Effective Elimination of HIV-1 Infected CD4+ T-Cells by NKG2C+ "Memory-Like" NK Mediated by TRAIL 443

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BACKGROUND

We previously observed that "Memory-like" NKG2C+ Natural Killer (NK) cells from people with HIV-1 (PLWH) on ART induced in the presence of dendritic cells (DC) are associated with a more effective elimination of autologous latently HIV-1 infected CD4+ T cells and lower expression of TIGIT (Figure 1A). TRAIL is a TNF family receptor that can induce cytotoxic function (Figure 1B).

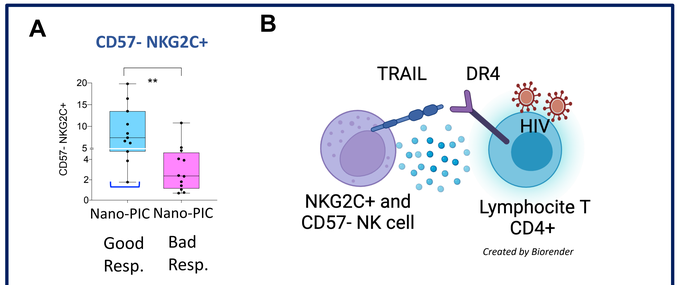


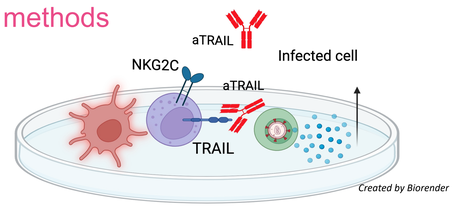
Figure 1. Induction of NKG2C+ cells in response to DC-based immunotherapy: (A) Proportions of NKG2C+ CD57- cells within NK cells from PLWH showing functional (good resp.) or dysfunctional characteristics (Bad Resp.) eliminating autologous HIV-1 infected CD4+ T cells in vitro. (B): Schematic function of TRAIL receptor. Two-tailed paired Wilcoxon t test. **p<0.01.

Hypothesis: *In this study, we asked whether Trail expression on NKG2C+ cells mediate elimination of HIV-1 infected CD4+ T cells.*

METHODS

NK cells were isolated from the blood of n=19 PLWH on ART and activated in the presence of autologous monocyte-derived DC pre-treated with nanoparticles loaded poly I:C (Nano-PIC DC). Expression of TRAIL and TIGIT in different memory and effector NK cell subsets defined by NKG2C vs CD57 expression was tested and associated with functional ability to kill HIV-1 infected cells. In addition, expression of the TRAIL ligand DR4 was also tested in PHA+IL-2 reactivated p24+ isolated CD4+ T cells. Finally, impact of anti-TRAIL, anti-NKG2C and anti-NKG2D or isotypic mAbs in NK cell-mediated elimination of p24+ HIV-1 infected CD4+ T cells from PLWH was tested in the presence of Romidepsin and Raltegravir.

Schematic methods



TRAIL expression on NKG2C+ "memory-like" NK cells mediate effective elimination of HIV-1 infected CD4+ T cells in presence of activated nano-poly I:C dendritic cells

RESULTS

CD56dim and CD56- CD16+ NK cells from PLWH exposed to nano-PIC DC displayed increased levels of TRAIL compared to cells stimulated with control nano DC (p=0.0001) (Figure 2).

Figure 2

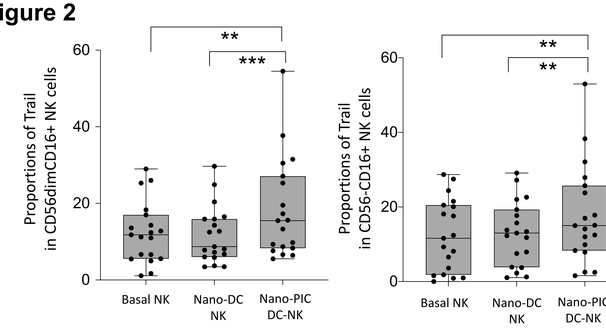


Figure 2. Proportions of TRAIL in CD56dim/low CD16+ NK cells in presence of immunotherapy: Proportions of TRAIL in CD56dim/low CD16+ NK cells after co-culture without and with DCs activated with nano-PIC plus autologous NK cells in the presence of IL-2. Significande was calculated using a two-tailed paired Wilcoxon t test. **p<0.01; ***p<0.001.

Induction of TRAIL expression was higher in CD56dim and CD56- CD16+ NK cells from PLWH characterized by effective elimination of infected CD4+ T cells at baseline (p=0.0076; p= 0.0133) and after activation with nano-PIC DC (p=0.0076; p= 0.0133), respectively (**Figure 3 A and B**).

Figure 3

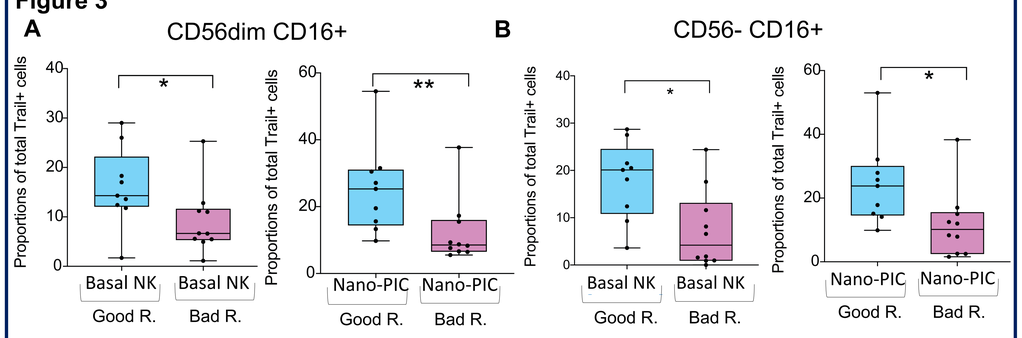


Figure 3. Proportions of TRAIL+ cells within CD56dim/low CD16+ NK cells in good and bad responders to immunotherapy. (A,B): proportions of TRAIL in CD56dim/low CD16+ NK cells in medium (basal) and after co-culture with Nano-PIC DC in PLWH with effective (blue) or bad (pink) abilities to eliminate autologous infected CD4+ T cells. Two-tailed paired Wilcoxon t test. *p<0.05; **p<0.01.

Figure 4

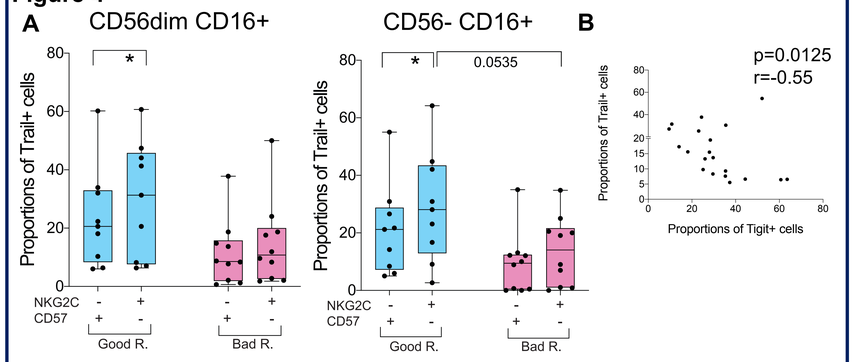


Figure 4. Expression of TRAIL into NKG2C+ CD57- and NKG2C- CD57+ memory-like CD56dim/low CD16+ NK cell subsets. (A): proportions of TRAIL+ cells into NKG2C+ CD57- and NKG2C- CD57+ memory-like in CD56dim/low CD16+ NK cell subsets in presence of Nano-PIC DC immunotherapy from PLWH with effective elimination of infected CD4+ T cells and bad responders. **(B):** Spearman correlation between proportions of TRAIL+ cells and TIGIT+ cells in CD56dim CD16+ NK cells. Significance was calculated using a two-tailed paired Wilconxon t test and unpaired Mann-Whitney t test. Spearman Correlation p and R values are also shown. *p<0.05

Notably, higher levels of TRAIL were enriched in NKG2C+ cells from good compared to bad PLWH responders after nano-DC stimulation. Moreover, TRAIL was enriched in NKG2C+ CD57- NK cells, compared to NK cells expressing CD57 (p=0.0391) (**Figure 4A**). In this regard, TRAIL negatively associated with expression of TIGIT in CD56dim CD16+ NK cells (**Figure 4B**) (p=0.0125; r=-0.55).

Figure 5

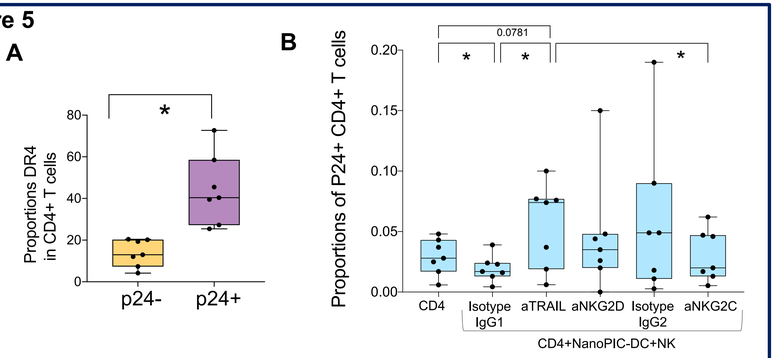


Figure 5. Impact of TRAIL, NKG2C and NKG2D blockade in elimination of p24+ CD4+ T cells by NK cells. (A): Proportions of p24+ CD4+ T cells after culture with Nano-PIC DC plus NKs immunotherapy in presence of aTRAIL, aNKG2C and aNKG2D antibodies compared with isotype control antibodies. **(B):** proportions of cells expressing activating ligands DR4 TRAIL receptor on p24+ vs p24- subpopulations of CD4+ T cells. Significance was calculated using a two-tailed paired Wilcoxon t test. *p<0.05.

By the other hand, p24+ CD4+ T cells from PLWH showed higher expression of the TRAIL ligand DR4 than p24- CD4+ T cells (**Figure 5A**). Finally, cytotoxic NK function against HIV-1 infected p24+ CD4+ T cells was abrogated in presence of an anti-TRAIL blocking mAbs, in contrast to anti- NKG2C and anti-NKG2D (**Figure 5B**).

CONCLUSIONS

Cytotoxic function of memory-like NKG2C+ CD57- NK cells is associated with effective elimination of HIV-1-infected CD4+ T cells by TRAIL.

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