



A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection

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Abstract

Background: During natural killer (NK) cell development, NK receptors, such as KIR molecules, interact with their ligand in order to produce self-tolerant cells. These will exhibit functional competence in situations where receptor-ligand binding is disrupted, such as occurs in HIV infection when HLA-A and B expression is downregulated by Nef. Receptor-ligand interactions mediating strong inhibitory signals may eventually favor a more potent NK cell activation and better microbial or tumor control when inhibition is abrogated. In HIV infection, co-expression of different allelic combinations of KIR3DL1 subtypes with HLA-B influence disease outcome such that strong KIR-HLA interactions correlate with slower time to AIDS. The strongest influence on disease outcome in KIR3DL1 homozygotes (hzm) individuals is co-expression of HLA-B*57 and a KIR3DL1 genotype (3DL1*^h*) lacking low expressing alleles at this locus.

Hypothesis: Co-expression of HLA-B*57 and 3DL1*^h* not only affects HIV disease outcome, but also contributes to resistance to infection in individuals who are exposed repeatedly to HIV but remain uninfected (EU).

Methods: The study population included 39 EU and 186 subjects recently infected subjects enrolled in the Montreal Primary Infection (PI) cohort. All were KIR3DL1 hzm. Inclusion criteria for EU were at least 3 years of follow up in a high-risk cohort and at least 5 documented exposures to HIV. KIR3DL1 subtyping was performed by sequencing KIR3DL1 exons 3, 4, 5, 7 and 9. Single nucleotide polymorphisms corresponding to the KIR3DL1 subtypes KIR3DL1*001, *002, *004, *005, *007, *015, *019, and *020 were then identified. Sequence specific oligonucleotide typing and sequencing were used for HLA-genotyping. We compared the genetic distribution of KIR3DL1 subtypes and of HLA-B*57 genotypes in EU versus PI subjects using a Fisher's exact test.

Results: The frequency of HLA-B*57 allele in the EU and PI population was 0.06 (5/78 alleles) and 0.02 (8/372 alleles), respectively (OR = 3.116, p=0.0565) whereas KIR3DL1*^h*^y distribution was similar in both populations (EU, 26/39, 66.7%, PI, 106/186, 57.0%, p=0.29). However, the combined B*57-3DL1*^h*^y genotype was more frequent in EU (5/39, 12.8%) than PI subjects (5/186, 2.7%) (OR = 5.171, p=0.0154).

Conclusions: The 3DL1*^h*^y-B*57 combined genotype not only plays a role in HIV disease progression, but may be related to protection from infection.

Background

• Some individuals remain HIV-seronegative despite repeated high-risk behavior. These are called EU (exposed uninfected).

• Understanding the resistance seen in these subjects could lead to better vaccine design.

• Certain aspects of NK cell function and NK receptor-ligand genotype have been linked to EU status

—Scott-Algara et al. 2003, Montoya et al. 2006, Jennes et al. 2006, Ballan et al. 2007, Boulet et al. In Press.

NK cells are regulated by surface receptors

• Surface receptors mediate activating and inhibitory signals determining state of activation of the cell. These include:

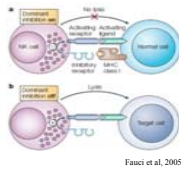
- NCR
- NKG
- ILT-2
- KIR

• KIR: Killer cell-immunoglobulin-like receptors.

• Some KIR are activating, e.g. KIR3DS1 and others are inhibitory, e.g. KIR3DL1.

• Ligand for KIR is MHC I

• Ligand for KIR3DL1 is HLA-Bw4. This includes HLA-B*57



Fauci et al. 2005

Figure 1 NK cell activation paradigm

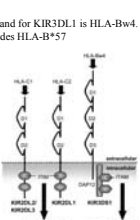


Figure 2 KIR structure and signaling

KIR3DL1 subtypes affect HIV disease progression

- There are many subtypes of KIR3DL1. These subtypes differ in surface expression levels and in inhibition signal strength.
 - high: KIR3DL1*001, 002, 008, 009, 015, 020.
 - low: 005, 006, 007.
 - null: 004.

• An individual with no low expression KIR3DL1 is defined as KIR3DL1*^h*^y

• KIR3DL1*^h*^y defines individuals with at least one low expressing KIR3DL1 subtype.

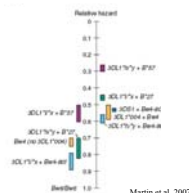


Figure 3 Continuum of protection against HIV disease progression confirmed by KIR and HLA-Bw4 genotypes.

Hypothesis

- The combination of KIR3DL1*^h*^y and HLA-B*57 associated with decreased viral load may also be associated with resistance from infection in EU

Population

All study subjects are KIR3DL1 homozygotes in order to eliminate any possible confounding effects of the allelic KIR3DS1, which is also known to influence HIV outcome (Martin et al. 2002, Barbour et al. 2007, Boulet et al. In press).

- EU
 - Seronegative despite at least 5 documented direct intravenous or mucosal HIV exposures.
 - No CCR5Δ32 homozygous individuals.
- PI
 - Primary infection of HIV
 - Selected as a control population for these analyses because any effect that disease progression would have on skewing the distribution of KIR or MHC class I allele expression has yet to occur.

Racial background	EU (%) N = 41	PI (%) N = 186	p
Caucasian	38 (92.7)	171 (91.9)	1.0000
African	1 (2.4)	5 (2.7)	1.0000
Asian	0 (0)	1 (0.5)	1.0000
Latino	0 (0)	6 (3.2)	0.5947
Native American	0 (0)	1 (0.5)	1.0000
Other	2 (4.9)	2 (1.1)	0.1503

Methods: sequencing

- Exons 2, 3, 4, 5, 7-9 of the KIR3DL1 gene were sequenced and single nucleotide polymorphisms were used to identify the following KIR3DL1 subtypes:
 - KIR3DL1*001, *002, *004, *005, *007, *008, *015, *019, *020.

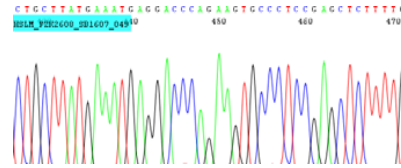


Figure 5 KIR3DL1 sequencing data

Results I: KIR3DL1 subtype frequency is similar in EU vs. PI

- Although the 3DL1*009 allele had a higher frequency in the EU population, a single 3DL1*009 homozygous individual among the EU group accounted for this between-group difference. Furthermore, following Bonferroni's correction for multiple comparisons, this difference was no longer significant.

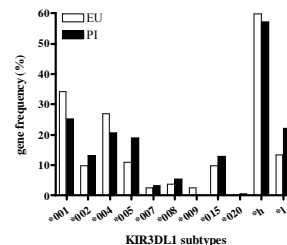


Figure 6. Frequency of each KIR3DL1 subtypes in the HIV exposed uninfected (EU) and subjects in primary HIV infection (PI) populations that are KIR3DL1 homozygotes. Grouped high (*) and low (*) expressing alleles are also shown for each population.

Results II: KIR3DL1*^h*^y+B*57 is more frequent in EU vs. PI

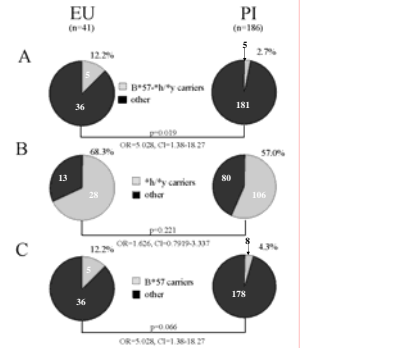


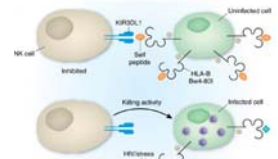
Figure 7. Distribution of KIR3DL1*^h*^y and B*57 in the EU and PI populations that are KIR3DL1 homozygotes. Pie charts depict for each population: A) the proportion of combined KIR3DL1*^h*^y and B*57 carriers, B) the proportion of KIR3DL1*^h*^y carriers and C) the frequency of the HLA-B*57 allele. Numbers in the pie charts indicate the n for each subset of the corresponding population.

- All other between group comparisons of KIR3DL1 alleles or genotypes (3DL1*^h*^y, 3DL1*^h*^x, 3DL1*004) and their receptors (HLA-Bw4, HLA-Bw4-80I, HLA-B*57 or HLA-B*27) taken individually or in combination were not significantly different (data not shown).

Conclusion

- The combined genotype of KIR3DL1*^h*^y and HLA-B*57 is more frequent in EU than in PI.
- This could provide a level of resistance to infection due to a process termed licensing. This would lead to more potent NK cells in individuals with highly inhibitory KIR3DL1 receptors in the presence of a strong ligand (HLA-B*57).
- The consequences of this genotypic distribution should be studied:
 - Increased NK cell activation (secretion of cytokines, degranulation, ...).
 - Surface expression of KIR
 - Possible effect on the adaptive immune response through maturation of DC.

Model



Ahfeld et al. 2007

Figure 8. NK cells from individuals with a 3DL1*^h*^y genotype inside alleles expressed on the NK cell surface at high levels in terms of abundance per cell and the percentage of NK cells expressing the allele. In addition, the 3DL1*^h*^y alleles tested thus far have a higher affinity for HLA-Bw4 80I alleles such as HLA-B*57 than HLA-Bw4 80T alleles. Therefore, individuals expressing this combination of alleles would have NK cells that are potentially inhibited under normal circumstances. As HIV target cells are exposed to the virus, MHC class I downregulation will lead to interruption of the inhibitory signal mediated by 3DL1 receptors resulting in strong NK activation.

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