Recombinant RRV Vaccine Vectors Replicate in Lymph Nodes of Rhesus Macaques and Elicit Low Levels of CXCR5+ SIV-Specific CD8+ T-Cells

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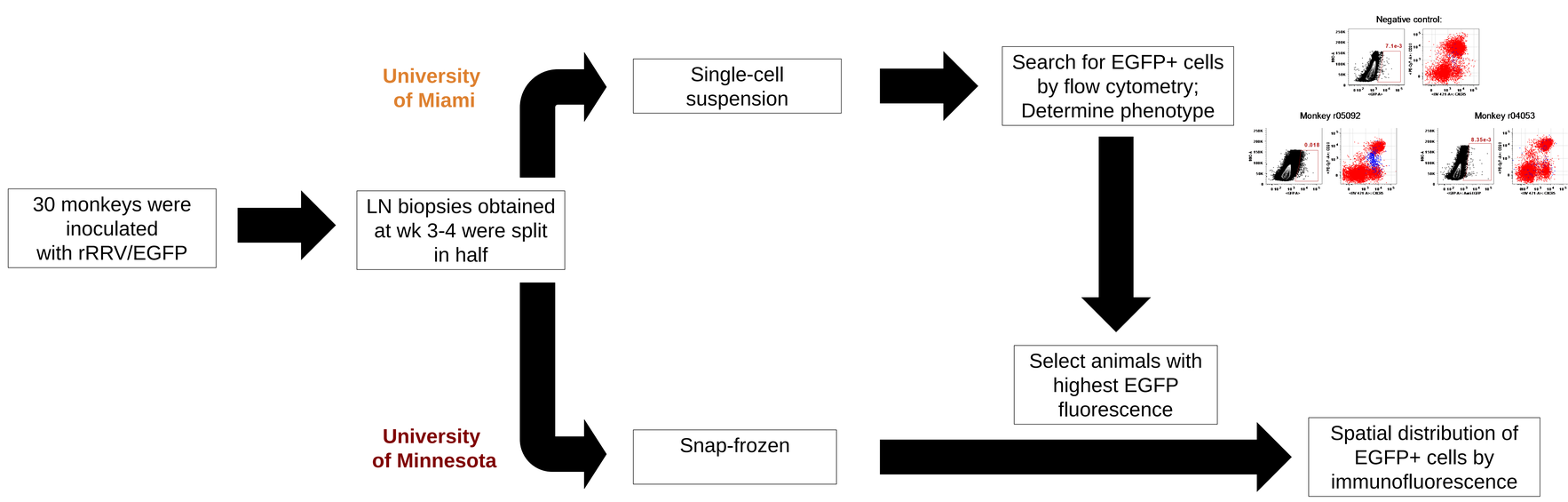
Abstract

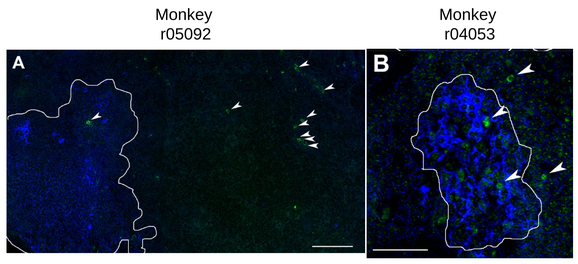
B-cell follicles in secondary lymphoid tissues are crucial sites for HIV/SIV replication as they are densely populated with follicular T helper cells–a major compartment for virus production and persistence. Notably, B-cell follicles have been considered immune-privileged sites since they harbor high numbers of virus-producing cells during HIV/SIV infection and yet CD8+ T-cells are largely excluded from these areas. Given the rapidity with which SIV reaches lymph nodes (LN) following transmission, vaccines that target effective CD8+ T-cells to follicular areas could improve control of AIDS virus replication. Vaccine approaches based on live recombinant (r) herpesvirus-based vaccine vectors have received considerable attention lately due to their ability to elicit effector memory CD8+ T-cell responses and afford significant control of viral replication after pathogenic SIV challenge. One of such vectors is rhesus monkey rhadinovirus (RRV), a γ2-herpesvirus that is closely related to the Kaposi's sarcoma-associated herpesvirus (KSHV). Notably, KSHV and other members of the *γ-herpesvirinae* subfamily can establish persistent infection in B-cell follicles. Based on the similarities between these viruses and RRV, we hypothesized that rRRV/SIV vectors can also replicate in B-cell follicles from rhesus monkeys and attract vaccine-induced SIV-specific CD8+ T-cells to these sites. To address this possibility, we utilized LN biopsies from rhesus macaques that were inoculated with rRRV constructs expressing either enhanced green fluorescent protein (EGFP) or SIV inserts. In collaboration with Dr. Pam Skinner at the University of Minnesota, we determined the spatial distribution of EGFP+ cells in LN sections. We also investigated if SIV-specific CD8+ T-cells elicited by rRRV/SIV vaccination express the B-cell follicle homing receptor CXCR5.

Spatial Distribution and Phenotype of rRRV-Infected Cells in LNs of rRRV-Vaccinated Monkeys



Results:

- EGFP fluorescence was dim and often indistinguishable from negative controls.
- When detectable, EGFP+ cells displayed a CD20^{int}CXCR5^{int} phenotype.



Results:

- Low numbers of EGFP+ cells (green; indicated by white arrows) found in LN sections from rRRV/EGFP-immunized animals.
- When detected, most EGFP+ cells were found outside B-cell follicles (blue).

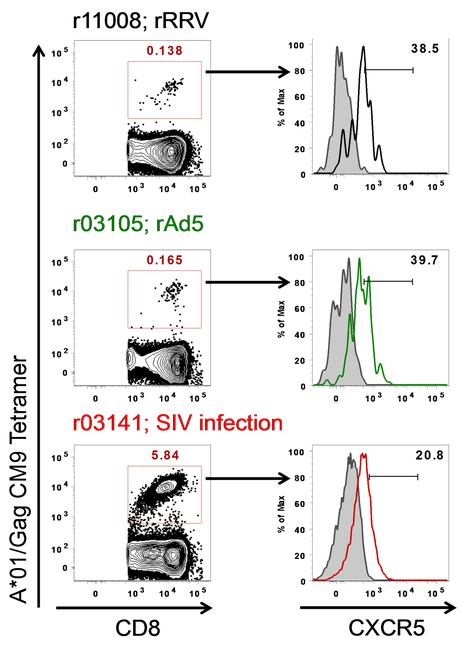
Analysis of SIV Gag CM9-Specific CD8+ T-Cells In LNs from *Mamu-A*01*+ Monkeys Using Fluorochrome-Labeled Tetramers

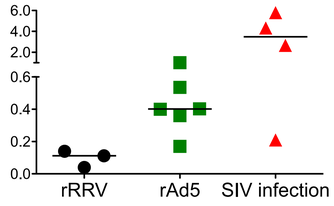
Sample Characteristics
- 3 monkeys vaccinated with rRRV/*gag*
- 6 monkeys primed or boosted with rAd5/*gag*
- 4 monkeys with uncontrolled SIV infection

LN biopsies obtained at wk 3-4 were split in half

Single-cell suspension

Goals:
- Determine % of tetramer+ CD8+ T-cells
- Measure CXCR5 expression in tetramer+ CD8+ T-cells





ositive for CXCR5

CXCR5 Levels on SIV-specific CD8+ T-Cells Induced by rRRV Were Not Significantly Different than those Elicited by rAd5 Vaccination or SIV Infection

Conclusions

- EGFP+ cells were rarely detected in LNs from rRRV/EGFP-inoculated animals
  - Pre-existing immunity to RRV may have reduced take of rRRV/EGFP
  - Most EGFP+ cells localized to extra-follicular regions and displayed a CD20^{int}CXCR5^{int} phenotype
- Low levels of Gag CM9-specific CD8+ T-cells in LNs from rRRV/Gag vaccinees, likely due to poor immunogenicity of rRRV/Gag vector
- *Can CD8+ T-Cells Induced by rRRV/SIV Access B-Cell Follicles?*
  - Flow cytometric analysis indicated that a few CD8+ T-cells were CXCR5+ and therefore may be able to enter follicles
  - Need IST staining to confirm if CXCR5 expression predicts anatomic distribution of tetramer+ CD8+ T-cells (pending)
  - SIV-specific CD8+ T-cells induced by rRRV appear to be similar to those elicited by rAd5 or primary SIV infection in their ability to access B-cell follicles

Acknowledgments

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