RESEARCH ARTICLE

HIV exposed seronegative (HESN) compared to HIV infected individuals have higher frequencies of telomeric Killer Immunoglobulin-like Receptor (KIR) B motifs; Contribution of KIR B motif encoded genes to NK cell responsiveness

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Abstract

Previously, we showed that Killer Immunoglobulin-like Receptor (KIR)3DS1 homoyzogotes (hmz) are more frequent in HIV exposed seronegative (HESN) than in recently HIV infected (HIV+) individuals. KIR3DS1 encodes an activating Natural Killer (NK) cell receptor (NKR). The link between KIR genotype and HIV outcomes likely arises from the function that NK cells acquire through expression of particular NKRs. An initial screen of 97 HESN and 123 HIV+ subjects for the frequency of KIR region gene carriage observed between-group differences for several telomeric KIR region loci. In a larger set of up to 106 HESN and 439 HIV+ individuals, more HESN than HIV+ subjects were KIR3DS1 homozygotes, lacked a full length KIR2DS4 gene and carried the telomeric group B KIR haplotype motif, TB01. TB01 is characterized by the presence of KIR3DS1, KIR2DL5A, KIR2DS3/5 and KIR2DS1, in linkage disequilibrium with each other. We assessed which of the TB01 encoded KIR gene products contributed to NK cell responsiveness by stimulating NK cells from 8 HIV seronegative KIR3DS1 and TB01 motif homozygotes with 721,221 HLA null cells and evaluating the frequency of KIR3DS1+/h KIR2DL5+/h, KIR3DS1+/h KIR2DS1+/h, KIR3DS1+/h KIR2DS5+/h.
cells secreting IFN-γ and/or expressing CD107a. A higher frequency of NK cells expressing, versus not, KIR3DS1 responded to 721.221 stimulation. KIR2DL5A⁺, KIR2DS1⁺ and KIR2DS5⁺ NK cells did not contribute to 721.221 responses or modulate those by KIR3DS1⁺ NK cells. Thus, of the TB01 KIR gene products, only KIR3DS1 conferred responsiveness to HLA-null stimulation, demonstrating its ligation can activate ex vivo NK cells.

Introduction

Natural killer (NK) cells are a lymphocyte subset involved in early defenses to virus infected and transformed cells [1]. They contribute to the elimination of these "altered self" cells, in the absence of prior antigen sensitization, by direct cytotoxicity and by secreting cytokines such as IFN-γ and TNF-α and chemokines such as CCL3, CCL4 and CCL5 [2–5]. NK cells also act to bridge innate and adaptive immunity, by contributing to the activation of T and B cells through dendritic cell activation and cytokine production [6].

NK cell activity is regulated by an array of cell surface receptors. The most diverse of these are the structurally related polymorphic Killer Immunoglobulin-like Receptors (KIR) [7]. The KIR gene cluster is located on the long arm of chromosome 19 (19q13.4) within the leukocyte receptor complex [8]. KIR genes are organized into group A or B haplotypes [9–11]. The group A haplotypes are comprised of four framework genes present in most KIR haplotypes (KIR3DL3 at the centromeric end, KIR3DL2 at the telomeric end and KIR2DL4 and the pseudogene KIR3DP1 in the middle) plus KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4 and KIR2DP1. The KIR2DS4 locus encodes several variants having a frameshift mutation that prevents cell surface expression [12, 13]. These are present at a high frequency in certain populations, such that many individuals homozygous for the KIR group A haplotype have no activating KIR (aKIR) [12]. The more diverse group B haplotypes include the framework genes with various combinations of KIR2DL2, KIR2DL5A/B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1 [14–16]. Most KIR region haplotypes are composed of one of 3 centromeric and one of 3 telomeric KIR motifs that include combinations of KIR genes in linkage disequilibrium (LD) with each other [17]. The centromeric region is delimited by the framework genes KIR3DL3 and KIR3DP1 while the telomeric region is delimited by framework genes KIR2DL4 and KIR3DL2 [18].

The engagement of inhibitory KIR (iKIR) by surface major histocompatibility complex class I (MHC-1) or HLA antigens on neighboring cells during development is required for NK cell education, a process that confers NK cells with functional competence [19, 20]. In mature educated NK cells, the engagement of iKIR by HLA results in inhibitory signals. Virus-infected and transformed cells with altered cell surface HLA expression can drive NK cell activation by altering MHC-1 expression that reduces or interrupts inhibitory signaling through iKIR and by inducing ligands that engage activating NK cell receptors (aNKR) [21].

Epidemiologic studies have found that some KIR and KIR/HLA genotype combinations are associated with protection from HIV infection in HIV exposed seronegative (HESN) individuals. For example, co-expression of the high expression homozygous KIR3DL1 genotype KIR3DL1⁺h⁺h⁺ and HLA-B⁺57 occurs at a higher frequency in HESN than in HIV-susceptible seropositive subjects as does the KIR3DS1 homozygous genotype [22, 23]. KIR2DS4 codes for an aKIR. Alleles at this locus can be broadly grouped into those encoding cell-surface expressed (KIR2DS4⁺001-like) and truncated, non-cell-surface expressed (KIR2DS4⁺003-like) variants [12, 13]. KIR2DS4⁺001 has been associated with HIV transmission.
in HIV discordant couples in Zambia, independently of its association with higher HIV viral load in index transmitting partners [24]. Carriage of these alleles has also been associated with poor outcomes such as low CD4 counts and/or high viral load in a cohort of HIV-infected American youth and in HIV+ individuals in Lima, Peru [25, 26]. It is notable that the KIR2DS4 and KIR3DL1/S1 genes are in LD with each other and with other KIR genes in telomeric KIR region motifs [17]. The KIR2DS4 and KIR2DS1 genes are in negative LD, suggesting they may be alleles at the same locus [27, 28]. Carriage of KIR2DS1 and absence of a KIR2DS4 gene is a hallmark of the telomeric TB01 motif [17].

KIR2DL5 is presumed to be an iKIR based on its long immunoreceptor tyrosine-based inhibitory motif (ITIM) containing cytoplasmic tail [29, 30]. The ligand for KIR2DL5 remains unknown. The gene encoding KIR2DL5 is duplicated in some KIR group B haplotypes [31]. KIR2DL5A lies in the telomeric region next to KIR3DL1/S1, while KIR2DL5B is in the centromeric region [11, 32]. KIR2DL5 genes mark centromeric and telomeric group B haplotypes [14]. While many KIR2DL5B gene products are not cell surface expressed, KIR2DL5A receptors are expressed on the surface of CD56dim NK cells [29]. Based on LD with KIR2DL5A and KIR2DL5B, KIR2DS3 and KIR2DS5 can also be present in the telomeric and centromeric group B haplotypes [33, 34]. The KIR2DS3 and KIR2DS5 genes are in negative LD with each other and have been proposed to be allele groups at the same locus [27, 28].

Given the LD between genes located in the telomeric group B KIR region and the previously reported higher frequency of KIR3DS1 homozygotes (hmz) among HESN compared to HIV+ subjects, we investigated the differential frequency of other KIR region genes in these two populations. The TB01 motif of linked KIR genes was found more frequently among HESN than HIV+ subjects. We took advantage of the stochastic expression of KIR gene products on the NK cells to investigate the contribution of KIR3DS1, KIR2DL5A, KIR2DS1 and KIR2DS5 to NK cell responses to the HLA null cell line 721.221 (221).

Materials and methods
Ethics statement
This study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Institutional Review Boards of the Comité d’Éthique de la Recherche du Centre Hospitalier de l’Université de Montréal and the Research Ethics Committee of the McGill University Health Centre. All individuals provided written informed consent for the collection of samples and subsequent analyses.

Study population
The study population for KIR region typing included a total of 545 individuals, of which 106 were HESN and 439 were HIV-infected individuals enrolled in the Montreal Primary Infection (PI) cohort. HESN were recruited from the St. Luc cohort, a prospective cohort of active HIV-negative injection drug users (IDU) at high risk for HIV acquisition [35] (n = 87), and among HIV-negative partners of serodiscordant couples followed in medical clinics in Montreal (n = 19). Information collected at follow-up visits included assessment of the frequency of high-risk behavior for HIV acquisition, blood draws and monitoring of HIV serostatus. All HESN subjects maintained a negative HIV enzyme immunoassay (HIV EIA) test despite at least five reported HIV exposures. Parenteral exposure was defined as sharing needles with known HIV-infected partners and mucosal exposure was defined as unprotected sex with a known HIV-infected partner. None of the HESN subjects were CCR5Δ32 homozygotes, a genotype known to confer resistance to HIV infection [36, 37]. The Montreal PI cohort enrolls individuals within 6 months of infection and follows them an average of every 3 months for up
to 4 yrs. At each visit CD4, CD8 and plasma viral load measurements are done and peripheral blood mononuclear cells (PBMC) and plasma are frozen and stored.

For functional assays, we studied 8 HIV–uninfected KIR3DS1 hmz, including 7 with at least 1 Bw4 allele and 1 who was a Bw6 hmz with no Bw4 alleles at the HLA-A locus. All the KIR3DS1 hmz were positive for KIR2DL5A and KIR2DS1 genes, 6 carried a KIR2DS5 gene and none carried a gene encoding an expressed KIR2DL5B variant. All were negative for KIR2DS4.

Table 1 shows the HLA and KIR information for each of these 8 study participants.

### Table 1. KIR/HLA information on KIR3DS1 homozygotes providing cells for functional studies.

| Donor | HLA-A          | HLA-B          | HLA-C          | Bw4 | KIR2DL5A | KIR2DS1 | KIR2DS5 |
|-------|----------------|----------------|----------------|-----|----------|---------|---------|
| 1     | 01:01/02:03    | 37:01/46:01    | 01:02/06:02    | Yes | Yes      | Yes     | No      |
| 2     | 02:01/24:02    | 39:01/51:01    | 07:02/07:02    | Yes | Yes      | Yes     | Yes     |
| 3     | 02:01/03:01    | 27:05/47:01    | 02:02/06:02    | Yes | Yes      | Yes     | Yes     |
| 4     | 02:05/03:01    | 44:02/44:02    | 06:02/07:01    | Yes | Yes      | Yes     | Yes     |
| 5     | 24:02/68:01    | 18:01/18:01    | 05:01/07:01    | Yes | Yes      | Yes     | Yes     |
| 6     | 24:02/68:01    | 18:01/57:01    | 06:02/07:01    | Yes | Yes      | Yes     | Yes     |
| 7     | 02:01/32:01    | 27:05/51:01    | 05:01/14:02    | Yes | Yes      | Yes     | Yes     |
| 8     | 03:01/11:01    | 07:02/40:02    | 02:02/07:02    | No  | Yes      | Yes     | No      |

Table 1. KIR/HLA information on KIR3DS1 homozygotes providing cells for functional studies.

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Genotyping

Genomic DNA was extracted from PBMCs or Epstein-Barr virus (EBV)-transformed cells using a QIAamp DNA blood kit (QIAGEN, Inc., Mississauga, Ontario, Canada). KIR region typing was performed on 97 HESN and 123 HIV+ subjects using commercially available reagents (KIR Genotyping SSP kit, OneLambda, Canoga Park, CA) according to manufacturer’s instructions. The presence of the following KIR genes was detected: KIR2DL1-2DL5, KIR2DS1-2DS5, KIR3DL1-3DL3, KIR3DS1 and the pseudogenes KIR2DP1 and KIR3DP1. All subjects carried the framework KIR genes, a KIR3DL1/S1 and a KIR2DL2/L3 gene. The subjects who were KIR region typed, as well as an additional 9 HESN and 316 HIV+ subjects for a total of 106 HESN and 439 HIV+ subjects, were typed for generic genotypes at the KIR3DL1/S1 locus using 2 sets of primers specific for KIR3DL1 and KIR3DS1 as previously described [23, 38]. A total of 105 HESN and 438 HIV+ subjects were tested for the presence of a KIR2DS4 gene and, if present, for KIR2DS4’001-like and KIR2DS4’003-like alleles by either KIR region typing and/or using 2 sets of primers specific for KIR2DS4 and conditions described by Kulkarni et al. [38]. The presence of KIR2DL5, KIR2DS3, KIR2DS5 and KIR2DS1 genes was assessed in 105 HESN and in 431, 321, 321 and 435 HIV+ subjects, respectively, by KIR region typing and/or using 2 sets of primers specific for these genes [38]. All subjects positive for a KIR2DL5 gene and, if present, for KIR2DS4’001-like and KIR2DS4’003-like alleles by either KIR region typing and/or using 2 sets of primers specific for KIR2DS4 and conditions described by Kulkarni et al. [38]. The presence of KIR2DL5, KIR2DS3, KIR2DS5 and KIR2DS1 genes was assessed in 105 HESN and in 431, 321, 321 and 435 HIV+ subjects, respectively, by KIR region typing and/or using 2 sets of primers specific for these genes [38]. All subjects positive for a KIR2DL5 gene were typed for the presence of a telomeric KIR2DL5A, a centromeric KIR2DL5B gene or both using a modification of methods described by Du et al. [33]. KIR2DL5A and KIR2DL5B genes were distinguished at 3 single nucleotide polymorphisms (SNP) at positions (-97, -84, and +16) [33]. Additionally, when a KIR2DL5B gene was present, the SNP present at position -97 was used to deduce whether it encoded an expressed gene product or one that was epigenetically silenced [32, 39]. Testing for the presence of KIR2DS3 and KIR2DS5 genes was performed on all individuals who carried KIR3DS1, KIR2DL5A or KIR2DS1 genes to ascertain whether one of these was present, as would be expected of carriers of a canonical TB01 motif. The presence of non-canonical telomeric motifs (i.e. other than TA01, TA02 or TB01 motifs) were verified by repeat typing.
Cells

Cryopreserved HLA-null 221 cells were thawed and cultured in RPMI medium supplemented with 10% Fetal Bovine Serum (FBS); 2mM L-glutamine; 50 IU/mL penicillin and 50 μg/mL streptomycin (R10) (all from Wisent, St Jean Baptiste, QC, Canada). PBMCs were isolated by density gradient centrifugation (Lymphocyte Separation Medium, Wisent) and cryopreserved in 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St Louis, MO); 90% FBS (Wisent).

NK cell activation by 221 cells

Cryopreserved PBMCs were thawed and co-cultured in R10 with 221 HLA-null cells at a 10:1 ratio for 6 hr at 37˚C in a humidified 5% CO₂ incubator. Unstimulated PBMCs were cultured alone as a negative control and PBMCs stimulated with 1.25μg/mL phorbol 12-myristate 13-acetate; 0.25μg/mL ionomycin (P/I) were used as a positive control to ensure that the NK cells were viable and functional. CD107a-BV421 (clone H4A3, BioLegend, San Diego, CA) was added at the start of the stimulation; Brefeldin A (5 μg/ml; Sigma-Aldrich) and monensin (6 μg/ml, Golgi Stop; BD Biosciences, Mississauga, ON, Canada) were added 30 min after starting the co-culture. After stimulation, cells were stained using the UV Live/Dead Fixable Dead Cell Stain Kit (Invitrogen, Burlington, ON, Canada) to assess viability. Nonspecific interactions with antibodies in the staining panel were minimized by using TruStain FcX reagent (BioLegend), as per the manufacturer’s instructions. Cells were surface stained with one of 2 fluorochrome-conjugated antibody panels. Panel 1 included antibodies to the following specificities: CD3-BV785 (OKT3), CD56-BV711 (HCD56, both from BioLegend), KIR3DL1/S1-PE (Z27, Beckman Coulter, Mississauga, ON, Canada) and KIR2DL5-PE-Vio 770 (Miltenyi Biotec, Cambridge, MA). Panel 2 included antibodies to the following specificities: CD3-BV785 (OKT3), CD56-BV605 (HCD56) and KIR2DL1/KIR2DS1/KIR2DS3/KIR2DS5-FTTC (HP-MA4, all from BioLegend), KIR2DL1-VioBlue (REA284) and KIR2DL1/KIR2DS1-APC-Vio 770 (11PB6) (all from Miltenyi Biotec) and KIR3DL1/S1-PE (Beckman). After surface staining, cells were fixed and permeabilized using Fix and Perm Kit (Invitrogen) reagents and stained for intra-cellular IFN-γ with anti-IFN-γ-BV510 (B27, BD Biosciences, San Jose, CA). Samples were washed, fixed with 2% paraformaldehyde (Santa Cruz Biotechnology, Santa Cruz, CA), and acquired within 24 hrs.

Flow cytometry analysis

Between 4.0 x 10⁵ and 1.0 x 10⁶ total events were acquired for each sample using a calibrated LSRFortessa™ X-20 flow cytometer (BD). Single stained control beads (CompBead; BD) were used in every experiment to calculate compensation. Boolean gating was used to identify the frequency of KIR3DS1+/− KIR2DL5+/−, KIR3DS1+/− KIR2DS1+/− and KIR3DS1+/− KIR2DS5+/− expressing CD3-CD56dim NK cells positive for all possible functional subsets defined by CD107a expression and IFN-γ secretion. For stimulations with 221 cells, PBMCs cultured in R10 served as background controls. All data obtained were corrected for background. Flow cytometry results were analyzed using FlowJo software (V9.8; TreeStar, Ashland, OR).

Statistical analysis

Statistical analysis and graphical presentation of genotyping results were performed using GraphPad InStat 3.10 and GraphPad Prism 6 (GraphPad Software Inc, La Jolla, CA). Fisher’s exact tests were used to compare proportional between-group differences for selected genes and genotypes in HESN and HIV+ subjects. Results are reported in the following format: (Odds Ratio [95% confidence intervals], p-value) unless otherwise specified. A p-value of less
than 0.05 was considered significant. The Holm-Bonferroni method was used to adjust p-values for multiple comparisons. For functional studies, Friedman tests were used to test the significance of differences between the frequency of four within-subject KIR3DS1+/− KIR2DL5+/−, KIR3DS1+/− KIR2DS1+/− and KIR3DS1+/− KIR2DS5+/− CD3 CD56dim NK cell populations responding to 221 cells. Wilcoxon tests were used to assess the significance of comparisons for within-subject paired data sets for functional NK cell populations.

Results

KIR region typing

The KIR region is polygenic and thus varies in gene content from one individual to another [9, 11]. To determine whether the frequency of certain KIR genes within this region differed between HESN and HIV+ subjects we screened for their presence in a subset of 97 HESN and 123 HIV+ individuals by KIR gene region typing. The frequency of KIR2DL2 and KIR3DL3 was considered separately as was the frequency of KIR3DL1 and KIR3DS1, which are allele groups at the KIR2DL2/L3 and KIR3DL1/S1 loci, respectively. We also considered the presence of a KIR2DS4 gene, and whether alleles at this locus belonged to the KIR2DS4'001-like and/or KIR2DS4'003-like groups. As expected, the framework genes KIR2DL4, KIR3DL2, and KIR3DL3 and the pseudogene KIR3DP1 were present in all subjects tested. Fig 1 and S1 Table

![Graph](https://doi.org/10.1371/journal.pone.0185160.g001)

Fig 1. Killer Immunoglobulin-like Receptor (KIR) gene and KIR allele group frequencies in 97 HIV exposed seronegative (HESN) and 123 recently infected HIV positive (HIV+) subjects. Shown on the y-axis are the percentage of HESN and HIV+ individuals carrying each KIR gene. Percentage refers to the number of subjects positive for each variable divided by the total number of subjects tested for that variable. The framework genes KIR2DL4, KIR3DL2, KIR3DL3, and the pseudogenes KIR3DP1 were present in all study subjects and are not shown in this this figure. Each gene shown on the x-axis is named without the “KIR” designation, i.e. 2DS1 = KIR2DS2, etc. ** = p’<0.01. This p-value refers to p-value corrected for multiple comparisons. This p’-value is shown over the bar linking the 2 groups being compared.

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show the frequency of each of these genes and allele groups in HESN and HIV+ subjects. The only significant between-group differences noted was the frequency of KIR2DS4*001-like alleles, which was lower in HESN than in HIV+ subjects (0.33 (0.18, 0.59), p = 0.0002, corrected p (p') = 0.003, Fisher’s exact test). The frequency of several other telomeric KIR genes differed between HESN and HIV+ subjects, though these differences did not achieve statistical significance. This prompted us increase the size of the study population to provide increased power to observe significant between-group differences in telomeric KIR gene frequency.

We previously showed that the distribution of the KIR3DL1/S1 generic genotypes were in Hardy-Weinberg equilibrium in HIV+ but not in HESN subjects [23]. The skewed distribution of KIR3DL1/S1 generic genotypes in HESN was due to an over representation of KIR3DS1 hmr among HESN. This observation was confirmed in a larger group of 106 HESN and 439 HIV+ subjects, however the significance of this finding did not survive correction for multiple comparisons (Fig 2A and S2 Table).

Fewer HESN than HIV+ individuals carried a KIR2DS4 gene, though the statistical significance of this difference did not survive correction for multiple comparisons (p = 0.03, p’ = 0.36, Fisher’s) (Fig 2B, S2 Table). Fewer HESN than HIV+ subjects carried at least 1 copy of a full length KIR2DS4*001-like allele, which encodes cell surface expressed receptors that have the potential to exert an effect on NK cell function. There were no significant between-group differences in the frequency of carriage of unexpressed KIR2DS4*003-like alleles (Fig 2B, S2 Table). KIR2DS4 and KIR2DS1 are genes in the telomeric KIR region that are in strong negative LD suggesting and that may be alleles at the same locus. Based on whether a KIR2DS1 gene was also present it was possible to deduce whether 1 or 2 copies of full length KIR2DS4*001-like or truncated KIR2DS4*003-like alleles were present in subjects that typed...

Fig 2. Killer Immunoglobulin-like Receptor (KIR) generic genotype and allele group frequencies in HIV exposed seronegative (HESN) and recently infected HIV positive (HIV+) subjects. Shown on the y-axis is the frequency of (A) HESN (n = 106) and HIV+ (n = 439) subjects positive for the three KIR3DL1/S1 generic genotypes, (B) HESN (n = 105) and HIV+ (n = 438) subjects positive for a KIR2DS4 gene and carrying at least 1 copy of a full length KIR2DS4*001-like allele, which encodes cell surface expressed receptors that have the potential to exert an effect on NK cell function. There were no significant between-group differences in the frequency of carriage of unexpressed KIR2DS4*003-like alleles (C) KIR2DS4*001-like or KIR2DS4*003-like allele groups among the 210 and 876 KIR haplotypes from HESN (n = 105) and HIV+ (n = 438) subjects, (D) HESN (n = 105) and HIV+ (n = 431) subjects positive for a KIR2DL5, KIR2DL5A and KIR2DL5B gene, (E) HESN (n = 105) and HIV+ (n = 435) positive for a KIR2DS1 gene, (F) TB01 motifs among the 210 and 846 KIR haplotypes from HESN (n = 105) and HIV+ (n = 423) subjects and (G) HESN (n = 105) and HIV+ (n = 423) subjects positive for a homozygous TB01 motif. P’-values over the lines linking groups being compared are corrected for multiple comparisons.

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for only one of these allele groups. As shown in Fig 2C and S2 Table the frequency of
KIR2DS4*001-like alleles, was significantly lower in HESN than in HIV+ persons (p<0.001,
p’-value p = 0.01, Fisher’s) while that of KIR2DS4*003-like alleles did not differ between
groups. The frequency of KIR2DS1 carriers did not differ significantly between groups (1.38
[0.89, 2.13] p = 0.15, Fisher’s) (Fig 2D and S2 Table). The proportion of HESN and HIV+
individuals positive for a KIR2DL5 gene was not significantly different (p = 0.82, Fisher’s, Fig 2E,
and S2 Table). A KIR2DL5 gene can be present in either centromeric or telomeric group B KIR
haplotypes. Although the frequency of telomeric KIR2DL5A was higher in HESN than HIV+
subjects, this difference did not achieve statistical significance. (Fig 2E and S2 Table).

In summary, we found that absence of an expressed KIR2DS4*001-like allele was associated
with a reduced risk of HIV infection and confirmed a trend towards an association between
KIR3DS1 homozygosity and a reduced risk of HIV infection.

The telomeric KIR3DS1, KIR2DL5A, KIR2DS3/5 and KIR2DS1 gene
grouping is more frequent in HESN than HIV+ subjects

The most common KIR haplotypes are derived from combinations of three centromeric and
two telomeric motifs linked to each other by a recombination hotspot located between
KIR3DP1 and KIR2DL4 [17, 40, 41]. Fig 3 shows that KIR3DS1 is positioned within the
telomeric group B haplotype TB01 motif, in LD with KIR2DL5A, KIR2DS3/S5 and KIR2DS1 [17,
40, 41].

HESN (n = 105) and HIV+ (n = 423) subjects were typed for
KIR3DL1/S1 genotypes, and
for the presence of KIR2DL5A, KIR2DS1 and KIR2DS4 genes. All subjects positive for
KIR3DS1, KIR2DL5A and/or KIR2DS1 were also typed for the presence of KIR2DS3 and
KIR2DS5, one of which should also be present in TB01 motifs. While most study subjects car-
rried canonical TA01/02 and/or TB01 motifs, 6 (5.7%) HESN and 58 (13.7%) HIV+ subjects
carried non-canonical telomeric KIR motifs. S3 Table provides information on the number of
individuals who carried non-canonical motifs and identifies how they diverged from canonical
telomeric motifs. All the non-canonical telomeric motifs were classified as non
TA01.

Of the 210 and 846 telomeric KIR region motifs in 105 HESN and 423 HIV+ subjects 52
(24.8%) and 126 (14.9%), respectively were canonical TB01 motifs, a proportional between-
group difference that was statistically significant (p = 0.0003, p’ = 0.004, Fisher’s) (Fig 2F
and S2 Table). HESN were more likely to carry a homozygous TB01 motif than HIV+ subjects (11
of 105 [10.48%] HESN versus 15 of 423 [3.5%] HIV+ persons were TB01 homozygotes [3.18
(1.42, 7.15), p = 0.009, Fisher’s). However, the significance of this between-group difference did not survive correction for multiple comparisons (Fig 2G and S2 Table).

The contribution of TB01 encoded gene products to NK cell responses to HLA-null cell stimulation

Although the KIR3DS1, KIR2DL5A, KIR2DS1 and, when present, KIR2DS5 genes are in LD they are stochastically expressed on NK cell populations. This prompted us to question the contribution of NK cells expressing various combinations of these receptors to stimulation with 221 cells. Of 8 KIR3DS1 hmz, all were positive for KIR2DL5A and KIR2DS1 and 6 were positive for KIR2DS5. NK cells from these individuals were investigated for functional responses to 221 cell stimulation. Fig 4A shows the gating strategy used to identify KIR3DS1+ and KIR2DL5+ NK cell populations that were stained with panel 1 antibodies. We used Boolean analysis to examine the frequency of the 4 possible NK populations defined by KIR3DS1+/− and KIR2DL5+/− receptors before and following stimulation with 221 cells (Fig 4B). No significant

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**Fig 4.** The frequency of NK cells expressing all possible combinations of KIR3DS1 and KIR2DL5 before and after stimulation with 721.221 (221) HLA-null cells. (A) Live singlet lymphocytes were gated on. From this population CD3−CD56dim NK cells were examined for the frequency of cells expressing KIR3DS1 and/or KIR2DL5 or neither. (B) The frequency of NK cells on the y-axis expressing all possible combinations of KIR3DS1 (3DS1) and KIR2DL5 (2DL5) on NK cells before and after stimulation with 221 cells (B). Each point represents results from a single individual. Bar heights and error bars represent the median and inter-quartile range of each group.

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difference in the frequency of expression of the 4 NK cell populations was noted indicating that HLA-null stimulation did not alter the expression of these KIR on the NK cell surface.

We also used Boolean analysis to ascertain the functional responses to 221 cell stimulation characterized by degranulation, as measured by CD107a, and IFN-γ secretion of the 4 possible NK populations defined by KIR3DS1+/− KIR2DL5+/− receptor expression (S1 Fig). The frequency of the double positive (3DS1+/−2DL5−) and KIR3DS1+ (3DS1+/−2DL5+) NK cells responding to 221 stimulation was significantly higher than that of the single KIR2DL5+ (3DS1−2DL5+) and double-negative (3DS1 2DL5−) NK cell populations (Fig 5). This was the case for all the functional subsets examined (Fig 5A, 5B and 5D), with the exception of the total CD107a+ functional profile, where between-group comparison did not achieve statistical significance (Fig 5C). There was no significant difference in the frequency of responding cells characterized by any of the functional subsets for the double positive and single 3DS1+ NK cell populations. A higher frequency of 3DS1+/−2DL5− than 2DL5+ NK cells responded to 221 stimulation for all functional subsets except total CD107a expression. The frequency of single 2DL5+ and double-negative 3DS1 2DL5− NK cells responding to 221 stimulation was not significantly different for any of the functional subsets examined.
Fig 6 shows the gating strategy used to identify KIR3DS1+, KIR2DS1+ and KIR2DS5+ NK cells following staining with panel 2 antibodies. Boolean analysis was used to examine the frequency of the 4 possible NK populations defined by KIR3DS1+/− KIR2DS1+/− and by KIR3DS1+/+ KIR2DS5+/− expression and for ascertaining the frequency of functional NK cells responding to 221 stimulation by expressing CD107a and secreting IFN-γ within each of these phenotypic NK cell populations (S1 Fig). A higher frequency of double and single KIR3DS1+ than KIR3DS1− NK cells responded to stimulation by exhibiting the sum of all functions tested whether KIR3DS1 was expressed with KIR2DS1 or not (Fig 5E). This was also the case for total IFN-γ secretion. Single KIR2DS1+ (3DS1−2DS1+) NK cells responded no better to 221 stimulation than did double negative 3DS1−2DS1− NK cells. A higher frequency of single and double KIR3DS1+ NK cells than KIR3DS1− NK cells responded to stimulation by secreting IFN-γ whether KIR3DS1 was expressed with KIR2DS5 (2DS5) or not (Fig 5F). Single KIR2DS5+ (3DS1−2DS5+) NK cells responded no better to 221 stimulation than double negative 3DS1−2DS5− NK cells.

Together, these results support the conclusion that a higher frequency of KIR3DS1+ than KIR3DS1− NK cells responded to 221 cells and indicate that KIR3DS1+ NK cells contributed...
more to NK functional responses to HLA-null cell stimulation than did KIR2DL5A⁺, KIR2DS1⁺ or KIR2DS5⁺.

Discussion

HESN, compared to HIV+ subjects, were more likely to carry 1 or 2 copies of a telomeric TB01 motif and were less likely to carry a full length KIR2DS4*001-like allele. As most KIR3DS1 hmz were also KIR2DL5A, KIR2DS1 and KIR2DS5 positive we investigated the functional potential of NK cells expressing various combinations of the KIR receptors encoded by these genes to stimulation with the HLA null cell line 221. Functional studies revealed that NK cells expressing KIR3DS1 alone or with any one of the KIR2DL5A, KIR2DS1 or KIR2DS5 receptors responded better to 221 stimulation than NK cells expressing one of these KIRs alone without KIR3DS1 or none of these receptors. These results suggest that of the KIR receptors encoded by genes present within TB01 motifs, KIR3DS1 contributes most to NK cell responsiveness to 221 HLA null cells.

NK cells from subjects with no KIR2DS4 gene, or who carry only KIR2DS4*003-like alleles do not express this receptor on their cell surface. On the other hand, NK cells from carriers of KIR2DS4*001-like alleles do express a KIR2DS4 receptor on a subset of their NK cells. Merino et al. reported that expressed KIR2DS4 was associated with poor outcome in the context of HIV infection such as higher viral load, HIV transmission in HIV discordant couples and low CD4 counts [24, 25]. This observation was confirmed by Olvera et al. in an HIV+ population from Lima, Peru and may have depended on the co-carriage of HLA-Cw4, a presumed ligand for this receptor [26]. Our results suggest that expressed KIR2DS4 is also associated with reduced resistance to HIV infection. The mechanisms underlying these outcomes are not understood. KIR2DS4 is an aKIR that is the product of gene conversion with the KIR3DL2 gene that has led to a reduced ability to recognize HLA-C ligands characteristic of KIR2D receptors and an increased ability to recognize HLA-A*11:02 and HLA-A*03 ligands, the presumed ligands for KIR3DL2 [43]. Further investigations are needed to understand whether, and if so how, KIR2DS4 expression is associated with negative outcomes in the context of HIV infection and HIV exposure. The possibility that the impact of expression of KIR2DS4 on poor HIV outcomes is due to other genes in LD with KIR2DS4 has not been excluded.

HESN cohorts have been studied to identify mechanisms underlying resistance to HIV. In a cohort of Vietnamese HESN injection drug users (IDU), NK cells were found to be more active than those from HIV uninfected persons who eventually seroconverted [44]. A study comparing 25 HESN with 19 HIV+ IDU and 26 HIV uninfected persons found that HESN had KIR and KIR/HLA expression profiles consistent with a lower threshold of NK cell activation and higher ratios of KIR3DS1:KIR3DL1 transcripts [45]. A higher prevalence of KIR3DS1 or lower frequency of KIR3DL1 in HESN than HIV susceptible subjects has been reported in several studies, and this, often in the absence of an association with HLA-Bw4 [23, 45–48]. Overall, these studies support the interpretation that HIV resistance may be due to NK cells that are more easily activated, which is consistent with carriage of a group B KIR haplotype in which larger numbers of genes encoding aKIR are present.

A study of HIV discordant and concordant couples found a role for alloreactive NK cells in protection from sexual HIV transmission [49]. The implication that alloreactive NK cells may play a role in HIV resistance could not be investigated here because the HIV+ index partner to which HESN and HIV+ subjects in this study were exposed is unknown. However, these studies highlight mechanisms other than carriage of aKIR that may be responsible for HIV susceptibility/resistance. For example, carriage of KIR3DL1 high expression genotypes with HLA-B*57 has been associated with a reduced risk of HIV infection in HESN and NK cells.
from individuals carrying this KIR/HLA combination are particularly responsive to stimulation by HLA-null cells and by autologous HIV infected cells. NK cells from carriers of this KIR/HLA combination have a superior ability to inhibit HIV replication compared to those from carriers of the receptor or ligand alone or neither [22, 50, 51].

*KIR* genes in the telomeric and centromeric *KIR* regions are present in LD with each other. This confounds the identity of the KIR gene product responsible for effects on HIV outcomes. One would expect that if *KIR* genotypes influence HIV exposure outcome they would do so through their effects on NK cell function. One way to test for NK cell functional potential is to stimulate with HLA null cells. When this was done with NK cells from *KIR3DS1* hzm who co-carried *KIR2DL5A, KIR2DS1* and *KIR2DS5*, but no *KIR2DS4*, genes the NK cells that responded with the highest frequency were *KIR3DS1*+. Co-expression of *KIR2DL5, KIR2DS1* or *KIR2DS5* did not modulate the frequency of responding *KIR3DS1*+ NK cells nor did *KIR2DL5*, *KIR2DS1*+ and *KIR2DS5*+ NK cells respond to this stimulus any better that their *KIR2DL5*, *KIR2DS1* or *KIR2DS5* counterparts. In these study subjects, expression of *KIR2DS4* would not have played a role in NK cell responsiveness since none carried a *KIR2DS4* gene. Although some subjects were positive for a *KIR2DL5B* gene the alleles they carried at this locus encoded unexpressed gene products, eliminating any role for *KIR2DL5B*+ NK cells in responses to 221 cells [32, 39]. The contribution of *KIR2DS3* to NK cell functionality is likely limited by its low expression on the cell surface [52]. Together these findings support the notion that, of the *KIR* genes in *TB01* motifs, *KIR3DS1* encodes a receptor that confers NK cells with functionality, at least to HLA-null 221 cells.

HLA-F has recently been identified as a ligand for *KIR3DS1* [53]. HLA-F is present on the surface of 221 cells, which may explain why these cells stimulate *KIR3DS1*+ NK cells. Attempts to find ligands for *KIR3DS1* among HLA-Bw4 or among the HLA-Bw4 alleles having an isoleucine at position 80 of the HLA heavy chain (HLA-Bw4*80I*) have largely failed [54–56]. An exception to this are results reported by O’Connor et al. showing that *KIR3DS1* can interact with the HLA-Bw4*80I* antigen HLA-B*75* when certain peptides, including some HIV derived peptides, are present. [57]. However, if such interactions were contributing to NK cell education they should tune down *KIR3DS1*+ NK cell responsiveness to 221 stimulation. Thus, it is more likely that the interactions between *KIR3DS1* and HLA-F is responsible for NK cell stimulation by 221 cells. Since HLA-F is usually intracellular in resting cells it would not be expected to contribute to NK cell education and tune down the responsiveness of *KIR3DS1*+ NK cells. However, it is cell surface expressed on activated cells, including HIV-infected CD4+ T cells. The interaction of *KIR3DS1* on NK cells with HLA-F in HIV infected cells may explain why *KIR3DS1*+ NK cells are superior to *KIR3DL1*+ NK cells in suppressing HIV replication [53, 58]. This interaction may provide a mechanistic explanation for the association of *KIR3DS1* with protection from HIV infection [53]. More needs to be done to explore the role of *KIR3DS1/HLA-F* interactions in the recognition of HIV infected cells.

The ligand for *KIR2DL5* has not been identified, though this receptor is likely an iKIR based this receptor having intra-cellular ITIM motifs [30]. Other iKIR usually participate in NK cell education resulting from their interaction with HLA antigen ligands during NK cell development. Education is required for the development of functional potential and the transmission of inhibitory signals to NK cells required for tolerance to self. These inhibitory signals are interrupted when the ligand for iKIR are absent, which occurs in the setting of several viral infections or cellular transformation. *KIR2DS1* interacts with HLA-C2 group antigens, though with a lower affinity than its inhibitory counterpart *KIR2DL1* [59]. It can participate in NK cell education, but tunes down NK cell activation potential if present with an HLA-C2 ligand, presumably to avoid reactivity to self [60]. *KIR2DS5* is cell surface expressed and appears to
transmit activating signals when cross linked with antibodies binding this receptor [61]. However, the identity of its ligand is unknown.

The background level of functionality in the single positive KIR2DL5⁺, KIR2DS1⁺ and KIR2DS5⁺ and double negative NK cell populations may either be due to co-expression of other iKIR such as KIR2DL1/L2/L3 participating in NK cell education in subjects co-carrying HLA-C ligands for these receptors or to inhibitory NK cell receptors such as NKG2A, which interacts with ubiquitously expressed HLA-E complexed with epitopes from the leader sequence of several HLA antigens [20, 62–64]. These receptor ligand interactions would be expected to be more or less evenly distributed among the 4 KIR3DS1⁺/⁻/KIR2DL5⁺/⁻ subsets as well as among the 4 KIR3DS1⁺/⁻/KIR2DS1⁺/⁻ and KIR3DS1⁺/⁻/KIR2DS5⁺/⁻ subsets.

One of the limitations of this study is that autologous HIV infected cells have not been used to stimulate NK cell populations defined by various combinations of their TB01 motif encoded gene products. Although we know that HIV infected cells express HLA-F, the ligand for KIR3DS1 it is possible, though as yet unknown, whether they express ligands for KIR2DL5, KIR2DS1 and/or KIR2DS5 that could account for NK cell functionality that plays a role in HIV control. This possibility requires further exploration.

In summary, despite this limitation, the higher frequency of TB01 motifs among HESN than HIV+ subjects studied here would be consistent with the interpretation that their resistance to HIV infection is related to their NK cells having a lower activation threshold due to the presence of aKIRs whose genes map to this region and by expressing the KIR3DS1 receptor able to mediate activating signals upon interacting with HLA-F on HIV infected CD4 T cells and HLA-null 221 cells. This does not explain resistance to HIV in all HESN. Thus, further investigation is needed to uncover other possible mechanisms underlying reduced susceptibility to HIV infection.

Supporting information

S1 Fig. The gating strategy for obtaining the frequency of NK cells expressing all possible combinations of KIR3DS1 (3DS1) and/or KIR2DL5 (2DL5) secreting IFN-γ and/or expressing CD107a or neither.

(TIF)

S1 Table. Comparison of the frequency of KIR genes and allele groups in HESN and HIV infected subjects.

(DOCX)

S2 Table. Comparison of the frequency of KIR genes in HESN and HIV infected subjects.

(DOCX)

S3 Table. Description of deviations from canonical telomeric KIR motifs.

(DOCX)

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References

1. Trinchieri G. Biology of natural killer cells. Adv Immunol. 1989; 47:187–376. PMID: 2683611
2. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol. 1999; 17:169–220. https://doi.org/10.1146/annurev.immunol.17.1.169 PMID: 10358757
3. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001; 22(11):633–40. PMID: 11698225
4. Kiessling R, Klein E, Wizigl H. “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol. 1975; 5(2):112–7. https://doi.org/10.1002/eji.1830050208 PMID: 1234049
5. Oliva A, Kinter AL, Vaccarezza M, Rubbert A, Catanzaro A, Moir S, et al. Natural killer cells from human immunodeficiency virus (HIV)-infected individuals are an important source of CC-chemokines and suppress HIV-1 entry and replication in vitro. J Clin Invest. 1998; 102(1):223–31. https://doi.org/10.1172/JCI2323 PMID: 9649576

6. Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural killer cells and dendritic cells: “l’union fait la force”. Blood. 2005.;

7. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol. 2005; 5(3):201–14. https://doi.org/10.1038/nri1570 PMID: 15719024

8. Trowsdale J. Genetic and functional relationships between MHC and NK receptor genes. Immunity. 2001; 15(3):363–74. PMID: 11567627

9. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity. 1997; 7(6):753–63. PMID: 9430221

10. Martin AM, Freitas EM, Witt CS, Christiansen FT. The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. Immunogenetics. 2000; 51(4–5):268–80. PMID: 10803839

11. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. Proc Natl Acad Sci U S A. 2000; 97(9):4778–83. https://doi.org/10.1073/pnas.080588597 PMID: 10781084

12. Middleton D, Gonzalez A, Gilmore PM. Studies on the expression of the deleted KIR2DS4*003 gene product and distribution of KIR2DS4 deleted and nondeleted versions in different populations. Hum Immunol. 2007; 68(2):128–34. https://doi.org/10.1016/j.humimm.2006.12.007 PMID: 17321903

13. Maxwell LD, Wallace A, Middleton D, Curran MD. A common KIR2DS4 deletion variant in the human that predicts a soluble KIR molecule analogous to the KIR1D molecule observed in the rhesus monkey. Tissue Antigens. 2002; 60(5):254–8. PMID: 12445308

14. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, et al. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. Hum Immunol. 2003; 64(6):648–54. PMID: 12770798

15. Martin AM, Kulski JK, Gaudieri S, Witt CS, Freitas EM, Trowsdale J, et al. Comparative genomic analysis, diversity and evolution of two KIR haplotypes A and B. Gene. 2004; 335:121–31. https://doi.org/10.1016/j.gene.2004.03.018 PMID: 15194195

16. Uhrberg M, Parham P, Wernet P. Definition of gene content for nine common group B haplotypes of the Caucasian population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics. 2002; 54(4):221–9. https://doi.org/10.1007/s00251-002-0463-7 PMID: 12136333

17. Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ, et al. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. PLoS One. 2010; 5(12):e15115. https://doi.org/10.1371/journal.pone.0015115 PMID: 21206914

18. Trowsdale J, Barten R, Haude A, Stewart CA, Beck S, Wilson MJ. The genomic context of natural killer receptor extended gene families. Immunol Rev. 2001; 181:20–38. PMID: 11513141

19. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by major histocompatibility complex class I molecules. Nature. 2005; 436(7051): https://doi.org/10.1038/nature03847 PMID: 16079848

20. Anfossi N, Andre P, Guia S, Falk CS, Roeyntck S, Stewart CA, et al. Human NK cell education by inhibitory receptors for MHC class I. Immunity. 2006; 25(2):331–42. https://doi.org/10.1016/j.immuni.2006.06.013 PMID: 16901727

21. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005; 23:225–74. https://doi.org/10.1146/annurev.immunol.23.021704.115526 PMID: 15771571

22. Boulet S, Kleyman M, Kim JY, Kamya P, Sharafi S, Simic N, et al. A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS. 2008; 22(12):1487–91. https://doi.org/10.1097/QAD.0b013e3282fde7e PMID: 18614872

23. Boulet S, Sharafi S, Simic N, Bruneau J, Routy JP, Tsoukas CM, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS. 2008; 22(5):595–9. https://doi.org/10.1097/QAD.0b013e3282f5eb23 PMID: 18317000

24. Merino A, Malhotra R, Morton M, Mulenga J, Allen S, Hunter E, et al. Impact of a functional KIR2DS4 allele on heterosexual HIV-1 transmission among discordant Zambian couples. J Infect Dis. 2011; 203(4):487–95. https://doi.org/10.1093/infdis/jiq075 PMID: 21216870

25. Merino AM, Dugast AS, Wilson CM, Goepfert PA, Alter G, Kaslow RA, et al. KIR2DS4 promotes HIV-1 pathogenesis: new evidence from analyses of immunogenetic data and natural killer cell function. PLoS One. 2014; 9(6):e93953. https://doi.org/10.1371/journal.pone.0093953 PMID: 24901871
26. Olvera A, Perez-Alvarez S, Ibarrondo J, Ganoza C, Lama JR, Lucchetti A, et al. The HLA-C*04:01/ KIR2DS4 gene combination and human leukocyte antigen alleles with high population frequency drive rate of HIV disease progression. AIDS. 2015; 29(5):507–17. PMID: 25715101

27. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. Immunol Rev. 2002; 190:40–52. PMID: 12493005

28. Manser AR, Weinhold S, Uhrberg M. Human KIR repertoire s: shaped by genetic diversity and evolution. Immunol Rev. 2015; 267(1):178–96. https://doi.org/10.1111/imr.12316 PMID: 2684478.

29. Estefanía E, Flores R, Gomez-Lozano N, Aguilar H, Lopez-Botet M, Vilches C. Human KIR2DL5 is an inhibitory receptor expressed on the surface of NK and T lymphocyte subsets. J Immunol. 2007; 178(7):4402–10. 178/7/4402. PMID: 17371997

30. Yusa S, Catina TL, Campbell KS. KIR2DL5 can inhibit human NK cell activation via recruitment of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2). J Immunol. 2004; 172(12):7385–92. 172/12/7385. PMID: 15187115

31. Gomez-Lozano N, Gardiner CM, Parham P, Vilches C. Some human KIR haplotypes contain two KIR2DL5 genes: KIR2DL5A and KIR2DL5B. Immunogenetics. 2002; 54(5):314–9. https://doi.org/10.1007/s00251-002-0476-2 PMID: 12185535

32. Vilches C, Gardiner CM, Parham P. Gene structure and promoter variation of expressed and non-expressed variants of the KIR2DL5 gene. J Immunol. 2000; 165(11):6416–21. PMID: 11086080

33. Du Z, Sharma SK, Spellman S, Reed EF, Rajalingam R. KIR2DL5 alleles mark certain combination of activating KIR genes. Genes Immun. 2008; 9(5):470–80. https://doi.org/10.1038/gene.2008.39 PMID: 18509341

34. Ordonez D, Meenagh A, Gomez-Lozano N, Castano J, Middleton D, Vilches C. Duplication, mutation and recombination of the human orphan gene KIR2DS3 contribute to the diversity of KIR haplotypes. Genes Immun. 2008; 9(5):431–7. https://doi.org/10.1038/gene.2008.34 PMID: 18480828

35. Bruneau J, Daniel M, Abrahamowicz M, Zang G, Lamothe F, Vincelette J. Trends in human immunodeficiency virus incidence and risk behavior among injection drug users in montreal, canada: a 16-year longitudinal study. Am J Epidemiol. 2011; 173(9):1049–58. https://doi.org/10.1093/aje/kwq479 PMID: 21362739

36. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996; 86(3):367–77. PMID: 8756719

37. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996; 382(6593):722–5. https://doi.org/10.1038/382722a0 PMID: 8751444

38. Kulikarni S, Martin MP, Carrington M. KIR genotyping by multiplex PCR-SSP. Methods Mol Biol. 2010; 612:365–75. https://doi.org/10.1007/978-1-60761-362-6_25 PMID: 20033654

39. Gomez-Lozano N, Trompete HI, de PR, Estefanía E, Uhrberg M, Vilches C. Epigenetic silencing of potentially functional KIR2DL5 alleles: implications for the acquisition of KIR repertoires by NK cells. Eur J Immunol. 2007; 37(7):1954–65. https://doi.org/10.1002/eji.200737277 PMID: 17557377

40. Trowsdale J, Jones DC, Barrow AD, Traherne JA. Surveillance of cell and tissue perturbation by receptors in the LRC. Immunol Rev. 2015; 267(1):117–36. https://doi.org/10.1111/imr.12314 PMID: 26284474

41. Jiang W, Johnson C, Jayaraman J, Simecek N, Noble J, Moffatt MF, et al. Copy number variation leads to considerable diversity for B but not A haplotypes of the human KIR genes encoding NK cell receptors. Genome Res. 2012; 22(10):1845–54. https://doi.org/10.1101/gr.137976.112 PMID: 22948769

42. Beziat V, Traherne J, Malmberg JA, Ivarsson MA, Bjorkstrom NK, Retiere C, et al. Tracing dynamic expansion of human NK-cell subsets by high-resolution analysis of KIR repertoires and cellular differentiation. Eur J Immunol. 2014; 44(7):2192–6. https://doi.org/10.1002/eji.201444464 PMID: 24723455

43. Graef T, Moesta AK, Norman PJ, Abi-Rached L, Vago L, Older Aguilar AM, et al. KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced specificity for HLA-A*11 while diminishing avidity for HLA-C. J Exp Med. 2009; 206(11):2557–72. https://doi.org/10.1084/jem.20091010 PMID: 19858347

44. Scott-Algara D, Truong LX, Versmisse P, David A, Luong TT, Nguyen NV, et al. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravenous drug users. J Immunol. 2003; 171(11):5663–7. PMID: 14634071

45. Ravel S, Scott-Algara D, Bonnet E, Tran HK, Tran T, Nguyen N, et al. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. Blood. 2007; 109(10):4296–305. https://doi.org/10.1182/blood-2006-08-040238 PMID: 17272507
46. Tallon BJ, Bruneau J, Tsoukas CM, Routy JP, Kiani Z, Tan X, et al. Time to seroconversion in HIV-exposed subjects carrying protective versus non protective KIR3DS1/L1 and HLA-B genotypes. PLoS One. 2014; 9(10):e110480. https://doi.org/10.1371/journal.pone.0110480 PMID: 25330014

47. Habegger de SA, Sinchi JL, Marincic K, Lopez R, Illovich E. KIR-HLA-A and B alleles of the Bw4 epitope against HIV infection in discordant heterosexual couples in Chaco Argentina. Immunology. 2013; 140(2):273–9. https://doi.org/10.1111/imm.12137 PMID: 23789883

48. Guerini FR, Lo CS, Gori A, Bandera A, Mazzotta F, Uglietti A, et al. Under Representation of the Inhibitory KIR/HLA- A and B alleles of the Bw4 epitope compatible with sexual partners confers protection against HIV-1 transmission. Blood. 2013; 121(7):1157–64. blood-2012-09-455352 https://doi.org/10.1182/blood-2012-09-455352 PMID: 23243280

49. Jennes W, Verheyden S, Mertens JW, Camera M, Seydi M, Dieye TN, et al. Inhibitory KIR/HLA incomparability in HIV exposed seronegative individuals. J Infect Dis. 2011; 203(9):1235–9. https://doi.org/10.1093/infdis/jrt020 PMID: 21983938

50. Boulet S, Song R, Kamya P, Bruneau J, Shoukry NH, Tsoukas CM, et al. HIV protective KIR3DL1 and HLA-B genotypes influence NK cell function following stimulation with HLA-devoid cells. J Immunol. 2010; 184(4):2057–64. https://doi.org/10.4049/jimmunol.0902621 PMID: 20061407

51. Song R, Lisovsky I, Lebouche B, Routy JP, Bruneau J, Bernard NF. HIV Protective KIR3DL1/S1- HLA-B Genotypes Influence NK Cell-Mediated Inhibition of HIV Replication in Autologous CD4 Targets. PLoS Pathog. 2014; 10(1):e1003867. https://doi.org/10.1371/journal.ppat.1003867 PMID: 24453969

52. VandenBussche CJ, Mulrooney TJ, Frazier WR, Dakshnamurthy S, Hurley CK. Dramatically reduced surface expression of NK cell receptor KIR2DS3 is attributed to multiple residues throughout the molecule. Genes Immun. 2009; 10(2):162–73. https://doi.org/10.1038/gene.2008.91 PMID: 19005473

53. Garcia-Beltran WF, Holzerma A, Martin G, Chung AW, Pacheco Y, Simomeau CR, et al. Open conformers of HLA-F are high-affinity ligands of the activating NK-cell receptor KIR3DS1. Nat Immunol. 2016; 17(9):1067–74. https://doi.org/10.1038/nature21531 PMID: 27455421

54. Gillespie GM, Bashirova A, Dong T, McVicar DW, Rowland-Jones SL, Carrington M. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8+ T cell epitopes. AIDS Res Hum Retroviruses. 2007; 23(3):451–5. https://doi.org/10.1089/aid.2006.0165 PMID: 17411378

55. O’Connor GM, Guinan KJ, Cunningham RT, Middleton D, Parham P, Gardiner CM. Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. J Immunol. 2007; 178(1):235–41. PMID: 17182560

56. Vivian JP, Duncan RC, Berry R, O’Connor GM, Reid HH, Beddoe T, et al. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. Nature. 2011; 479(7373):401–5. https://doi.org/10.1038/nature10517 PMID: 22020283

57. O’Connor GM, Vivian JP, Gostick E, Pymm P, Lafont BA, Price DA, et al. Peptide-Dependent Recognition of HLA-B*57:01 by KIR3DS1. J Virol. 2015; 89(10):5213–21. https://doi.org/10.1128/JVI.03586-14 PMID: 25740999

58.Alter G, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneiderwind A, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med. 2007; 204(12):3027–36. https://doi.org/10.1084/jem.20070695 PMID: 18025129

59. Stewart CA, Laugier-Anfoisi F, Vely F, Saulquin X, Riedmuller J, Tisserant A, et al. Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. Proc Natl Acad Sci U S A. 2005;.

60. Fauriat C, Ivarson MA, Ljunggren HG, Malmberg KJ, Michaelson J. Education of human natural killer cells by activating killer immunoglobulin-like receptors. Blood. 2010; 115(6):1166–74. https://doi.org/10.1182/blood-2009-09-245746 PMID: 19903900

61. Della CM, Romeo E, Falco M, Balsamo M, Augugliaro R, Moretta L, et al. Evidence that the KIR2DS5 gene codes for a surface receptor triggering natural killer cell function. Eur J Immunol. 2008; 38(6):2284–9. https://doi.org/10.1002/eji.200838434 PMID: 18624290

62. Liano M, Lee N, Navarro F, Garcia P, Albar JP, Geraghty DE, et al. HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. Eur J Immunol. 1998; 28(9):2854–63. PMID: 9754572

63. Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc Natl Acad Sci U S A. 1993; 90(24):12000–4. PMID: 8265660

64. Korner C, Granoff ME, Amero MA, Sirignano MN, Vaidya SA, Jost S, et al. Increased frequency and function of KIR2DL1-3(+) NK cells in primary HIV-1 infection are determined by HLA-C group haplotypes. Eur J Immunol. 2014; 44(10):2938–48. https://doi.org/10.1002/eji.201444751 PMID: 25043727