221 - SYSTEMIC ACTIVATION OF NOVEL NATURAL KILLER-LIKE B CELLS IN HIV/SIV INFECTIONS

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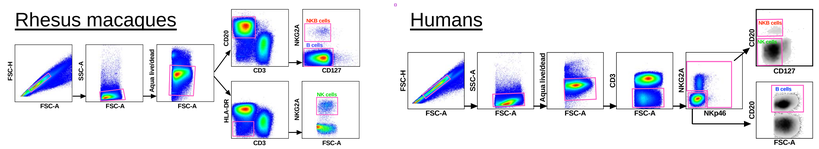
BACKGROUND

- Recently, multiple subpopulations of B cells (innate like B cells, ILB) have been shown to exhibit innate functions in addition to adaptive functions in mice. ILB have highly cross-reactive BCRs and/or TLRs that results in robust cytokine production and/or enhanced production of natural antibodies against virus and bacterial antigens.
- A novel subset of ILB, natural killer-like B (NKB) cells that share features of natural killer (NK) and B cells have been identified at early phases of microbial infection in mice to play critical roles in eradication of infection via cytokine secretion and lymphocyte priming.
- While B cell dysfunction has been reported in HIV/SIV infections, the role of NKB and other subsets of ILB have been largely unexplored.
- In order to help clarify the existence of the proposed NKB population, we investigated whether the putative phenotype exists in blood and tissues of humans and rhesus macaques, and if chronic HIV and SIV infection may perturb this unique cell niche.

METHODS

Using surface and intracellular markers, NKB cells from naïve and infected primates were phenotypically characterised using polychromatic flow cytometry

Flow cytometric gating strategy



Samples used-

- Rhesus macaques: blood, spleen, mesenteric lymph nodes (MLN) and colon of naïve and SIV infected macaques (post 140 days of infection)
- Humans: PBMC of naïve and ART naïve HIV infected samples; naïve spleen samples

SUMMARY & CONCLUSIONS

- Natural Killer–like B cells were identified both in rhesus macaques and humans
  - ✓ $CD3^{-}CD20^{+}CD127^{-}NKG2A^{+}$ in rhesus macaques
  - ✓ $CD3^{-}CD20^{+}CD127^{-}NKG2A^{+}NKp46^{+}IgA^{+}$ in humans
- Elevated NKB frequency in PBMC and colon of SIV infected rhesus macaques compared to naïve animals.
- Differential expression of NK and B cell markers on NKBs from SIV/HIV infected primates
  - ✓ Constitutive surface expression of IgA and IgM altered to elevated IgG expression in lentivirus infection

RESULTS

Phenotypic characterization of NKB in macaques

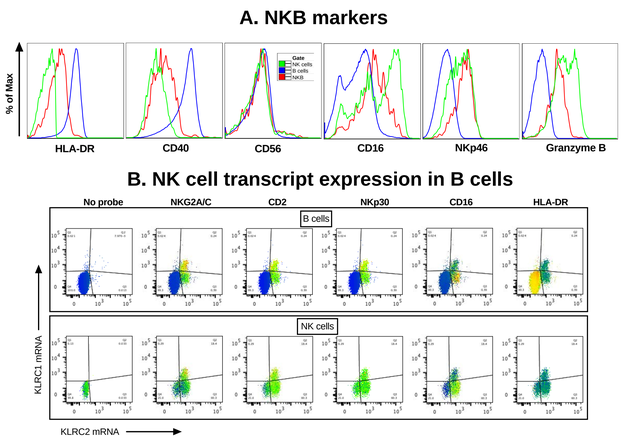


Fig 1. Phenotypic characterization of NKB cells in rhesus macaques. (A) Comparative expression of markers to delineate NKB cells from NK and B cell markers by histogram overlay analysis, (B) B cells and NK cells were gated for KLRC1 mRNA (NKG2A) and KLRC2 mRNA (NKG2C) and further characterized for the expression of NKG2A, CD2, NKp30, CD16 and HLA-DR in splenic mononuclear cells by flow cytometry.

Altered NKB frequency and phenotype in SIV infection

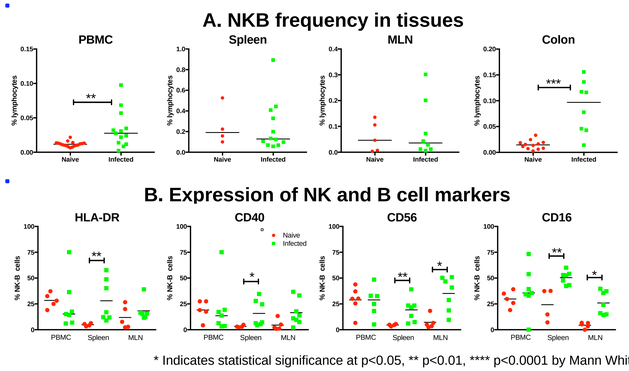
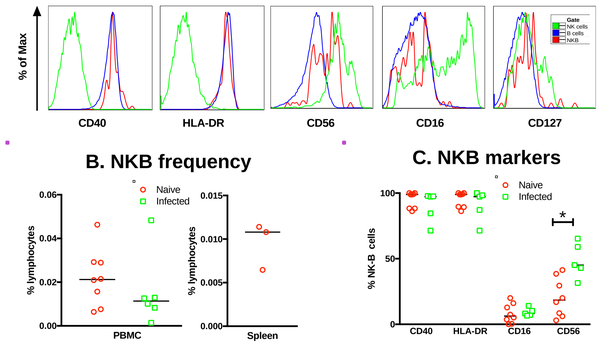


Fig 2. Elevated activated NKB cell frequencies in SIV-infected macaques. (A) Percentages of NKB cells in PBMC, spleen, colon and mesenteric lymph nodes (MLN) and (B) Expression of NK and B cell specific markers namely HLA-DR, CD40, CD56 and CD16 are shown for naïve and SIV-infected macaques.

* Indicates statistical significance at p<0.05, ** p<0.01, **** p<0.0001 by Mann Whitney U test

Altered NKB phenotype in HIV infection



* Indicates statistical significance at p<0.05 by Mann Whitney U test

Fig 3. NKB phenotype in humans. (A) Comparative expression of markers to delineate NKB from NK and B cell markers by histogram overlay analysis cells in human PBMC, (B) percentages of NKB cells in human PBMC and spleen and (C) median expression of NK and B cell specific markers CD40, HLA-DR, CD56 and CD16 in naïve and HIV infected PBMC samples.

Modulated Ig expression on NKB cells in infection

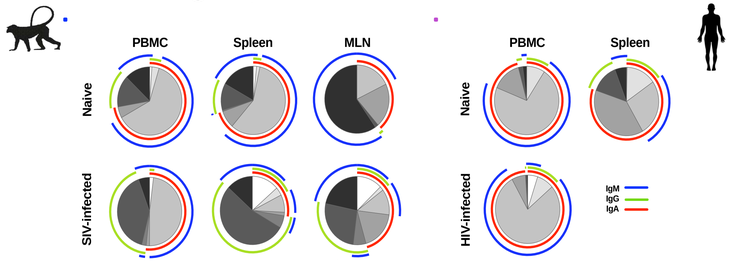


Fig 4. Immunoglobulin expression on NKB cells in rhesus macaques and humans. Distribution frequencies of IgA, IgG, and IgM calculated using Boolean gates of Ig positive samples averaged over 4 to 18 animals/human subjects per group, depending on tissue, using SPICE (v.5.3) Shaded pie slices indicate individual populations and pie arcs show overlapping expression of Ig molecules.