The prospective community cohort study Cohort Development (CODE) (Mbeya, Tanzania) is described in detail elsewhere [2, 10]. Briefly, 3096 consented individuals were enrolled, and seroprevalence at entry was 16.6%. During the 42-month study, 104 individuals seroconverted. Carriage of KIR2DL2, KIR2DL3, KIR3DS1, KIR3DL1, and their class I HLA ligands was determined using a validated sequence-specific priming, real-time polymerase chain reaction (PCR) assay [11]. Samples available for KIR/HLA genotyping came from 97 seroincident and 174 seronegative participants. Full-length HIV-1 genome sequencing from plasma viral RNA was performed by near-endpoint dilution reverse-transcription (RT)-nested PCR and followed by phylogenetic analysis, as previously reported [12]. Full-genome sequences were retrieved from 91 incident cases. A comprehensive questionnaire that included questions regarding lifetime and recent sexual behavior was administered. There were no indications that the HIV seronegative controls presented in our study would have had less exposure to HIV-1 than those who seroconverted (data not shown).

NK-cell inhibition assays were performed using fresh cells, as previously described [8], with clade A (KNH1088) and C clade (TZBD9/11) HIV molecular clones (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, from Dr Victoria Polonis). Consented healthy donors were recruited at Massachusetts General Hospital.

Odds ratios were determined using logistic regression (JMP, SAS Institute). Time-to-event analysis was determined using Kaplan-Meier life table methods, with significance assessed using log-rank tests. Differences in subtype-specific NK-cell-mediated HIV inhibition were assessed using paired *t* tests after verifying normality (Prism, GraphPad).

RESULTS

We compared the frequencies of KIR3DL1/S1 and HLA ligands in HIV-1 seroincident (*n* = 97) and seronegative (*n* = 174) individuals from the CODE cohort (Supplementary Table 1). While neither KIR nor HLA ligand independently influenced HIV-1 acquisition, carriage of KIR3DS1 in combination with HLA-Bw4-80Ile was significantly associated with increased risk of acquisition (odds ratio [OR] = 3.46; 95% confidence interval [CI], 1.12–10.63; *P* = .04; Figure 1A). The HLA locus that contributed most significantly to this effect was HLA-A (OR = 9.40; 95% CI, 1.08–81.68; *P* = .02), even in logistic regression models using carriage of HLA-B Bw4-80Ile as covariate (OR = 9.66; 95% CI, 1.52–187.0; *P* = .04). HLA-A*23:01 was the most common HLA-A Bw4(+) allele, but it was not independently associated with HIV-1 acquisition [10] nor did it affect the association between KIR3DS1/HLA-A Bw4-80Ile and HIV-1 acquisition in multivariate analysis (data not shown). Variation in KIR2DL2/3 and their HLA-C1 ligand showed no effect on HIV-1 acquisition (Supplementary Table 2).

At first glance, these results are in contrast with the expected protective effect of the epistatic KIR3DS1/HLA-Bw4-80Ile interaction, with the caveat that previous observations on the beneficial effect of this genotype were evidenced in the analysis of disease progression and in epidemics dominated by a single clade, that is, subtype B. Here, we hypothesized that the underlying reason for the discrepancy was the differences in HIV-1 subtypes circulating in East Africa. The cocirculation of multiple subtypes and their recombinants in Tanzania allowed us to explore the effects of KIR3DS1/HLA-Bw4-80Ile carriage on the infecting HIV-1 strain.

Ninety percent of seroincident cases were noncarriers of KIR3DS1/HLA-Bw4-80Ile, and their subtype distribution coincided with that of contemporaneous seroprevalent individuals from the same community (ie, subtype C > subtype A, A/C, C/D, and A/C/D recombinants > subtype D and A/D recombinants [2]; Figure 1B). On the other hand, seroincident carriers of KIR3DS1/HLA-Bw4-80Ile exhibited a trend for enrichment for subtype A or A-containing recombinant strains (78% vs 46% in noncarriers; OR = 4.05; 95% CI, .91–28.30; *P* = .09) at the expense of subtype C (11% vs 43% in noncarriers; OR = 0.17; 95% CI, .01–.97; *P* = .08; Figure 1B). The small number of KIR3DS1(+)/HLA-Bw4-80Ile(+) individuals precluded further analysis of the separate contribution of HLA-A and HLA-B ligands. Other KIR/ligand genotypes were explored, but no other statistically significant effects on subtype distribution were observed (data not shown). No statistically significant differences in viral load set point were evident when stratified based on KIR/HLA or HIV-1 subtype (data not shown). Thus, the initial observation of the detrimental effect of KIR3DS1/HLA-Bw4-80Ile on HIV-1 acquisition could be ascribed to a greater protection against subtype C

Figure 1. Killer immunoglobulin receptor (KIR)3DS1/HLA-Bw4-80Ile is associated with subtype-specific human immunodeficiency virus type 1 (HIV-1) acquisition in Mbeya, Tanzania. *A*, Time-to-event analysis of HIV-1 seroconversion shows increased risk of viral acquisition for carriers of KIR3DS1/HLA-Bw4-80Ile compared with noncarriers. “Other genotypes” combines KIR3DS1(–)/HLA-Bw4-80Ile (+), KIR3DS1(+)/HLA-Bw4-80Ile (–), and KIR3DS1(–)/HLA-Bw4-80Ile (–). *B*, Comparison of the subtype distribution among seroincident patients stratified based on carriage of KIR/HLA ligand shows enrichment of subtype A and A-containing recombinants (combined in dotted box) at the expense of subtype C among carriers of KIR3DS1/HLA-Bw4-80Ile. The genetic diversity in seroprevalent patients sampled contemporaneously from the same cohort [2] is illustrated to provide the molecular context of the local epidemic. For details on HLA, KIR, and HIV-1 genotyping, see Materials and Methods.

acquisition, a diminished protection against subtype A, or a combination of the two. Overall, these results suggest a subtype-specific effect of KIR3DS1/HLA-Bw4-80Ile on HIV-1 acquisition.

To determine whether some functional difference in NK-cell-mediated recognition of these 2 viral clades could account for the observed subtype selectivity, we performed in vitro NK-cell viral inhibition assays, where cells from healthy donors with defined KIR/HLA genotypes were infected with viral

Figure 2. Carriage of killer cell immunoglobulin-like receptor (KIR)3DS1/HLA-Bw4-80Ile is associated with enhanced natural killer (NK)-cell-mediated inhibition of human immunodeficiency virus type 1 (HIV-1) subtype C in vitro replication compared with subtype A. *A*, The capacity of NK cells from donors that either lack the HLA ligand [ie, HLA-Bw4-80Ile(–)], carry the HLA ligand in the absence of KIR3DS1 [ie, KIR3DS1(–)/HLA-Bw4-80Ile(+)], or carry both KIR3DS1 and its ligand [ie, KIR3DS1(+)/HLA-Bw4-80Ile(+)] to inhibit the replication of HIV-1 subtype A and subtype C–infected autologous CD4 T cells was compared. *B*, The differential inhibition of subtype A vs subtype C by NK cells based on their KIR/HLA genotype is reiterated when the contributions of the HLA-A and HLA-B loci are analyzed separately. The statistical significance of the difference in inhibition could not be calculated for KIR3DS1(+)/HLA-A Bw4(+) due to the few carriers of this genotype. Abbreviation: n.d., not determined.

isolates from HIV-1 subtypes A and C in the absence or presence of autologous NK cells. In noncarriers of HLA-Bw4-80Ile, no appreciable differences in NK-cell-mediated inhibition of subtype A vs subtype C viruses were apparent, and a similar pattern was observed among donors who carried HLA-Bw4-80Ile in the absence of KIR3DS1 (Figure 2*A*). However, in KIR3DS1(+)/HLA-Bw4-80Ile(+) donors, the NK-cell-mediated inhibition of HIV-1 subtype C was 2-fold higher than that of subtype A ($P = .01$). These results remained significant when the analysis was limited to donors who were KIR3DS1(+)/HLA-B Bw4-80Ile(+) ($P = .02$; Figure 2*B*). While too few donors were KIR3DS1(+)/HLA-A Bw4(+), they showed a concordant trend for higher

inhibition of subtype C. Thus, the observed enrichment of HIV-1 subtype A and A-containing recombinants at the expense of subtype C among carriers of KIR3DS1/HLA-Bw4-80Ile in Tanzania was recapitulated in vitro by the higher capacity of NK cells from donors with this genotype to inhibit HIV-1 subtype C compared with subtype A.

DISCUSSION

This is the first study to implicate innate immune responses against HIV-1 that exert a protective effect in a subtype-specific manner, as supported by convergent lines of evidence from molecular epidemiology and functional immunology. At the population level, this phenomenon was manifested by a differential risk of acquisition of subtype C vs subtype A and A-containing recombinant HIV-1 in carriers of KIR3DS1/HLA-Bw4-80Ile. It is noteworthy that most of the previous KIR/HLA studies have been conducted in epidemics where a single HIV-1 subtype constituted the dominant strain [6, 13, 14]. It was only in a multisubtype epidemic, like the one in East Africa, that this phenomenon could be revealed.

Given the low efficiency of HIV-1 mucosal acquisition (1/100 to 1/1000 coital acts) and the strong viral genetic bottleneck at mucosal transmission (80% of heterosexual infections are established by a single viral variant), it is widely believed that incoming viruses are presented with several lines of host defense that frequently are effective at limiting the establishment of new viral infections [15]. Here we demonstrate that one of these putative innate immunity barriers, the NK cells that abundantly patrol the mucosa and whose antiviral responses are governed by genetically polymorphic receptors and ligands, exert differential effector function depending on HIV-1 subtype, in essence creating an innate sieve effect. In light of the extensive global genetic diversity in host defense factors [4, 5], our findings support the hypothesis that the circulation of particular HIV-1 subtypes or recombinants in a given geographic area may reflect not only founder effects but also the population-level balance between the diverse genetic background of human populations and the varying capacities of HIV-1 strains from different subtypes to subvert the host antiviral defenses.

This study has several limitations. It is widely recognized that the KIR3DS1/HLA-Bw4-80Ile genotype is minimally represented in East Africa [4], which has limited the statistical power of the current study. Through observation of 7471 person-years, the prospective CODE cohort was able to characterize only 9 HIV-1 seroincident individuals with this genotype. However, the fact that we were able to replicate the epidemiological observation in a well-established in vitro system supports our results. Also, previous work on HIV-exposed seronegatives (HESNs) identified KIR3DS1 homozygosity as protective for HIV-1 acquisition [13, 14]. However, this genotype is very infrequent in East Africa in general [4] and specifically in our study (2/97

seroincident and 1/174 seronegative individuals), which precluded the assessment of its impact in our cohort.

Another limitation derived from the current cohort design is that we only know what HIV-1 strains were able to establish new infections; we were unable to identify those viruses that were successfully contained by protective mechanisms. Nevertheless, the fact that the subtype distribution among KIR3DS1/HLA-Bw4-80Ile noncarrier seroincident individuals was similar to that of contemporary seroprevalent cases [2] supports the concept that the latter constitute a valid approximation of the pool of viral strain to which individuals were exposed. Also, this study was limited to analysis of viral variation at the subtype level. The elucidation of the HIV-1 sequence motifs that may be differentially presented by HLA or recognized by KIR is underway. Moreover, it is possible that different subtypes will lead to the differential expression of host stress peptides that are recognized by KIR/HLA. Finally, KIR/HLA epistatic interaction explains only a portion of the observed viral genetic variation in East Africa. Thus, other factors with subtype-specific protective capacity, probably acting in concert, might be responsible for shaping the HIV-1 subtype ecology in the region.

In summary, the prospect of genetic variation in innate immunity affecting subtype-specific acquisition of HIV-1 calls for re-examination of our understanding of host–pathogen interaction. The study of the implications of host and viral genetic variation on innate immunity may inform HIV vaccine development.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). 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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Ethics Statement. Laboratory and field work was done in accordance with the Helsinki Declaration of 1975 as revised in 2000 and was also approved by the ethics committees of the Mbeya Referral Hospital, the Tanzanian National Institute of Medical Research, and the Medical Center of the University of Munich as well as the Institutional Review Board of the Walter Reed Army Institute of Research. The Massachusetts General Hospital Institutional Review Board approved the NK viral inhibition study. All volunteers provided written informed consent.

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Potential conflicts of interest. All authors: No reported conflicts.

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