Opinion

Synergy or Independence? Deciphering the Interaction of HLA Class I and NK Cell KIR Alleles in Early HIV-1 Disease Progression

Jason D. Barbour*, Uma Sriram, Stacy J. Caillier, Jay A. Levy, Frederick M. Hecht, Jorge R. Oksenberg

Introduction

Individual susceptibility to infectious disease, such as HIV-1, is strongly influenced by the genetic profile of the host. Allelic variants of the human major histocompatibility complex (human leukocyte antigen [HLA]) genes have been implicated repeatedly with susceptibility, course, and outcome of HIV-1 infection [1]. The HLA Class I loci is genetically diverse, and in addition to its functions in presenting peptide fragments of the invading pathogen to CD8+ T cells, may assume other functions, such as direct interaction with natural killer (NK) cells. Alleles of the Class I A and B loci form the Bw4/Rei6 cluster, a grouping based on an exposed epitope on the a1 helix, on the lip of the peptide binding cleft. This region of the HLA molecule is known to interact with NK cells via the killer Ig-like receptor (KIR). Expressed on the NK cell surface, KIR is allelic, with forms that, upon engagement with an HLA Class I ligand, may either inhibit or activate an NK cell. The KIR receptor alleles fall into two broad groups: forms with a long cytoplasmic tail (such as KIR2DL1) bear an immune tyrosine inhibitory motif and inhibit, while short-tail forms (such as KIR2DS2) activate NK cells [2]. Upon engagement with an HLA Class I ligand, short-tail forms of KIR may increase NK cell effector activity, inducing the release of perforin and granzyme, and secretion of IFN-γ [2].

Summary of Observational Studies on HLA and KIR on HIV-1 Disease Progression

Flores-Villanueva and colleagues noted that HIV-1-infected persons with superior control of viral replication in the absence of anti-retroviral treatment were more likely to be homozygous for a Bw4 allele than those who could not control infection [3]. The Bw4 cluster, in addition to bearing an epitope that interacts with certain KIRs, contains a number of HLA types known to confer protection against HIV-1 disease progression, such as B*57 and B*27. The Bw4 cluster does not contain the B*35 allele, of which the B*35pv variant [4] has been linked to accelerated disease progression. Hence, the association of Bw4 with improved outcomes may be linked to the inclusion of strongly protective alleles such as B*57, the exclusion of others such as B*35, and or interaction with a form of KIR.

The Bw4 epitope contains a subset of alleles known as Bw4Rei80, which bear an isoleucine in position 80 of the a1 helix, the lip of the HLA binding cleft. The Bw4Rei80 epitope may be important in T cell modulation [5] and immunomodulation of NK cell function [6]. The Bw4Rei80 epitope interacts with the KIR3DL1 inhibitory allele. KIR3DS1 is a short-tailed form of KIR, and has very high sequence homology to KIR3DL1 in its extracellular domains. KIR3DS1 was predicted to likewise interact with Bw4Rei80 molecules, although this interaction had not been directly observed. Martin et al. examined the epitastic interaction of KIR3DS1 and Bw4Rei80 for the effect on time to the onset of AIDS [7] in a cohort of HIV-1-infected adults in North America. They observed that Bw4Rei80/KIR3DS1 carriers experienced a significant delay in time to disease. The authors controlled their analysis for the known effects of the protective B*27, B*57, and the deleterious B*35 alleles. Hence, the Bw4Rei80/KIR3DS1 effect on disease progression may be due to a synergistic benefit of carriage of both the Bw4Rei80 and KIR3DS1 alleles, an effect which may be attributable to enhanced NK cell activity via KIR activation [2].

In a subsequent study by Qi et al. [8] based on same cohorts of HIV-1-infected persons studied by Martin et al., Bw4Rei80/KIR3DS1 was associated with a delayed risk of development of certain opportunistic infections. Qi et al. observed that Bw4Rei80/KIR3DS1 carriers had an average HIV-1 RNA level of 4.75 log_{10} copies/mL versus a viral load of 4.89 log_{10} copies/mL for those who did not carry both genes, and suggested this difference might at least partially explain the observed clinical benefits. This difference in viral replication is modest, and may not be sufficient to explain the benefit in long-term clinical outcome in HIV-1 disease that was observed among persons with Bw4Rei80/KIR3DS1.

Gaudieri et al. examined HLA and KIR effects in a cohort of HIV-1-infected persons in Australia [9]. The authors observed an increased relative rate of CD4+ T cell percent

**Editor:** Marianne Manchester, The Scripps Research Institute, United States of America

**Citation:** Barbour JD, Sriram U, Caillier SJ, Levy JA, Hecht FM, et al. (2007) Synergy or independence? Deciphering the putative interaction of HLA Class I and NK cell KIR receptor alleles on early HIV-1 disease progression. PLoS Pathog 3(4): e43. doi:10.1371/journal.ppat.0030043

**Copyright:** © 2007 Barbour et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Abbreviations:** HLA, human leukocyte antigen; KIR, killer Ig-like receptor; NK, natural killer; SE, standard error

Jason D. Barbour, Jay A. Levy, and Frederick M. Hecht are with the HIV/AIDS Division, Department of Medicine, San Francisco General Hospital, University of California San Francisco, San Francisco, California, United States of America. Uma Sriram, Stacy J. Caillier, and Jorge R. Oksenberg are with the Department of Neurology, University of California San Francisco, San Francisco, California, United States of America

* To whom correspondence should be addressed. E-mail: jbarbour@php.ucsf.edu
loss for those carrying KIR3DS1. In contrast to results by Martin et al., Gaudieri et al. did not find a significant protective effect against CD4+ T cell loss among Bw4Ile80/KIR3DS1 carriers, and in fact found these persons were at increased hazard (relative hazard of 14) for reaching AIDS (under the 1987 US Centers for Disease Control and Prevention definition).

In our contemporary cohort of recently HIV-1-infected persons, we had the opportunity to observe the association of Bw4Ile80 and KIR3DS1 alleles on HIV-1 RNA and CD4+ T cell levels during very early infection (see Protocol S1). These individuals were treatment-naïve adults with precisely known dates of seroconversion (within six months of seroconversion for 90% of cases). Of the 255 persons in this analysis, the median HIV-1 RNA level at study entry was 4.66 (interquartile range [IQR] 3.77, 5.29) log10 HIV-1 RNA copies/mL, and the median CD4+ T cell count was 534 (IQR 410, 674) cells/μL. These persons were observed for a total of 263.7 person-years prior to initiating potent anti-retroviral therapy. The study population was 88% Caucasian and 94% were male, reflecting the HIV-1 epidemic in San Francisco, where we are based. Of these 255 individuals, 94 (37%) carried at least one KIR3DS1 allele, 239 (94%) carried at least one KIR3DL1 allele, 114 (45%) carried at least one HLA Class I B Bw4Ile80 allele, and 43 (17%) carried both a KIR3DS1 and an HLA-B Bw4Ile80 allele. We considered only HLA Class I B alleles as part of the Bw4Ile80 cluster for this analysis.

With the benefit of multiple HIV-1 RNA and CD4+ T cell measures from very early in infection, we observed that the KIR3DS1-bearing individuals had higher CD4+ T cell counts (average + 58 cells/μL [standard error (SE) = 28.5] higher over time, p = 0.04, Figure 1A), but did not have lower HIV-1 RNA levels (~0.11 [SE = 0.12] log10 copies/mL, p = 0.34, Figure 1A). In contrast, those carrying the Bw4Ile80 allele had a significant reduction in viral load at study entry (~0.37 log10 [SE = 0.10] copies/mL, p = 0.0003, Figure 1B), but did not display a significant difference in CD4+ T cell counts (~1.5 [SE = 25.5] cells/μL, p = 0.95, Figure 1B). Those carrying both Bw4Ile80 and KIR3DS1 did not display a significant trend towards higher CD4+ T cell counts over the period of observation (+40 cells/μL [SE = 36.5], p = 0.27, Figure 1C), and displayed a trend towards a reduction in
viral loads ($-0.22 \log_{10} [SE = 0.15]$ lower HIV-1 RNA copies/mL, $p = 0.14$, Figure 1C) relative to those who did not carry both genes.

The reduction in viral load among Bw4Ile80/KIR3DS1 carriers was smaller ($-0.22 \log_{10} [SE = 0.15]$ copies/mL, $p = 0.14$; Figure 1C) than that reduction seen for all Bw4Ile80 ($-0.37 \log_{10}$ copies/mL, $p = 0.0003$, Figure 1B). And, the effect on CD4+ T cell count was smaller and not statistically significant for Bw4Ile80/KIR3DS1 compared to the effect for KIR3DS1 alone ($+40$ cells/μL versus $+58$ cells/μL, Figure 1C versus 1A).

That neither the viral load nor CD4+ T cell effects were augmented among carriers of Bw4Ile80/KIR3DS1 suggests that Bw4Ile80 and KIR3DS1 do not synergize to confer greater protective effects on HIV-1 disease markers in early infection, a period in which innate immunity ought to be active.

To further explore the possibility of synergy between Bw4Ile80 and KIR3DS1, we compared those with KIR3DS1 and lacking Bw4Ile80 to those who carried both genes. If Bw4Ile80 and KIR3DS1 were operating synergistically, we would expect to see a greater benefit on HIV-1 RNA levels, or CD4+ T cell counts, among Bw4Ile80/KIR3DS1 versus those carrying KIR3DS1 alone. The Bw4Ile80/KIR3DS1 carriers did not display a trend towards higher CD4+ T cell counts ($-0.10$ [SE = 48.3] cells/μL over time, $p = 0.9$) versus those who carried KIR3DS1 alone. Those with both Bw4Ile80 and KIR3DS1 demonstrated a marginally significant trend towards viral load reductions ($-0.29$ [SE = 0.19] $\log_{10}$ copies/mL, $p = 0.12$) versus those who carried KIR3DS1 alone. This magnitude of difference in viral load was similar to the deficit observed between those who did and did not carry Bw4Ile80 alone (Figure 1B), regardless of KIR3DS1 carriage (which was $-0.37 \log_{10}$ copies/mL, Figure 1B). This suggests that the effect on viral load reduction was due to the Bw4Ile80 gene itself, and not due to an interaction with KIR3DS1. Indeed, when persons bearing B*57 (a Bw4Ile80 allele known to confer lower viral loads) were removed from the analysis, the magnitude of the viral load reduction was reduced from $-0.37 \log_{10}$ copies/mL to $-0.25$ (SE = 0.10) $\log_{10}$ copies/mL, $p = 0.02$). The HLA B*57 allele, which is known to associate with very low viral loads during early infection, may confer a large share of the viral load reduction among Bw4Ile80 carriers, regardless of KIR3DS1 carriage. Taken together, these data suggest that KIR3DS1 may associate with elevated CD4+ T cell counts during early infection in a manner independent of viral load, with Bw4Ile80 having a modest additive benefit via a small reduction in HIV-1 RNA levels.

The reduction in viral load that we observed for the Bw4Ile80/KIR3DS1 group was slightly larger than that observed by Qi et al. ($-0.14 \log_{10}$ c/mL for Qi et al. versus our observation of $-0.22 \log_{10}$ c/mL). This magnitude of difference in HIV-1 RNA levels observed by Bw4Ile80/KIR3DS1 is small and may not be sufficient to explain differences in CD4+ T cell levels. Our results indicate that there is an effect of KIR3DS1 carriage on CD4+ T cell counts independent of Bw4Ile80 carriage in early HIV-1 infection. Bw4Ile80/KIR3DS1 joint carriage was associated with a modest reduction in HIV-1 RNA levels, which is likely due to independent activity of Bw4Ile80 member alleles, such as B*57. The effect of KIR3DS1 on CD4+ T cell counts during early infection may reflect higher CD4+ T cell counts prior to acquisition of infection, a sparing of CD4+ T cells during early infection, or both.

We performed our analyses considering only HLA Class I B loci alleles as part of the Bw4Ile80 cluster. This is consistent with the approach by Martin et al. [7] and facilitates comparison of results. When we included HLA Class I A loci alleles as part of the Bw4Ile80 cluster, our main inference—the absence of synergy between Bw4Ile80 and KIR3DS1 on either viral load or CD4+ T cell count—was unchanged.

Until recently, it has not been clear whether HLA Class I A loci alleles, which bear a Bw4Ile80 motif, interact with KIR alleles, such as KIR3DL1. Recent work has suggested that certain HLA Class I A Bw4Ile80 alleles, such as A*24, interact with differing KIR3DL1 allotypes [10]. Binding of A*2402 to certain allotypes was influenced by the nature of the peptide bound. HIV-1 Nef and p17 peptides were observed to enhance A*2402 binding to certain KIR3DL1 allotypes. Hence, sequence of the acquired viral strain, and peptide presentation patterns within the infected person, may influence the interaction of HLA and KIR molecules.

Recent experimental results may shed light on the question of Bw4 and KIR3DS1 interaction. O’Connor et al. recently verified that KIR3DS1 is in fact expressed on the NK cell surface, and was higher among homozygotes than heterozygotes. However, Carr et al. [11] and O’Connor et al. [12] each separately demonstrated that neither Bw4 nor Bw6 ligands recognize KIR3DS1 extracellular domains. KIR3DS1 extracellular domains bear high sequence homology to KIR3DL1 and were predicted to be recognized by Bw4 and Bw4Ile80 molecules. That it was not recognized may indicate that Bw4Ile80 molecules do not, as a group, recognize KIR3DS1. Alternatively, and as the authors suggest, it is possible that KIR allotype recognition by a Class I A or B Bw4Ile80 molecule is dependent on the peptide bound in the HLA cleft [10]. But, that has yet to be shown for KIR3DS1.

Our study focused on correlates of HIV-1 RNA and CD4+ T cell levels in the earliest stages of HIV-1 infection. Disease progression rates are established in early infection [13,14], and innate immune activity such as NK cell activity ought to be present in the earliest stages of infection. An NK cell-mediated effect on disease parameters such as CD4+ T cell counts or viral load is likely to be observed at that time. The study by Martin et al. noted that the effect of Bw4Ile80/KIR3DS1 carriage on disease outcome (the fraction remaining AIDS-free) was not present until year three of infection. A definition of clinical AIDS is reached at a time of severe CD4+ T cell loss late in infection. Hence, there may have been an observable effect on CD4+ T cell count and rate of loss or on elevated and increasing HIV-1 RNA levels during earlier time periods in the Martin et al. cohort.

The studies by Martin et al. and Qi et al. take a survival analysis or time-to-event approach to an historical cohort with extended pre-treatment follow-up. This approach to observational data does not make use of all available clinical data. In our cohort, we used longitudinal mixed effects modeling statistics (as did Gaudieri et al.) that allowed us to make use of all of our data over time. Our cohort has the advantage of being contemporary (enrollment 1996 to present) and having tightly spaced HIV-1 RNA and CD4+ T cell measurements during early infection. Our study is larger in enrollment than that by Gaudieri et al., and has more precisely known dates for seroconversion. However, our cohort, as has been under observation since the advent of effective anti-retroviral therapy, has less untreated follow-up.
than either of these prior studies. Therefore, our data do not extend to the effect of Bw4Ile80 and KIR3DS1 carriage beyond the first two years of HIV-1 infection.

Neither our study nor others described here have addressed the issue of KIR “dose” or expression patterns beyond whether an individual is an homozygotic or heterozygotic carrier of an allele. That is, individuals carry variable numbers of KIR genes (six to 19 genes) [2], which can lead to complex expression patterns. The number and pattern of KIR3DS1 or other activating alleles carried by an individual may have an impact on the clinical phenotypes described here, and may help explain the discrepancy in outcomes across studies.

Alternative explanations for a role of KIR3DS1 in delayed disease progression may exist. KIR molecules may be found on cell types other than NK cells. For example, KIR2DS2 molecules are expressed on T cells [15] among those suffering from chronic inflammatory conditions such as rheumatoid arthritis [16], and may influence the activation state and cytokine expression of these T cell populations. Snyder et al. observed that, upon antibody stimulation of surface KIR2DS2 on CD4+ CD28null DAP12-expressing T cells, the signaling protein Erk was phosphorylated and IFN-γ production increased [17]. In this way, KIR-activating alleles, of which KIR3DS1 is a member, may influence CD4+ and/or CD8+ T cell activation states in a manner independent of T cell receptor stimulation. Hence, it is possible the KIR3DS1 expression on lymphocytes influences the activity and activation state of memory CD4+ T cells in HIV disease and, in turn, influences bystander cell death or dysfunction of uninfected CD4+ T cells [18]. Again, the ligand for such an effect is not known.

While our data do not support an interaction for Bw4Ile80 and KIR3DS1, it remains possible that there is an interaction which manifests later in disease, dependent on an unknown ligand or on presentation of specific HIV-1 peptide fragments that are not processed until later in infection. Alternatively, KIR3DS1 expression alone may confer a protective effect on CD4+ T cell counts in HIV-1 disease absent interaction with a Bw4 ligand via direct modulation of CD4+ or CD8+ T cells that express KIR [17]. Further experimental work is needed to verify the regulation, expression, and localization of KIR3DS1, its interaction with Class I ligands, and dependence on the peptide fragment bound by a Class I molecule. ■

Supporting Information
Protocol SI. Materials and Methods
Found at doi:10.1371/journal.ppat.0030043.sd001 (38 KB DOC).

Acknowledgments
We thank the OPTIONS study participants for their involvement in this study. We thank the OPTIONS study staff for their work recruiting and following the participants in this study.

Author contributions. JDB, JAL, FMH, and JRO designed the study. US, SJC, and JRO performed experiments. JDB analyzed the data. US, SJC, FMH, and JRO contributed reagents or tools. JDB, JAL, FMH, and JRO wrote the paper.

Funding. This work was supported by grants from the National Institutes of Health (NIH U10 AI11531 to JAL and FMH). JDB is supported by K01 AI 066917 (National Institute of Allergy and Infectious Diseases/NIH).

Competing interests. The authors have declared that no competing interests exist.

References
1. Carrington M, O’Brien SJ (2003) The influence of HLA genotype on AIDS. Annu Rev Med 54: 535–551.
2. Williams AP, Bateman AR, Khakoo SI (2005) Hanging in the balance: KIR and their role in disease. Mol Interv 5: 226–240.
3. Flores-Villanueva PO, Yunis JI, Delgado JC, Vittinghoff E, Buchbinder S, et al. (2001) Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. Proc Natl Acad Sci U S A 98: 5140–5145.
4. Gao X, Nelson GW, Karacap I, Martin MP, Pfair J, et al. (2001) Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. N Engl J Med 344: 1668–1675.
5. Nossner E, Goldberg JE, Naftzger C, Lyu SC, Clayberger C, et al. (1996) HLA-derived peptides which inhibit T cell function bind to members of the heat-shock protein 70 family. J Exp Med 183: 339–348.
6. Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M (1994) NK3-specific, natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoeucine 80. J Exp Med 180: 1255–1242.
7. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, et al. (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 31: 429–434.
8. Qi Y, Martin MP, Gao X, Jacobson L, Goodlett JI, et al. (2006) KIR/HLA pleiotropism: Protection against both HIV and opportunistic infections. PLoS Pathog 2: e79. doi:10.1371/journal.ppat.0020079.
9. Gaudet S, De Santis D, McKinnon E, Moore C, Nolan D, et al. (2005) Killer immunoglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression. Genes Immun 6: 685–690.
10. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, et al. (2007) Cutting edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol 178: 35–37.
11. Carr WH, Rosen DB, Arase H, Nixon DF, Michaelsson J, et al. (2007) Cutting edge: KIR3DS1, a gene implicated in resistance to progression to AIDS, encodes a DAP12-associated receptor expressed on NK cells that triggers NK cell activation. J Immunol 178: 647–651.
12. O’Connor GM, Guinan KJ, Cunningham RT, Middleton D, Parham P, et al. (2007) Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. J Immunol 178: 235–241.
13. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, et al. (1998) CD8+ T lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. J Acquir Immune Defic Syndr Hum Retrovirol 18: 332–340.
14. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, et al. (2004) Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 104: 942–947.
15. Uhrlberg M, Valiantne NM, Young NT, Lanier LL, Phillips JL, et al. (2001) The repertoire of killer cell Ig-like receptor and CD94/NKG2A receptors in T cells: Clones sharing identical alpha beta TCR rearrangement express highly diverse killer cell Ig-like receptor patterns. J Immunol 166: 3923–3932.
16. Schmidt D, Martens PB, Weyand CM, Goronzy JJ (1996) The repertoire of CD4+ CD28- T cells in rheumatoid arthritis. Mol Med 2: 608–618.
17. Snyder MR, Nakajima T, Leibson PJ, Weyand CM, Goronzy JJ (2004) Stimulatory killer Ig-like receptors modulate T cell activation through DAP12-dependent and DAP12-independent mechanisms. J Immunol 173: 3725–3731.
18. Holm GH, Gabuzda D (2005) Distinct mechanisms of CD4+ and CD8+ T cell activation and bystander apoptosis induced by human immunodeficiency virus type 1 virions. J Virol 79: 6299–6311.