Abstract & Introduction

CD8+ lymphocytes are a critical part of cell mediated immune response, controlling viral infections and other intracellular pathogens. Currently, there is an insufficient understanding of how this immunity takes place in three-dimensional space. A better understanding for distribution of CD8+ T cells in mucous tissues is an important part of improving both the treatment and prevention of pathogens.

Unfortunately, there are few accurate three dimensional depictions of full organs, particularly the female reproductive tract (FRT), which is an important portal for sexually transmitted diseases. Typically tissue samples scatter light and make 3D imaging of whole organs difficult or impossible, however, various tissue-clearing techniques can facilitate 3D imaging of a tissue while preserving overall cell structure and organization.

Using LCMV infection of mice as a model we have been able to characterize the CD8+ T-cell distribution in the FRT, and within lymphocyte aggregates. These lymphocyte aggregates are an important, but still poorly defined dynamic structure in the context of the immune response, wherein dendritic cell present antigens to CD8+ lymphocytes. However, there is still much to be learned about the organization, interactions, and volume occupied by these aggregates.

In order to better understand the composition and structure of the aggregates we have 3D imaged whole murine FRT imaging with Thiodiethanol (TDE) clearance and two-photon microscopy. Using 3D imaging software we have calculated the volume occupied by these lymphocyte aggregates during effector CD8+ T-cell response and during the memory phase of the response 30 days after infection.

Procedure:

A transgenic CD8+ T-cell population specific for lymphocytic choriomeningitis virus LCMV (P14) was isolated from transgenic mice with Ub-R-GFP tags, allowing the visualization of CD8+ cells. 50,000 CD8+ cells were transferred I.V. into a CD11c-YFP expressing host. The CD11c-YFP mice were then infected with 1x10^5 PFU of LCMV and sacrificed at 7 and 30 days.

The female reproductive tract was harvested, and cleared with passive clarity technique (PACT), 3DISCO, and TDE. However, the simple TDE preparation allowed for sufficient penetration (650 micron) with 2-photon microscopy, and little structural change to the native organization of the organ.

Results and Conclusion

The volumes of lymphocytes within aggregates during the effector stage of infection was 1.467% +/-0.578% of the total volume of the FRT, lymphocytes occupied 12.354% +/- 2.991% of the FRT, notably, the aggregates represented a sizable portion of the total lymphocytes in the FRT (28.364% +/-4.630%).

These preliminary effector stage data demonstrate the substantial role that these aggregates play in the context of LCMV infection. Upon analysis of the memory stage data we will have a superior understanding of the presence and importance of these aggregates over the effector and the memory stages of infection.

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