The Frequency of NKG2C$^+$CD57$^+$ Adaptive NK Cells in HIV$^{+/-}$CMV$^+$ Subjects Declines with Age

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Background

90% of HIV+ persons in the Canadian HIV and Aging Cohort Study (CHACS) are cytomegalovirus (CMV) co-infected. CMV infection drives the expansion NKG2C$^+$CD57$^+$ adaptive-like Natural Killer (NK) (adapNK) cells to levels higher than that seen in CMV-mono-infected persons. However, these observations were made using samples from young individuals often aged <40 yrs. We questioned whether this was also the case for older HIV$^{+/-}$CMV$^+$ persons.

Hypotheses

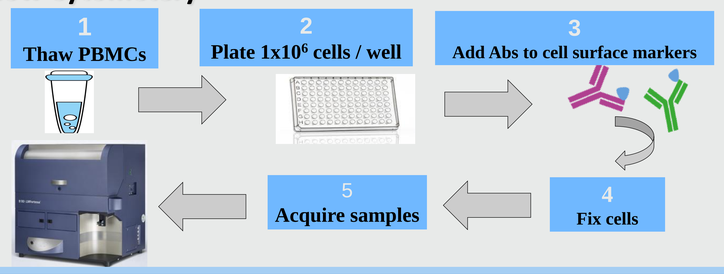
Does age impact adapNK cell frequency?

Objectives

To assess the frequency of NKG2C$^+$CD57$^+$ adapNK cells in HIV$^+$CMV$^+$ and HIV$^-$CMV$^+$ subjects above and below 40 years of age

Methodology

- **Flow Cytometery**



Results

- **Gating Strategy**

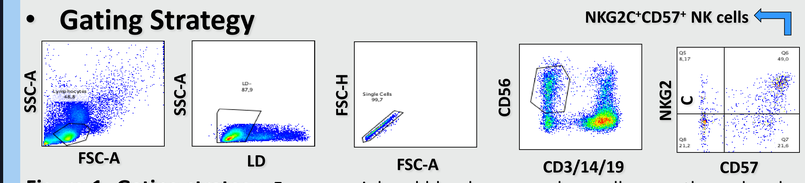


Figure 1. Gating strategy. Frozen peripheral blood mononuclear cells were thawed and stained with an antibody panel that included antibodies specific for CD3, CD56, CD57 and NKG2C. Using flow cytometry, CD3$^-$CD56$^+$ NK cells were gated on. From these, we determined the frequency of NKG2C$^+$CD57$^+$ adapNK cells .

Results

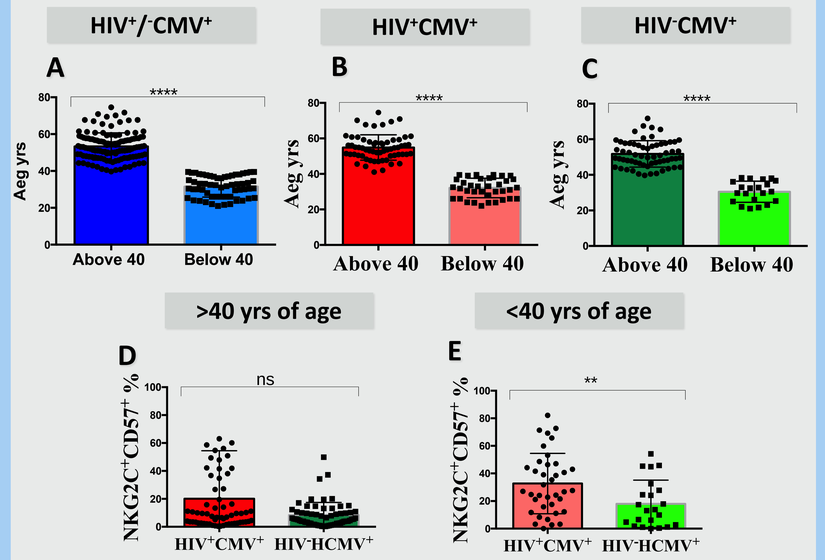


Figure 2. The frequency of NKG2C$^+$CD57$^+$ adapNK cells in HIV$^{+/-}$CMV$^+$ subjects. Panels A-C show the age distribution of the study population stratified by >40 versus <40 yrs of age for all (n=184) CMV$^+$ persons (A), n=100 CMV$^+$ PLWH (HIV$^+$CMV$^+$, B) and n=84 CMV mono-infected (HIV$^-$CMV$^+$, C) subjects. Frequency of NKG2C$^+$CD57$^+$ adapNK cells among HIV$^+$CMV$^+$ versus HIV$^-$CMV$^+$ subjects aged >40 (D) or <40 (E) yrs of age. Note that in those <40 yrs of age, HIV$^+$CMV$^+$ persons have a higher frequency of adapNK cells than HIV$^-$CMV$^+$ persons (D). While this is not the case for those >40 yrs of age (E). Bar graphs show medians and interquartile ranges (IQR). Mann-Whitney tests were used to assess the statistical significance of between-group differences for the frequency of NKG2C$^+$CD57$^+$ adapNK cells. "*" = p<0.05; "**" = p<0.01; "***" = p<0.001; "****" = p<0.0001.

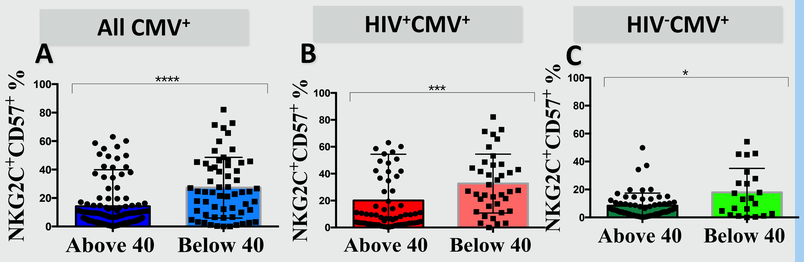


Figure 3. Comparison of the frequency of NKG2C$^+$CD57$^+$ NK cells from all CMV$^+$ subjects, HIV$^+$CMV$^+$ and HIV$^-$CMV$^+$ individuals aged >40 versus <40 yrs of age. Frequency of NKG2C$^+$CD57$^+$ cells in n=184 CMV$^+$ persons (A), n=100 HIV$^+$CMV$^+$ (B) and n=84 HIV$^-$CMV$^+$ (C) subjects >40 versus < 40 years of age. Note that the frequency of adapNK cells is significantly lower in both HIV$^+$CMV$^+$ and HIV$^-$CMV$^+$ persons aged >40 compared to those aged <40 yrs. Data bars graphs, statistical analyses and p-values designations as in Figure 2.

Results

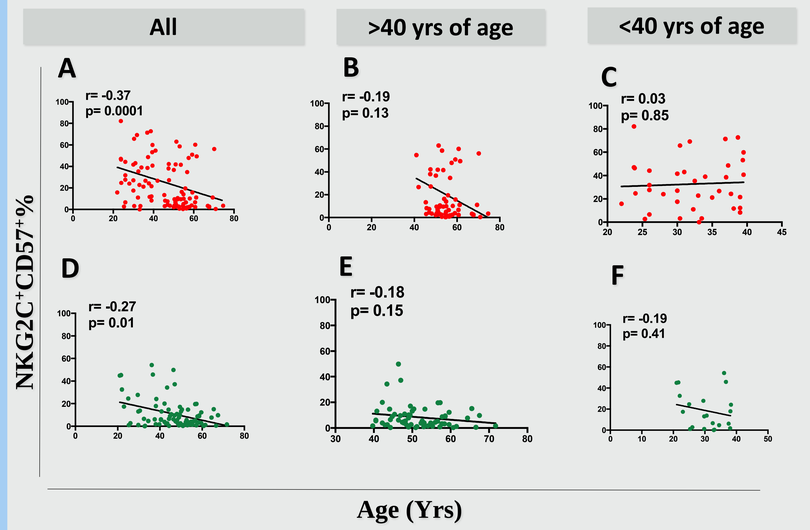


Figure 4. Analysis of the correlations between the frequency of NKG2C$^+$CD57$^+$ adapNK cells and age. Shown are the correlations between the frequency of NKG2C$^+$CD57$^+$ adapNK cells and age in HIV$^+$CMV$^+$ (A-C) and HIV$^-$CMV$^+$ (D-F) individuals. Panels A and D show these results for persons of all ages while Panels B and E show results for those >40 and Panels C and F for those <40 yrs of age. The frequency of adapNK cells declines with age in both HIV$^+$CMV$^+$ (A) and HIV$^-$CMV$^+$ (D) subjects. There is a steeper decline in adapNK cell frequency in those >40 versus <40 yrs of age as well as a steeper decline in HIV$^+$CMV$^+$ than in HIV$^-$CMV$^+$ persons aged >40 yrs.

Conclusion

- Many have reported that the frequency of adapNK cells in CMV$^+$ PLWH is significantly higher than in CMV mono-infected persons.
- We did not find such a difference in persons aged >40 yrs of age recruited to the CHACS.
- The frequency of adapNK cells was lower in both HIV$^+$CMV$^+$ and HIV$^-$CMV$^+$ people >40 than in those <40 yrs of age.
- The frequency of adapNK cells in both HIV$^+$CMV$^+$ and HIV$^-$CMV$^+$ people was negatively correlated with age.
- The slope of adapNK cells decline in HIV$^+$CMV$^+$ persons was steeper in in those >40 compared to those <40 yrs of age and also compared to HIV$^-$CMV$^+$ persons >40 yrs of age.
- The lack of significant differences in the frequency of adapNK cells in HIV$^+$CMV$^+$ compared to HIV$^-$CMV$^+$ CHACS participants aged >40 yrs is due to the decline in the frequency of these cells with age in HIV$^+$CMV$^+$ subjects.

Acknowledgements

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