

Dynamic *in vivo* high resolution imaging of immune cell behavior

Ronald N. Germain^{1*}, Alex Y. Huang¹, Hai Qi¹, Marcello Chieppa¹, Maria Rescigno⁴, Jackson Egen¹, Flora Castellino¹, Ina Ifrim¹, Clemens Scheinecker¹, Gregoire Altan-Bonnet¹, Anthony Rothfuchs², Alan Sher², Marc Bajénoff², Nicholas Glaichenhaus³, Katrin Eichelberg¹, Ze Wang¹ and Sabine Stoll¹

¹Laboratory of Immunology, NIAID, NIH, Bethesda, MD, USA

²Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD, USA

³INSERM, Université de Nice-Sophia Antipolis, Valbonne, France

⁴Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

*Presenting author

Abstract

Immune responses involve multiple cell-cell interactions, both among hematopoietic cells and between these cells and those of parenchymal tissues including tumors. We have used explant and intravital confocal or multiphoton microscopy to collect 4D (XYZ and time) data on the interactions of antigen (Ag)-specific T cells with Ag-bearing dendritic cells (DCs) in lymph nodes (LNs), on myeloid cell behavior during immune-mediated liver inflammation or granuloma formation, on NK cells in LNs, and on bacterial interaction with DCs in the gut. Some naive T cells move rapidly in the absence of Ag but show prolonged adherence to Ag-bearing DCs, accompanied by immunological synapse formation. Rapid movement of DC dendrites is readily visualized, as is T-DC contact through these processes, followed by movement of the T cell towards the DC body. Activation and detachment from the antigen-bearing DC follows, along with cell division. These data suggest that T cell activation follows from prolonged lymphocyte association with individual antigen-bearing DCs rather than summation of signals from brief encounters with such presenting cells. CD4 and CD8 T cells associate with a single DC when both antigens are present and very recent data has uncovered a cascade of inflammatory chemokines and cytokines that orchestrate the directed movement of naive CD8 T cells to sites of CD4 T cell-DC interaction for the delivery of "help". Quantitative analysis suggests an impact of self-MHC recognition on the time of naive T cell-DC interaction.

Intravital methods have permitted visualization of DC migration into LNs and the egress of lymphocytes from HEV for initial contact with DCs. Stable association of non-motile NK cells with DCs in LNs and differential migratory behavior of T lymphocytes and DCs in distinct regions of the lymph node has been documented. Fluorescent reporter constructs are revealing the consequences of T-DC interactions in real time within LNs (for example, the synthesis of cytokines). Differential migratory behavior of lymphocytes and DCs in distinct regions of the lymph node has been observed, as has the failure of rapidly moving T and B lymphocytes to cross rather strict borders between the T cell zone and B cell follicle. The accumulation of myeloid cells at locations of apoptotic hepatocytes during liver inflammation or in liver granulomas has been visualized, as has DC extension through small bowel epithelium for sampling of luminal contents. These studies are contributing to a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell interaction and signaling events in host immune

responses. Future work will involve visualization of the entry of effector cells into tumor sites, the interaction of these effectors with tumor cells and other cells such as macrophages and DCs within the tumor bed, the spatial relationship between Tregs and Tefs in LNs and in peripheral sites, and the functional consequences of this co-localization in terms of proliferation and effector cytokine production.