TIGIT Blockade Restores Functional NKG2C+ NK Cells and Limits Spread of HIV-1 *In Vivo*

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BACKGROUND

We previously observed that higher of TIGIT on Natural Killer (NK) from people living with HIV (PLWH) on ART associates with lower induction of adaptive NKG2C+ CD57- cells and lower functional abilities to eliminate HIV-1 infected cells after receiving dendritic cell (DC)-based immunotherapy (Figure 1).

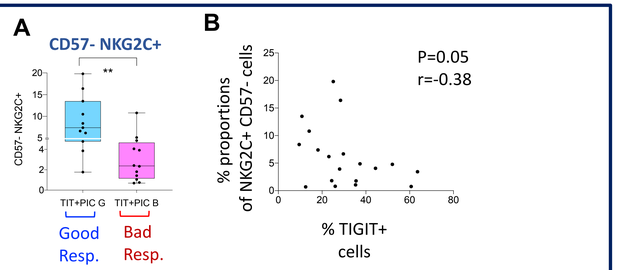


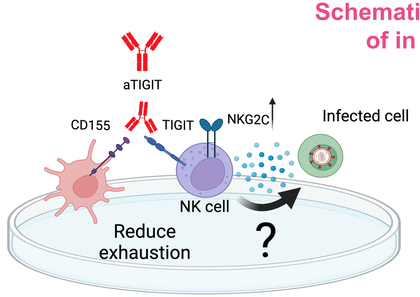
Figure 1. Induction of NKG2C+ cells in response to DC-based immunotherapy: (A) Proportions of NKG2C+ CD57- cells within NK cells from PLWH showing functional (good resp.) or dysfunctional characteristics (Bad Resp.) eliminating autologous HIV-1 infected CD4+ T cells in vitro. (B): Spearman Correlation between proportions of NKG2C+ CD57- vs TIGIT levels in PLWH. Two-tailed paired Wilconxon t test. **p<0.01.

Hypothesis: In this study, we asked whether blockade of TIGIT enhances functional adaptive NKG2C+ cells *in vitro* and *in vivo*.

METHODS

Monocyte-derived dendritic cells (DCs) from n=9 PLWH on ART and activated with nanoparticle-loaded poly I:C (Nano-PIC) were cultured with autologous NK cells in the presence of anti-TIGIT or isotypic control mAbs. Reduction of proportions of HIV-1 p24+ cells CD4+ T cells in the presence of NK cells, Romidepsin and Raltegravir was evaluated by FACS.

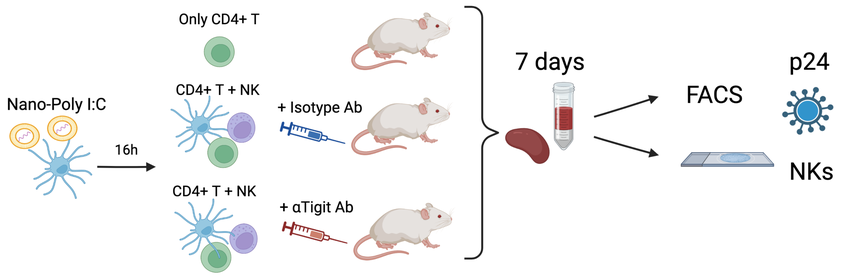
Schematic Representation of in vitro system



Combination of **DC immunotherapies and TIGIT blockade** is a promising strategy to enhance functional **NKG2C+** NK responses and limit HIV-1 replication ***in vivo***

In vivo efficacy of TIGIT blockade on NK cells was addressed by transplanting n=42 immunodeficient NSG mice with CD4+ T cells from the same PLWH alone (n=14) or with NK cells and Nano-PIC DC in combination with either anti-TIGIT (n=14) or Isotypic mAbs (n=14) in three independent experiments. After 7 days, mice were sacrificed and proportions of circulating human CD45+ p24+ CD4+ T cells were analyzed by FACS. In addition, histological distribution of p24+ infected cells in the spleen was analyzed by confocal microscopy.

Schematic representation of humanized mouse *in vivo* experimental system



RESULTS

Blockade of TIGIT combined with Nano-PIC DC enhanced the ability of NK cells from PLWH to significantly reduce by 70% the proportions of autologous HIV-1 p24+ CD4+ T cells ex vivo compared to treatment with Isotypic mAb (p=0.0078) or anti-TIM3 antibodies (Figure 2).

Figure 2

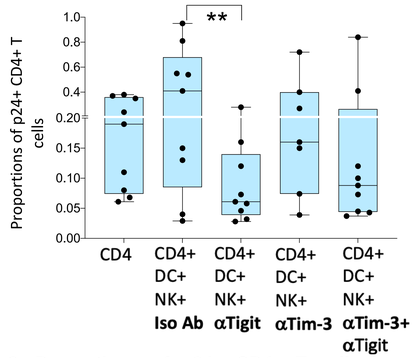


Figure 2. Proportions of p24+ CD4+ T cells in presence of immunotherapy: Proportions of p24+ CD4 T cells after co-culture with DCs activated with nano-PIC plus autologous NK cells in the presence of an anti-TIGIT or isotypic control mAbs, and of Raltegravir and Romidepsin. two-tailed paired Wilcoxon t test. **p<0.01.

Figure 3

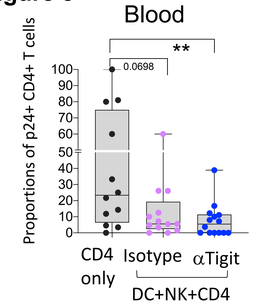


Figure 3. Detection of p24+ CD4+ T cells blood of NSG mice receiving anti-TIGIT and DC-NK immunotherapy. (A): proportions of p24+ infected cells in three groups of mice (14 per group). CD4 alone (black), isotype (pink) and aTigit (blue) mAbs combined with DC plus NK plus CD4 treatment tested flow cytomery

Importantly, i.p. administration of anti-TIGIT into NSG mice xenotransplanted with CD4+ T cells from PLWH and with autologous NK cell and nano-PIC-DC immunotherapy led to a more significant reduction of p24+ cells (mean reduction 57%, p= 0.0019) within human circulating CD4+ T cells compared to mice receiving only CD4+ T cells, in contrast to mice receiving isotypic control mAb (mean reduction 23.6%, p=0.0698) (Figure 3).

Figure 4

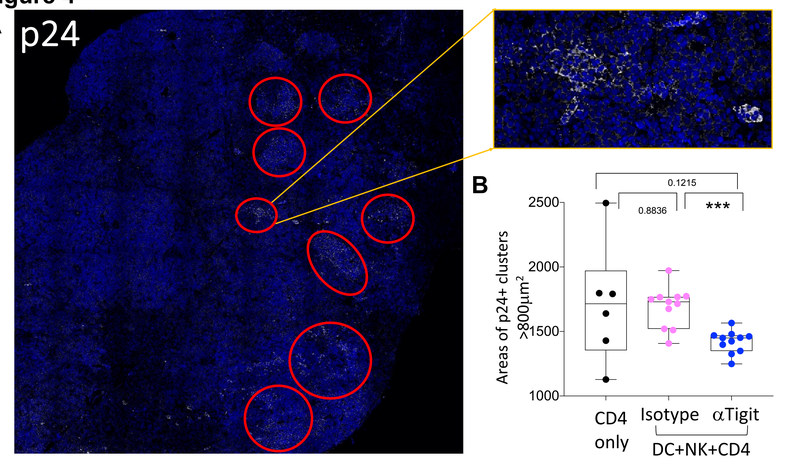


Figure 4. Quantification of p24+ CD4+ T cells tissue of NSG mice reciving anti-TIGIT and DC-NK immunotherapy. (A) Representative confocal (40X magnification) image of histological analysis of p24+ foci comparing three groups of NSG mice receiving only CD4 T cells or DC-NK immunotherapy in the presence of isotypic or anti-TIGIT antibodies. **(B):** Quantification of the p24 foci defined in (A) using image J software. Significance was calculated using a Mann-Whitney t test. ***p<0.001.

In addition, significantly lower areas of p24+ foci (Median 1,452 µm2 vs 1,766 µm2; p=0.0078) were detected in the spleen of mice injected with αTIGIT compared to Isotype mAbs (Figure 4).Finally, we observed a reduction of CD57 expression (38% αTIGIT vs 63% Isotype mAbs; p=0.0011) on adaptive NKG2C+ cells in this tissue (Figure 5).

Figure 5

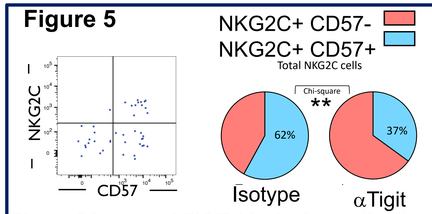


Figure 5.Impact of TIGIT blockade on distribution of memory-like NK cell subsets. Pie chart quantification of NKG2C+ CD57+ vs NKG2C+ CD57- NK cells (see left) in transplanted NSG mice treated with isotypic control or anti-TIGIT Ab. Significance was calculated using a Chi-square test. ***p<0.001.

CONCLUSIONS

combination of DC immunotherapy and TIGIT blockade is a promising strategy to enhance functional NKG2C+ NK responses and limit HIV-1 replication in tissue in vivo.

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