

# Analysis of T-cell Proliferation in Lymph Nodes of Lymphopenic Hosts Using 2-Photon Microscopy

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Lymphopenia, a condition in which the number of lymphocytes falls below normal levels, makes patients more susceptible to infection and disease, and can be caused by genetic predisposition or therapies such as chemotherapy for cancer. A lymphopenic immune system can be reconstituted by injecting lymphocytes that will proliferate in the host through a process known as 'homeostatic proliferation'. It is hypothesized that in the lymph node, homeostatic proliferation occurs as a result of T-cell interaction with dendritic cells (DCs), a key cell type to regulate T-cell activation. With the use of 2-photon laser-scanning microscopy, high-resolution intravital lymph node imaging was performed to gain insights into the dynamic interactions between T-cells and DCs during homeostatic proliferation within intact lymph node tissue of live animals. In addition, a mouse (CD11c-mCherry) expressing red fluorescence protein (RFP) in DCs was also used to visualize DCs by 2-photon microscopy. A lymphopenic CD11c-mCherry mouse was injected with  $1 \times 10^7$  green fluorescently labeled naïve CD4<sup>+</sup> T-cells, and the popliteal lymph node was imaged up to 60 minutes. The computer software Imaris was used to analyze the images, followed by Excel for statistical analysis. The speed of T-cell movement, frequency and duration of T cell-DC contacts were calculated. On average, T-cells contacted DCs for  $8.8 \pm 2.3$  minutes in control wild type animals compared to  $26.9 \pm 2.3$  minutes in lymphopenic animals. Taken together, the data supports the hypothesis that T cell-DC interaction is significantly prolonged under lymphopenic conditions, possibly leading to homeostatic proliferation. Further examination will define DC interactions with other T-cell subtypes within the lymphoid tissues.