CD56^{neg}CD16^{+} NK Cells are Mature NK cells with Impaired Function during HIV-1 Infection

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ABSTRACT

HIV-1 infection has a significant impact on the phenotype and function of Natural Killer (NK) cells. A subset of CD3^{neg}CD56^{neg}CD16^{+} Natural Killer (NK) cells is highly expanded during chronic HIV-1 infection, and the role of this subset in HIV-1 pathogenesis remains unclear.

The lack of NK cell lineage-specific markers has complicated the study of minor NK cell subpopulations. Using CD7 as an additional NK cell marker, we found that CD3^{neg}CD56^{neg}CD16^{+} cells are a mixed population of CD7^{+} NK cells and CD7^{neg} myeloid cells. We compared CD7^{+}CD56^{neg}CD16^{+} NK cells between healthy individuals, HIV-1 acute and HIV-1 chronic patients that were not on Anti-Retroviral-Therapy when their cells were assessed. CD7^{+}CD56^{neg}CD16^{+} NK cells are significantly expanded in HIV-1 infection. They are mature NK cells and express KIRs, NKG2A, NKG2C, and natural cytotoxicity receptors similar to CD7^{+}CD56^{+}CD16^{+} cells, however they express a higher amount of CD95, a marker of NK cell activation. CD7^{+}CD56^{neg} NK cells in healthy donors produced minimal IFNγ following K562 target cell or IL-12 plus IL-18 stimulation; however, they did degranulate in response to K562 cells similar to CD7^{+}CD56^{+} NK cells. HIV-1 infection resulted in a slight decrease of NKp30 and NKp46 expression, reduced IFNγ secretion following K562 or cytokine stimulation by both NK cell subsets compared to healthy donors. Significantly fewer CD7^{+}CD56^{neg} NK cells produced IFNγ compared to CD7^{+}CD56^{+} NK cells from HIV-infected donors. Decreased granzyme B and perforin expression and increased expression of CD107a in the absence of stimulation, particularly in HIV-1-infected subjects, suggests that CD7^{+}CD56^{neg}CD16^{+} NK cells may have recently engaged target cells. NK cells from HIV-infected subjects experience chronic exposure to pro-inflammatory cytokines and increased oxidative stress. Interestingly, in HIV-infected subjects CD7^{+}CD56^{neg}CD16^{+} NK cells have increased expression of the aging-related cyclin-dependent kinase inhibitors p16/INK4a and p21/Waf1, that are induced by oxidative stress.

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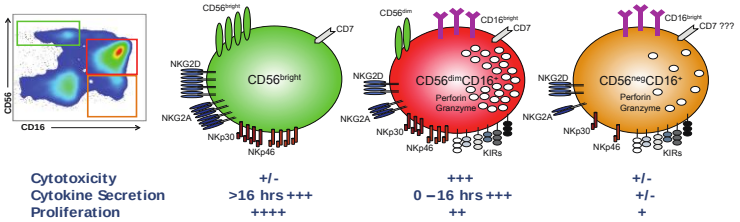
NK CELL BACKGROUND

- Constitute 5 - 20% of all peripheral blood lymphocytes
- Generally characterized as CD3^{neg}CD56^{+}CD16$^{+ \text{ or } -}$ lymphocytes
- CD3^{neg}CD56^{neg}CD16^{+} NK cells are expanded in chronic infectious diseases such as HIV-1 and hepatitis C virus infection and appear to have diminished effector functions.

Summary of Phenotypic and Functional Properties of NK cell Subsets



Cytotoxicity	+/-	+++	+/-
Cytokine Secretion	>16 hrs +++	0 –16 hrs +++	+/-
Proliferation	++++	++	+

NK Cell Gating Strategy

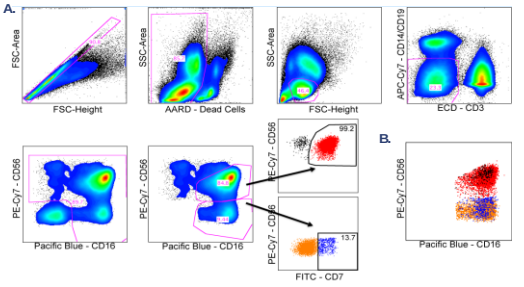
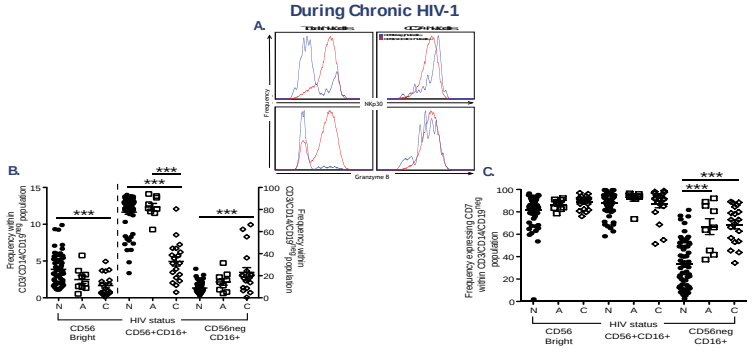


Figure 1. A) Using 12-color flow cytometry, the phenotype and functional characteristics of CD56^{+}CD16^{+} and CD56^{neg}CD16^{+} cells were assessed using the depicted gating strategy. To ensure we were assessing NK cells, CD7 was included in all staining panels. **B)** Overlaying CD7^{+} and CD7^{neg} cells indicates the utility of the CD7 marker in identifying CD7^{+} NK cells from CD7^{neg} cells.

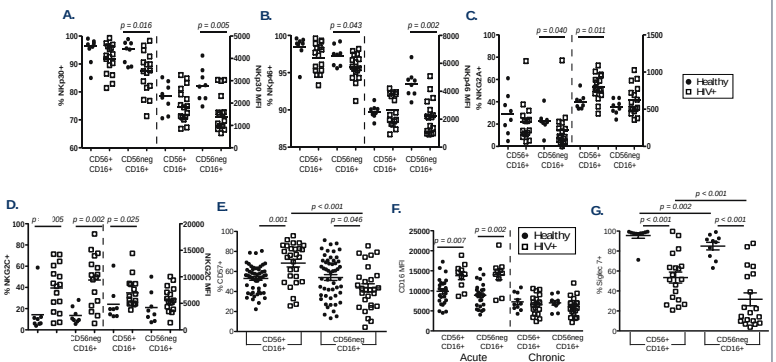
RESULTS

Figure 2 –CD56^{neg}CD16^{+} Cells are a Heterogenous population and CD7 Significantly Impacts the Interpretation Of their Cells Phenotype ; CD7^{+}CD56^{neg}CD16^{+} NK Cells Are Expanded During Chronic HIV-1



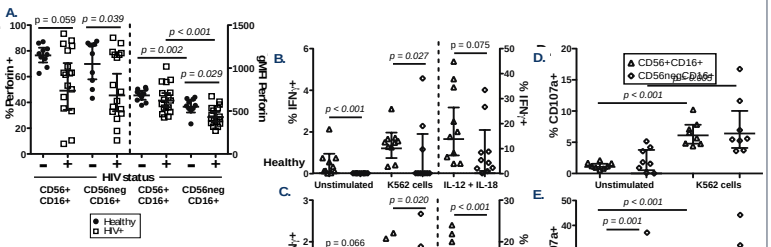
A) CD56^{+}CD16^{+} and CD56^{neg}CD16^{+} cells were gated as in Figure 1 and analyzed for NKp30 and Granzyme B expression prior to or following CD7 gating. When the analysis is performed without removing CD7^{neg} cells, few CD56^{neg}CD16^{+} cells appear to express NKp30 or Granzyme B. However, when CD7^{+} cells are analyzed, CD7^{+}CD56^{neg}CD16^{+} NK cells actually express higher levels of NKp30 and similar levels of Granzyme B compared to CD7^{+}CD56^{+}CD16^{+} NK cells. **B)** Frequency of classically defined NK within CD3^{neg} CD14^{neg} CD19^{neg} population in healthy donors ((●) N, n = 63) and acutely- ((□) A, n = 9) and chronically- ((◊) C, n = 19) HIV-1-infected subjects. **C)** Frequency of CD7^{+} NK cells in CD56^{bright}CD16^{neg}, CD56^{+}CD16^{+} and CD56^{neg}CD16^{+} cells within CD3^{neg} CD14^{neg} CD19^{neg} population. CD7 is expressed on ~40% and ~65% of CD56^{neg}CD16^{+} cells in healthy and HIV-1-infected subjects respectively.

Figure 3 –CD7^{+}CD56^{neg}CD16^{+} and CD7^{+}CD56^{+}CD16^{+} NK Cells Are Phenotypically Similar In Healthy Donors; However, HIV-1 Infection Significantly Alters The Phenotype Of Both NK Cell Subsets



In Healthy donors CD7^{+}CD56^{+}CD16^{+} and CD7^{+}CD56^{neg}CD16^{+} NK cells have a similar phenotype with minimal differences: CD56^{neg}CD16^{+} NK cells have a higher MFI for NKp46 and fewer express Siglec-7. In HIV-1-infected subjects the phenotype of both subsets is altered: NKG2C and CD16 expression are increased and Siglec-7 is decreased in both subsets; in CD7^{+}CD56^{neg}CD16^{+} NK cells the expression of NKp30, NKp46 and NKG2A is decreased, whereas CD57, a marker of maturation, is increased in CD56^{+}CD16^{+} NK cells and decreased in CD56^{neg}CD16^{+} NK cells. Frequency (left axis) of each NK cell subset expressing **(A)** NKp30, **(B)** NKp46, **(C)** NKG2A, **(E)** CD57, **(F)** CD16 and **(G)** Siglec 7; and **(A-G)** the density (right axis) of each marker assessed by mean fluorescence intensity (MFI).

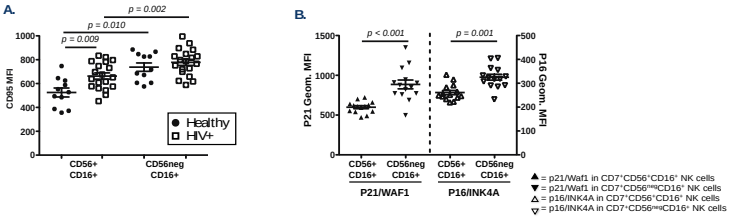
Figure 4 –HIV-1 Infection Has A Significant Impact On The Function Of Both CD7^{+}CD56^{+}CD16^{+} And CD7^{+}CD56^{neg}CD16^{+} NK Cells



A) Perforin expression in CD7^{+}CD56^{+}CD16^{+} and CD7^{+}CD56^{neg}CD16^{+} NK cells of healthy (n=10) and chronically HIV-1-infected subjects (n=17). Chronic HIV-1 infection results in decreased perforin expression in both NK cell subsets.

Assessment of IFNg **(B & C)** and CD107a **(D & E)** expression by CD7^{+}CD56^{+}CD16^{+} and CD7^{+}CD56^{neg}CD16^{+} NK cells following *in vitro* stimulation with K562 target cells or IL-12 + IL-18. In healthy controls and HIV-1-infected subjects, CD7^{+}CD56^{neg} NK cells fail to produce IFNg, however they degranulate similar to CD7^{+}CD56^{+} NK cells. HIV-1 infection decreases IFNg production, but increases CD107a degranulation.

Figure 5 –Increased activation and CDK expression in CD7^{+}CD56^{neg}CD16^{+} NK Cells in chronically HIV-1-infected patients



A) CD95 expression, a marker of NK cell activation, on CD7^{+}CD56^{neg}CD16^{+} NK cells was compared to CD7^{+}CD56^{+}CD16^{+} NK cells in healthy (n = 11) and chronically HIV-1-infected subjects (n = 19). CD95 expression is higher on CD56^{neg}CD16^{+} NK cells and is increased in both subsets by HIV-1 infection. **B)** Expression of the cyclin-dependent kinase inhibitors p21/Waf1 (left half) and p16/INK4a (right half) in CD7^{+}CD56^{+}CD16^{+} and CD7^{+}CD56^{neg}CD16^{+} NK cells from chronic HIV-1-infected patients. P21/Waf1 and p16/INK4a are expressed higher on CD7^{+}CD56^{neg}CD16^{+} NK cells.

CONCLUSIONS

CD7^{+}CD56^{neg}CD16^{+} NK cells are expanded in HIV-1 infection. We found increased expression of cyclin-dependent kinase inhibitors in CD7^{+}CD56^{neg}CD16^{+} NK cells from HIV-1 chronic subjects possibly resulting from oxidative stress and chronic exposure to pro-inflammatory cytokines. Thus CD7^{+}CD56^{neg}CD16^{+} NK cells are activated, mature NK cells that are likely generated from chronic immune activation associated with persistent viremia. Treatment strategies that include anti-oxidants to reduce oxidative damage or cytokine therapies, such as IL-2, may allow NK cell recovery during anti-retroviral therapy.