Fresh Tissue Migration Assay of Rhesus Macaque Tissue Incubated with CFSE Labeled PBMCs and Lymph Node Cells

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Introduction

During infection, HIV and SIV producing cells are highly concentrated inside B cell follicles in lymph nodes[2, 4], while virus specific cytotoxic T cells (CTL) are responsible for clearing viral infections are unable to enter B cell follicles in infected individuals[1]. One method of removing the virus from the infected B cell follicles would be to transduce SIV-specific CTL with the B cell follicle homing protein CXCR5, and introduce those cells to SIV infected tissue and observe whether or not the CTL are able to enter the B cell follicles and lead to decreased viral loads within that tissue[3]. In order to check whether or not the CXCR5 transduced CTL can migrate into lymph node tissues, a fresh tissue migration assay (FTMA) procedure was created. The goal of the FTMA was to observe if peripheral blood mononuclear cells (PBMCs) and disaggregated lymph node cells migrate into specific zones when introduced into new lymph node tissue. We hypothesized that the PBMCs would move into T-cell zones and that lymph node cells would move into B-cell follicles within the lymph node tissue. The experiment was performed using a rhesus macaque model. Some of the PBMCs express CXCR5 whereas many lymph node cells express CXCR5. Based on this we hypothesize that if our migration assay was successful, greater numbers lymph node cells compared to PBMCs would migrate into B cell follicles.

Methods

For this project, images were taken by Justin Jacques, and the tissues were prepared and stained by Preethi Haran.

Images of stained tissue were taken using a FluoView FV1000 confocal microscope, with images captured using the proprietary FluoView software.

Tissues were stored at -20°C. The tissues were viewed using a trans-illumination lamp to make a map of the tissue at 20x magnification, then observed under a 20x oil immersion lens to take pictures of the tissue sections. Tissue images were taken at 3um steps in order to properly visualize the cells in the tissue, in 10 step montages and projection images.

Results

Figure 1 is a projection image of the rhesus macaque tissue after incubation with green CFSE labeled PMBC's after three hours. The blue cells in the image were CD3+ T cells stained with Cy3, and the red cells are CD20+ B cells stained with Cy5.

Figure 2 is a projection image of the rhesus macaque tissue after incubation with green CFSE labeled lymph node cells after three hours. The blue cells in the image were CD3+ T cells stained with Cy3, and the red cells are CD20+ B cells stained with Cy5.

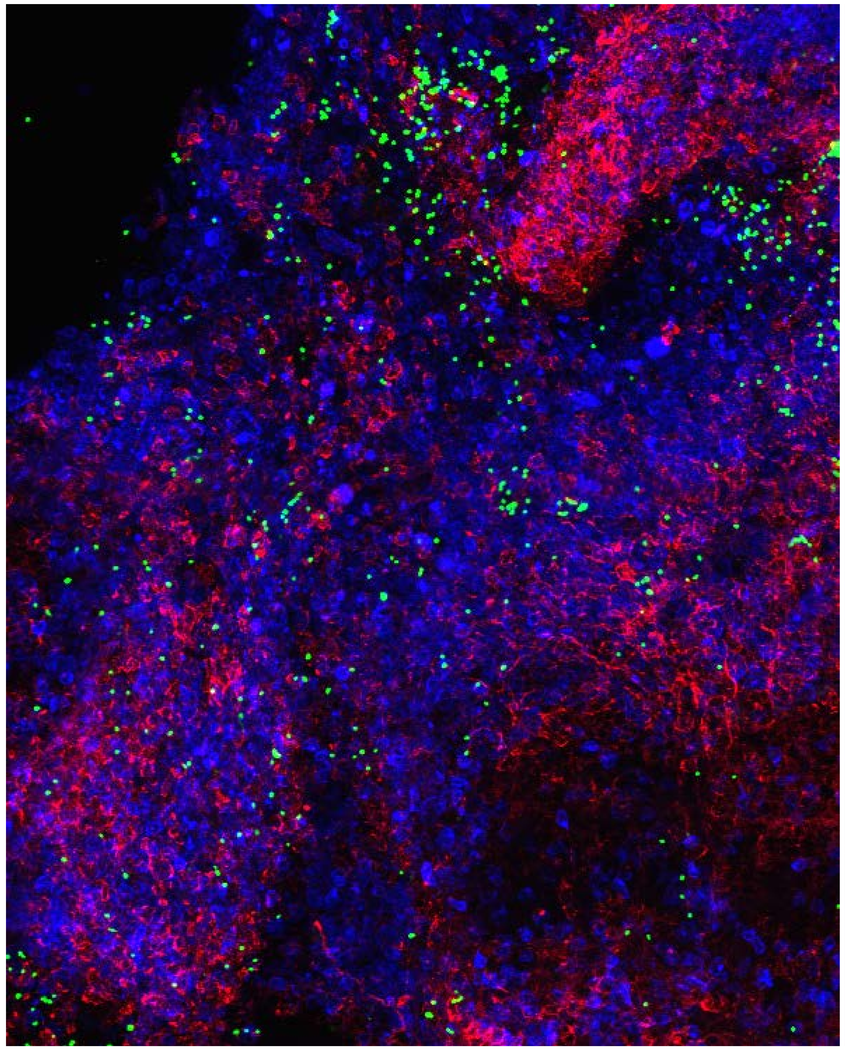

Figure 1: Projection image of RhMacBG-85 Lymph Node tissue incubated with CFSE labeled PBMCs for 3 hours taken under oil immersion confocal microscopy at 20x magnification.

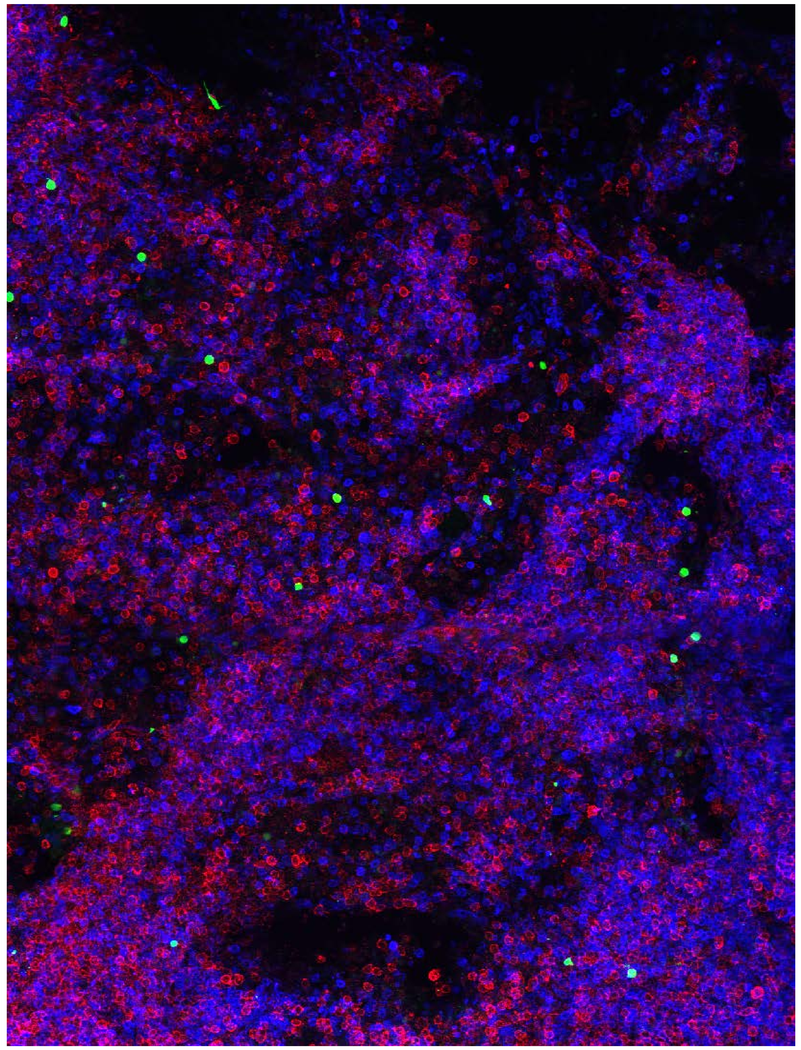

Figure 2: Projection image of RhMacBG-85 Lymph Node tissue incubated with CFSE labeled lymph node cells for 3 hours taken under oil immersion confocal microscopy at 20x magnification.

Conclusions

•Both CFSE labeled PBMCs and lymph node cells were able to migrate into lymph node tissue.

•Based on the location of CFSE labeled PBMCs and CFSE labeled lymph node cells, it appears that PBMCs are not localized to a specific area within the tissue itself.

•Low abundance of PBMCs and lymph node cells in tissue suggest that the migration assay was not very effective.

References

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