

## **Imaging T cell interactions with dendritic cells and B cells in the lymph node**

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Cellular interactions initiate the immune response and determine its outcome. We developed two preparations for immuno-imaging: lymphoid organs maintained in tissue culture; and intravital imaging of the inguinal node in an anesthetized mouse (1-3). Two photon microscopy has permitted us to visualize the following dynamic **processes** that initiate and sustain an adaptive immune response. Following the **entry** of lymphocytes into the node by homing from high endothelial venules (HEV), the “default” basal motility of naïve T and B cells consists of an amoeboid random walk within the diffuse cortex and follicle of a lymph node, respectively (1, 3). Antigen **scanning** takes place by random encounters between motile naïve T cells and actively probing dendritic cells (4). The **activation** choreography of T cells and dendritic cells during a specific antigen response evolves in stages from transient interactions to formation of stable clusters, followed by swarming and **T cell proliferation** (5). **Chemotaxis** positions antigen-engaged B cells at the follicle edge where they encounter and form stable conjugate pairs with CD4<sup>+</sup> T cells (6). Such conjugate pairs are highly motile, B cells leading the way as T cells provide **help** for antibody production at the start of the humoral immune response. T and B cells leave the lymph node to recirculate throughout the body by migrating across the endothelium that lines the lymphatic sinus. We recently investigated the role of S1P<sub>1</sub> receptors during lymphocyte **egress** from the lymph node by imaging the movement of lymphocytes into medullary sinuses while applying specific S1P<sub>1</sub> receptor agonist and antagonist compounds (7). Migration of T cells across the lymphatic endothelium and basal motility in the medulla were both inhibited by a specific S1P<sub>1</sub> receptor agonist; and this inhibition was reversed acutely by removal of the agonist or by addition of an antagonist in the maintained presence of the agonist. Our results are in accord with a barrier model, whereby agonist activity of S1P<sub>1</sub> receptors on the lymphatic endothelial cells is required for lymphocyte sequestration.

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