Early initiation of anti-retroviral therapy restores some but not all perturbations of natural killer cell functions and phenotypes in early HIV-1 infected men who have sex with men

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Abstract

Background: ART effectively restores CD4+ T cell counts and suppresses HIV-1 viral loads to undetectable levels. However, it is not clear whether the perturbations on NK cells are fully recovered or not. We hypothesized that early initiation of ART restores NK cell perturbations due to HIV-1 infection. We therefore sought to understand the phenotypic and functional changes that may occur to NK cells in early HIV-1 infection and whether early initiation of ART restores these possible changes.

Methodology: We longitudinally evaluated NK cell functional and phenotypic changes in early HIV-1 infected Men who have Sex with Men (MSM) in Nairobi, Kenya and who were recently initiated on ART. Blood samples were obtained fortnightly for 3 visits post seroconversion. Baseline blood samples collected in the SiVET study prior to seroconversion were also analysed. Frozen PBMCs were thawed and stimulated overnight with K562 cell line, IL-2 and IL-15 and stained with a cocktail of antibodies for evaluation of NK cell phenotype, activation and functionality.

Results: Compared to the pre-seroconversion time point, there were no changes in the total NK cell frequencies across the time points. We observed significant reductions in NK cell production of IFN-γ, expression of CD69 and NK cell inhibitory receptor siglec7. However, there were significant increases in NK cell degranulation and expression of cell exhaustion marker PD-1. Most of these changes were restored to near the pre-seroconversion level around 30 days post ART initiation. The reduction in expression of siglec7 receptor was, however not restored.

Conclusion: The impairment of NK functionalities in early HIV-1 infection may enhance disease progression. This could be one way in which the HIV-1 escapes the immune system. However, these impairments seem to be restored a few weeks after ART supporting the test and treat strategy. Evaluating the mechanisms by which HIV-1 impairs NK cell effector functions would inform designing an effective HIV-1 vaccine boosting innate immunity.

Introduction

HIV antiretroviral therapy (ART)

- ✓ Life long treatment
- ✓ Combination of drugs
- ✓ Prevents viral replication
- ✓ Effectively suppress HIV viremia
- ✓ Restores CD4+ T cells counts

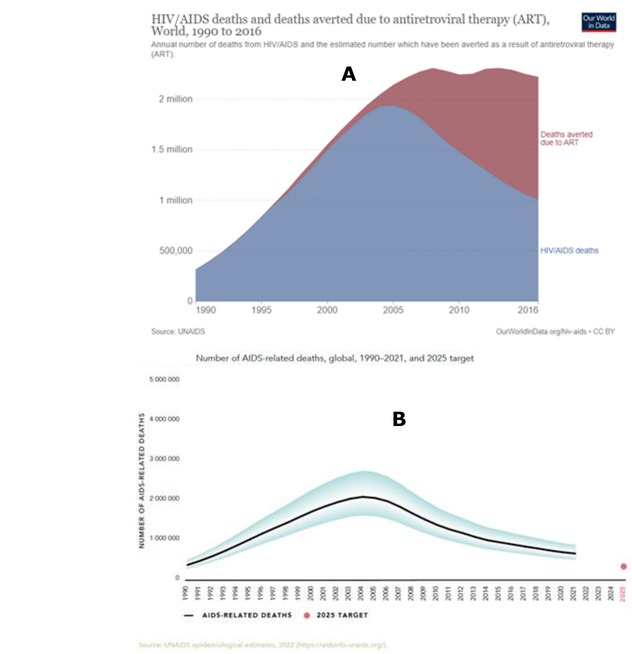

Figure 1: Deaths averted as a results of ART

Apart from CD4+ T cells HIV-1 infection indirectly affects the phenotype and/or functionality of innate immune cells such as monocytes and NK cells

NK cells play a critical role in control of viral replication by;

- ✓ production of antiviral cytokines such IFNγ and TNFα
- ✓ Production of granzymes and perforins hence killing target cells
- ✓ important in the recruitment and regulation of the adaptive immunity which has been shown to curtail run-away HIV replication

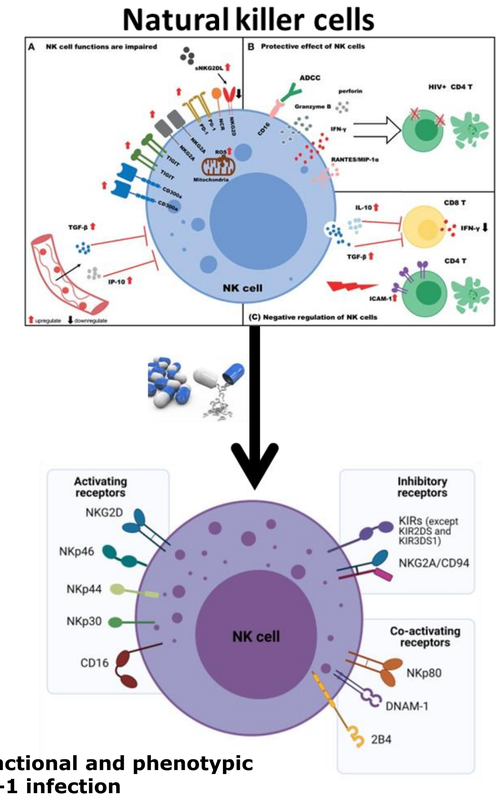

Figure 2: NK cell functional and phenotypic perturbation in HIV-1 infection

Methodology

Research question

Do ART restore NK cell phenotypic and functional perturbations in early HIV-1 infection?

Hypothesis

Early initiation of ART restore NK cell phenotypic and functional perturbations resulting from HIV-1 infection

Aims

Longitudinally evaluate the effects of ART on the phenotypes and effector functions of NK cells in early HIV-1 infected men who have sex with men (MSMs)

Study design

Longitudinal exploratory cohort study on recently seroconverted MSMs in Nairobi Kenya (rolled over from IAVI's SiVET study)

Ethical considerations

KNH/UoN/ERC (P61/02/2017)

Sampling

Convenient sampling (N=5)

Data analysis

- ✓ flowjo software (ver. 10.8.1) and graph pad prism (ver. 8.0.1)
- ✓ Statistical tests done: Paired t test and ANOVA
- ✓ Result calculated at 95% confidence interval ($p \leq 0.05$).

Study approach

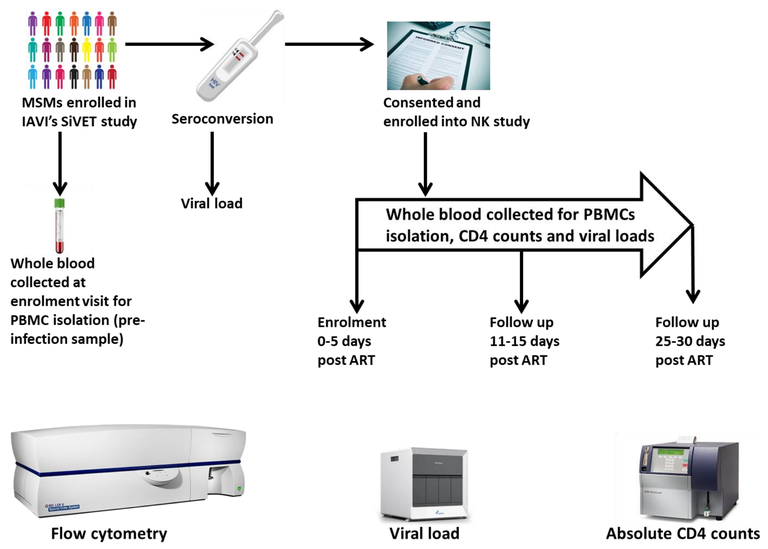

Figure 3: Study participants , sample collection and laboratory assays

Participants' Demographics	
N	5
Sex	Male
Median age (25th percentile/75th percentile)	30(21/32)
On HAART (Yes/No)	5/0
Median days post estimated date of infection (EDI) (25th/75th percentile)	78(50/81

Gating strategy for NK cell subsets

Figure 4: Gating strategy for total NK cells (a) and the different NK cell sub populations; CD56^{bright} NK cells (b), CD56^{dim} NK cells (c), CD56^{dim} NKG2A negative NK cells (d), CD56^{dim} NKG2A positive NK cells (e), CD56^{dim} naïve NK cells (f), CD56^{dim} intermediate NK cells (g) and CD56^{dim} fully differentiated NK cells (h)

Results

Total NK cell frequencies, absolute CD4 counts and the HIV viral loads [(a)]

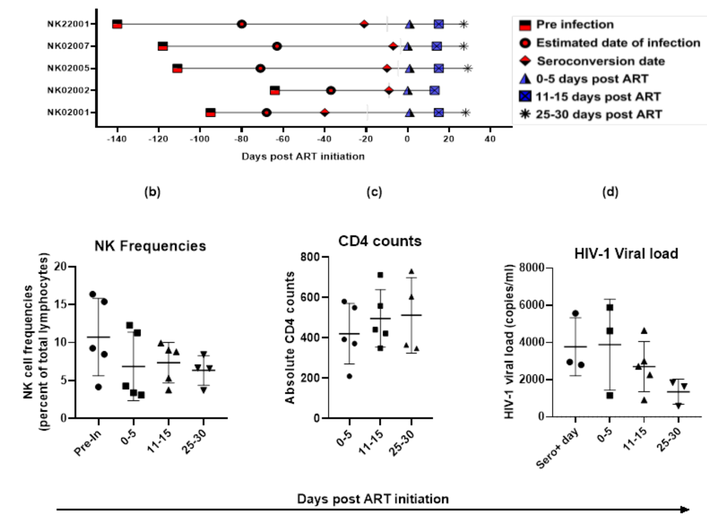

Figure 5: Series of events at different time points pre and post ART initiation (a) and changes in the frequencies of total NK cells from the total lymphocytes (b), absolute CD4 T cell counts (c) and HIV-1 viral loads (c) at different time points upon ART initiation.

Phenotypic changes of total NK cells in early HIV-1 infection

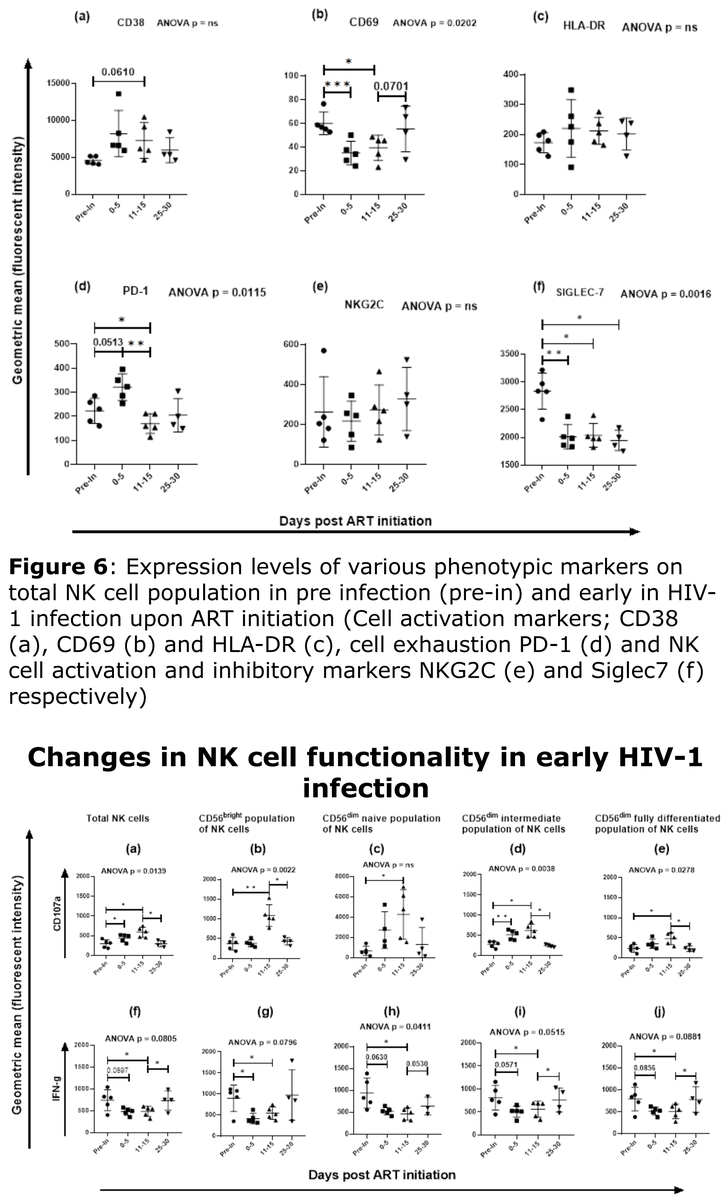

Figure 6: Expression levels of various phenotypic markers on total NK cell population in pre infection (pre-in) and early in HIV-1 infection upon ART initiation (Cell activation markers; CD38 (a), CD69 (b) and HLA-DR (c), cell exhaustion PD-1 (d) and NK cell activation and inhibitory markers NKG2C (e) and Siglec7 (f) respectively)

Changes in NK cell functionality in early HIV-1 infection

Figure 7: Expression levels of CD107a (a – e) and IFN-γ (f – j) in total NK cell population (a and f) and in the different NK cell sub populations; CD56^{bright} NK cell population (b and g), CD56^{dim} naïve NK cells (c and h), CD56^{dim} intermediate NK cells (d and i) and CD56^{dim} fully differentiated NK cells (e and j) across the different sample collection time points (Pre-In = pre infected)

Discussion

- ✓ Perturbation of NK cell phenotypes and functionality happens quite early in HIV-1 infection
- ✓ This seem to be among ways HIV-1 tries to evade the immune system (reduced expression CD69, increased expression of PD-1)
- ✓ ART restores most of the perturbations on NK cells resulting from HIV-1 infection
- ✓ This finding has implications for the broader field of HIV research, as it suggests that early initiation of ART could have benefits beyond the suppression of viral load and restoration of CD4+ T cell counts
- ✓ The study also highlights the importance of evaluating innate immunity in HIV-1 infected individuals, as it plays a critical role in both antiviral and adaptive immunity

- **Conclusion**: Early initiation of ART is effective and may be important in boosting the innate immunity and restoring some perturbations resulting from HIV-1 infection
- **Recommendations:** Future studies to evaluated the mechanism by which HIV-1 interferes with NK cells functionally in early HIV-1 infection will shed light on vaccine designs that will boost the innate immunity
- **Study limitations**: Small sample size and no sample for phenotypic and functional analysis on the day of seroconversion

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