NK CELLS ARE CRITICAL FOR IN VIVO CONTROL OF SARS-COV-2 REPLICATION AND DISEASE

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Harikrishnan Balachandran[1], Rhianna Jones[1], Kyle Kroll[1], Cordelia Manickam[1], Brady Hueber[1], Matthew Mosher[1], Andrew Hudson[1], Karen Terry[1], Amanda Martinot[2], Griffin Woolley[1], R. Keith Reeves[1]

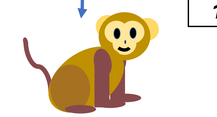
[1] Duke University School of Medicine, Durham, NC, USA , [2] Tufts University, Boston, MA, USA

BACKGROUND

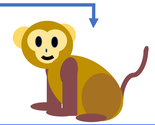
Natural killer (NK) cells play a critical role in the control of viral infections. In this study, we coupled a non-human primate (NHP) infection model with a validated NK cell depletion technique using anti-Interleukin15 (IL15) administration to investigate the role of NK cells in the pathogenesis and control of SARS-CoV-2 infection and evaluate if NK cell accumulation is beneficial or deleterious.

METHODS

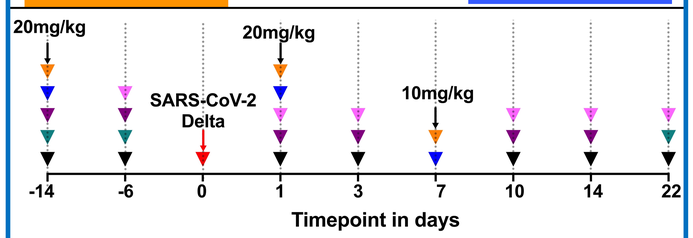
***Macaca fascicularis* (CM)**
12 NHPs – 75% female



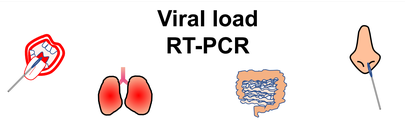
NK cell depleted n=6



Control n=6



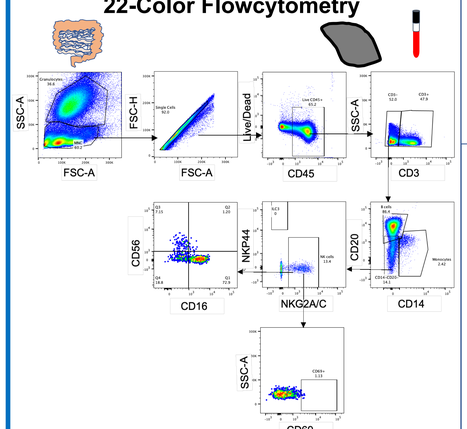
Viral load RT-PCR



Lung scores Pathology



NK cell frequencies 22-Color Flowcytometry



Cytokine analysis 37-Plex Luminex



Legend

- Colorectal
- Lymph node
- Blood
- Lungs
- Throat swab
- Nasal swab

- **Peak viral load in NHPs observed 1 - 3 days post SARS-CoV-2 infection**
- **Absence of NK cells causes higher viremia and delayed viral clearance**
- **Peak NK cell activity observed at 10 days post infection**
- **Increased expression of CD69 – an activation and tissue retention marker**
- **Cytotoxic NK cells positively associate with viral load**
- **Anti-IL15 administration leads to successful systemic and tissue level NK cell depletion**
- **NK cell depletion leads to increased inflammatory cytokine levels and tissue damage in lungs**

RESULTS

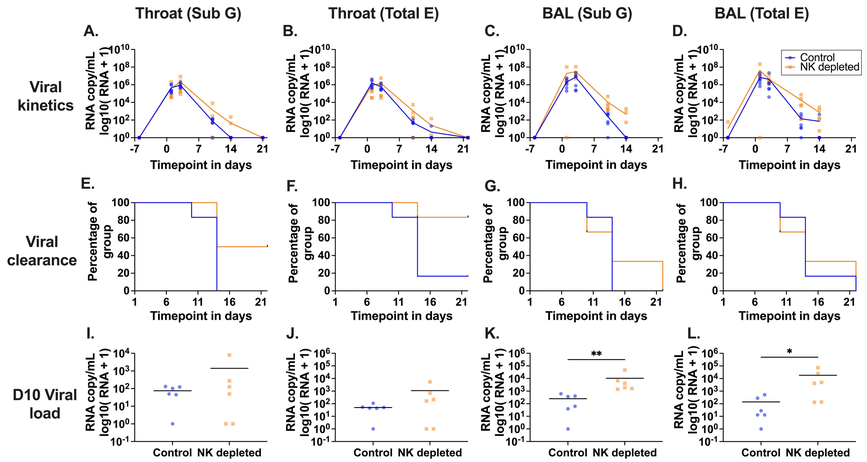


Figure 1. Viral load analysis. (A) – (D) Viral kinetics in Throat (Sub G), Throat (Total E), BAL (Sub G) and BAL (Total E). **(E) – (H)** Viral clearance in Throat (Sub G) , Throat (Total E), BAL (Sub G) and BAL (Total E). **(I) – (L)** Day 10 viral load in Throat (Sub G) , Throat (Total E), BAL (Sub G) and BAL. **Blue** – control group and **orange** – NK depleted, and black line indicates the mean. Mann-Whitney test * *p*value <0.05, ** *p*value<0.01 to 0.001.

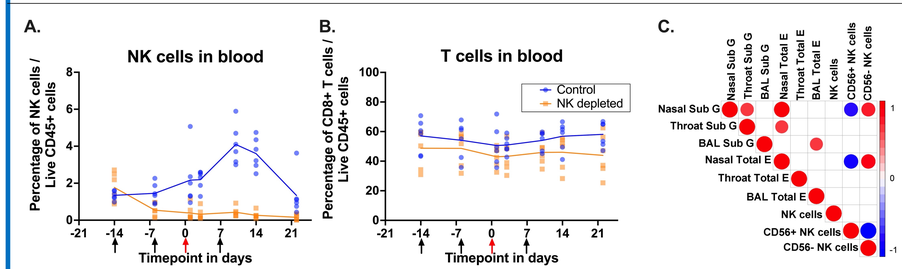


Figure 2. Cellular dynamics following NK cell depletion in acute SARS-CoV-2 infection in CM. (A) & (B) Longitudinal comparison of NK cell and T cell frequencies in CM PBMC from **NK depleted** and **control** groups. **(C)** Correlogram indicating the correlations between viral loads and NK cell frequencies measured on days 1 & 3 in **control** group. Pearson's correlation used.

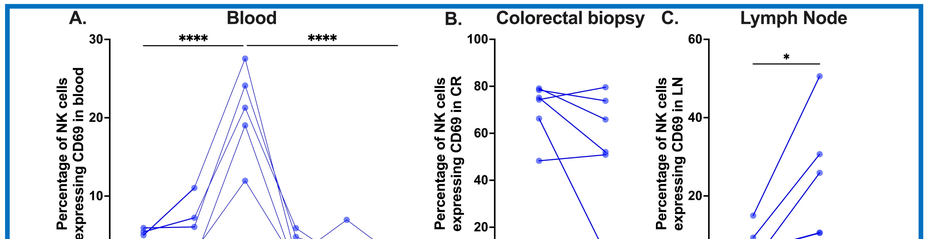


Figure 3. Longitudinal expression of activation and trafficking marker CD69 on NK cells in control group (A) Blood **(B)** Colorectal biopsy **(C)** Lymph Node. 2-way ANOVA; Wilcoxon matched-pairs signed rank test * *p*value<0.05,**** *p*value<0.0001.

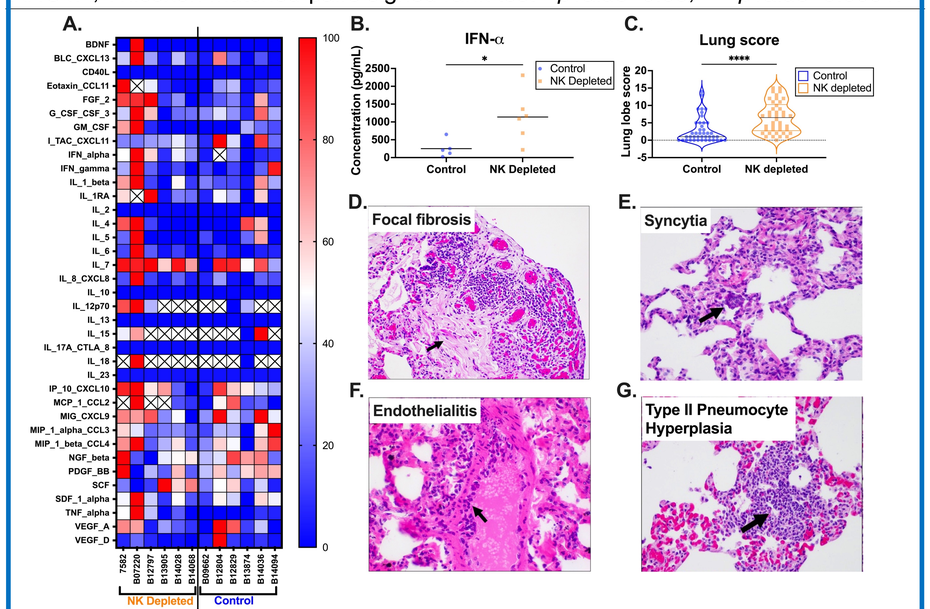


Figure 4. Lung cytokines and pathology. (A) Heatmap of cytokines analyzed in BAL at day 3 post-infection. 'X' indicates either over or under the detection limit. **(B)** Concentration of IFN-α in BAL at day 3 **(C)** Lung lobe score. **Blue** – control group and **orange** – NK depleted, black line indicates the mean and the lines in self color indicate quartiles. **(D)** – **(G)** H & E Staining on SARS-CoV-2 infected lungs of the NK depleted group. Arrows indicate focal fibrosis, syncytia, endothelialitis, and type II pneumocyte hyperplasia, respectively. Mann-Whitney test * *p*value <0.05, **** *p*value <0.0001.

CONCLUSIONS

- **NK cells are critical for reduced viral load and decreased duration of SARS-CoV-2 viremia.**
- **Lack of NK cells increase inflammation and may lead to lung damage.**

ADDITIONAL KEY INFORMATION

Author Contact Information
keith.reeves@duke.edu

Division of Innate & Comparative Immunology
NIH National Institute of Diabetes and Digestive and Kidney Diseases
R01DK130472
Duke Center for Human Systems Immunology
Duke University School of Medicine