

## Dynamic intravital imaging of immune cell migration, interaction, and effector function

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Immune responses involve multiple cell-cell interactions within lymphoid tissues, the trafficking of activated cells to sites of effector function, and the migration of such effector cells within peripheral tissues. To gain a more detailed appreciation of the dynamics of such cell behavior, we have used intravital multiphoton microscopy to collect 4D (XYZ and time) data on the interactions of antigen (Ag)-specific T and B cells with each other and with Ag-bearing dendritic cells (DC) in lymph nodes (LN). Our data show that T and B cells follow stromal pathways during their migration in LNs. In the fibroblastic reticular cell (FRC)-defined T zone, this constrained trafficking enhances interactions with dendritic cells that are also attached to the same FRC stromal network. Additional guidance cues also facilitate interactions among rare antigen-presenting and antigen-recognizing cells. For example, antigen-naïve CD8 T cells are actively attracted to DCs in lymph nodes that present antigen to CD4 T cells. These CD4-CD8-DC interactions result from a cascade of inflammatory signals and are critical to efficient development of CD8 T cell memory responses. These data have begun to build up a ‘fractal’ picture of how interactions among rare cells occur with the necessary pace and efficiency to support productive adaptive immune responses. New evidence suggests that adhesive interactions regulating the duration of cell-cell association once the proper cells locate each other are critical to effective delivery of the molecular signals required for adaptive immune response development. In particular, the lack of expression of the small adapter SAP in T cells has been shown to lead to a defect in humoral immunity and in the development of germinal centers. Our recent studies reveal that the primary effect of this genetic deficiency (equivalent to the human immunodeficiency XLP or X-linked lymphoproliferative disease) is to prevent stable adhesion between antigen-specific T and antigen-bearing B cells, but not between T cells and antigen-presenting dendritic cells. The lack of adhesion interferes with effective delivery of ‘help’ to the B cells in both the early interfollicular and late germinal center phases of the B cell response. Together, these observations show the power of in situ imaging in the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses.

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