NK Cell Subsets Responding to HIV-Infected Autologous CD4T Cell in Protective KIR/HLA Genotypes

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Background:

Genetic and epidemiological studies implicate NK cells in HIV resistance and slow disease progression in infected individuals. This protective effect is achieved by interactions between NK cell surface receptors called KIRs and their ligands, HLA molecules. These interactions are important for NK cells to gain functional potential during a developmental process known as education. The receptor KIR3DL1 with its ligand HLA-B*57 (*h/*y+B*57) has the strongest effect on slow time to AIDS and HIV viral load control compared to Bw6 homozygotes (Bw6hmz). *h/*y+B*57 carriers have a stronger effect on these outcomes than do carriers of *l/*x+B*57. Levels of KIR3DL1 on NK cells from *l/*x+B*57 carriers are lower *h/*y+B*57 carriers. NK cells from *h/*y+B*57 carriers receive potent educational signals through HLA-B*57 KIR3DL1 ligation leading to high functional potential. Since Bw6 antigens do not interact with KIR3DL1, NK cells from Bw6hmz are not educated through KIR3DL1. Here we investigated whether KIR/HLA genotype influences the potency of NK-mediated inhibition of viral replication and NK cell subset responses to autologous HIV-infected CD4 (iCD4) cells.

Methods:

The study population included carriers of *h/*y+B*57 (n=7), 3DS1+*80I (n=9), *l/*x+B*57 (n=4) and Bw6hmz (n=11). We measured NK cell mediated inhibition of HIV replication in autologous iCD4 cells by assessing the supernatant levels of HIV p24 longitudinally. We measured CC-chemokine secretion following stimulation with autologous infected CD4 cells by ELISA and multi-parametric flow cytometry.

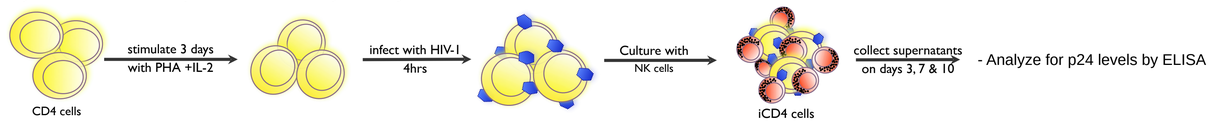


Figure 1: Schematic representation of the inhibition of viral replication assay. Purified CD4 T cells stimulated with PHA-P overnight are treated with media containing IL-2 (100U) for 3 days and infected with HIV-1 on day 4 for 4hrs. Unbound virus is washed off and iCD4 cells are cultured with autologous NK cells. Supernatants were collected on day 3, 7 & 10 and analyzed by ELISA for p24.

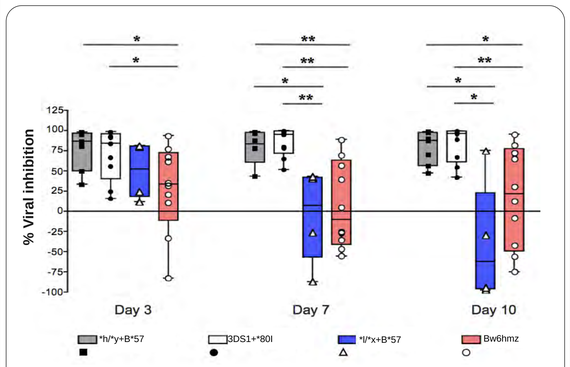


Figure 2: NK cell mediated inhibition of viral replication in autologous iCD4 cells. Co-cultures of NK-iCD4 from subjects with defined KIR/HLA genotypes were incubated for up to 10 days as described in Fig. 1. Culture supernatants were collected on days 3, 7 & 10 and assayed for p24 levels by ELISA. The lines through the box and whisker plots are the median values. Upper and lower limits of the box are the 25 and 75 percentile values and the error bars represent the maximum and minimum values. p-value <0.05 "*", p-value <0.01 "**".

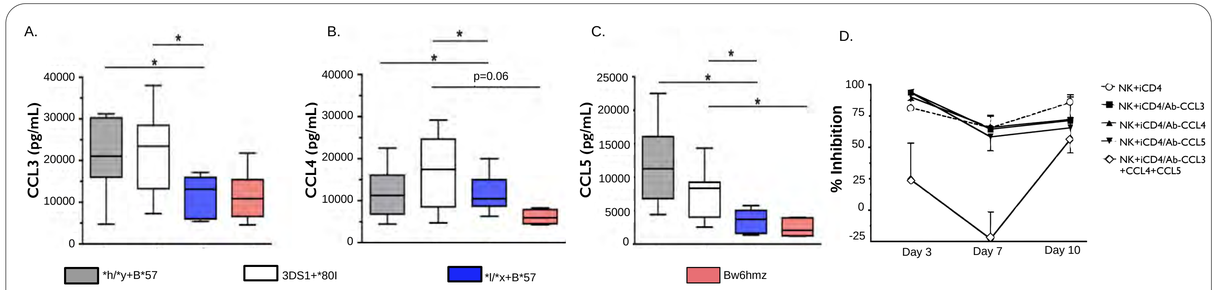


Figure 3: CC-chemokines secretion by autologous iCD4 stimulated NK cells from subjects with defined KIR/HLA genotype combinations. Levels of (A) CCL3, (B) CCL4 & (C) CCL5 secreted over 3 days were measured by ELISA. The median values are represented by the horizontal line in the box plot. p-value <0.05 "*". (D) %Viral inhibition of NK:iCD4 co-cultures in the absence of anti-CC-chemokine neutralizing antibodies or in the presence of anti-CCL3, anti-CCL4 or anti-CCL5 neutralizing antibodies, individually, or together.

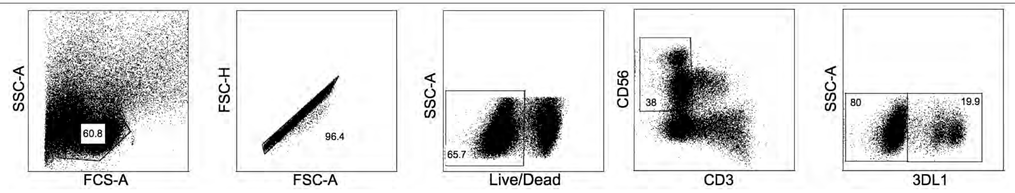


Figure 4: Gating strategy used to assess the percent of functional NK cells stimulated with iCD4 cells. Lymphocytes were gated on single cell events using the FSC-A and FSC-H parameters. Only live CD3⁻CD56⁺ NK cells were included in this analysis. 3DL1 subsets were examined for the following functional markers: CD107a, IFNγ , MIP-1α & MIP-1β. The representative subject shown in this figure carries a KIR3DL1 *l/*x genotype with 1 high and 1 low KIR3DL1 allele.

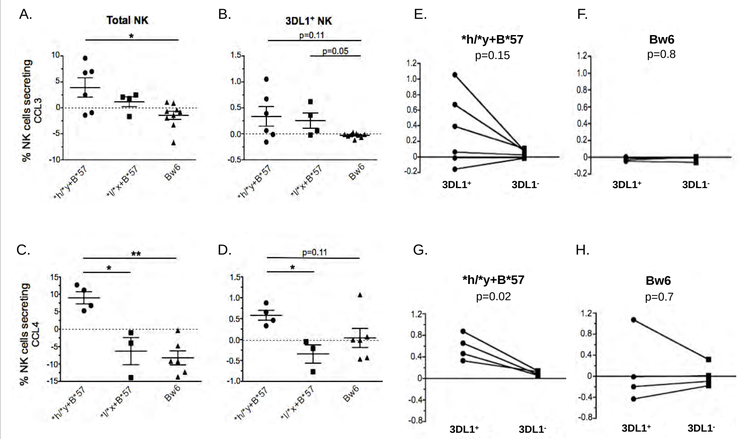


Figure 5: Intracellular CCL3 and CCL4 secretion in response to iCD4 cells by NK cells or NK cell subsets from subjects stratified by KIR/HLA genotype. The percent of NK cells (A, C) or KIR3DL1⁺ cells (B, D) secreting CCL3 (A, B) or CCL4 (C, D) from subjects who were *h/*y+B*57, *l/*x+B*57 or *Bw6hmz*. Comparison of the percent of CCL3 (E, F) or CCL4 (G, H) secreting KIR3DL1⁺ and KIR3DL1⁻ NK cell subsets following stimulation with autologous iCD4 for 24 hrs, with Brefeldin and Golgi Stop added for the last 5 hours of co-culture. Results for co-cultures of cells from carriers of *h/*y/+B*57 (E. G) and *Bw6hmz* (F, H) are shown.

Results Summary:

- NK cells from subjects carrying *h/*y+B*57 alleles suppress viral replication as well as those from *3DS1+*80I* carriers and better than those from carriers of *l/*x+B*57 and *Bw6hmz.*
- NK cells, and in particular KIR3DL1⁺ NK cells, from subjects with *h/*y+B*57 KIR/HLA genotypes secrete more CC-chemokines in response to autologous HIV infected CD4 cells than those from *Bw6hmz* and *l/*x+B*57 carriers.

Conclusions:

NK cells from carriers of *h/*y+B*57 inhibit HIV replication in autologous CD4 T cells more effectively than those from *l/*x+B*57 carriers and Bw6hmz. This suggests that NK cell education influences the potency of NK cell mediated inhibition of HIV replication. The inhibition of viral replication in autologous infected CD4 T cells involves responses by KIR3DL1 subset and is partially due to secretion of CC-chemokines.

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