Visualizing inflammatory chemokine-guided recruitment of naive CD8+ T cells to sites of antigen-dependent CD4+ T cell-dendritic cell interaction *in situ*

Alex Y. Huang[*], Flora Castellino, Grégoire Altan-Bonnet, Sabine Stoll, Clemens Scheinecker and Ronald N. Germain

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
[*]*Presenting author*

Abstract

CD8+ T cells play a crucial role in host resistance to many infectious agents and can contribute to the eradication of malignant cells. The immune function of this lymphocyte subset is not autonomous but dependent on antigen-specific CD4+ T cells chronic and memory CD8+ T cell responses. Co-operation between CD4+ and CD8+ T cells involves their recognition of antigens co-presented on the same cell membrane, but the frequencies of relevant T cells in the normal repertoire and of such antigen-bearing cells early in an infection are both quite low. Although this would suggest that some active mechanism facilitates the necessary cell-cell interactions so that they occur with sufficient efficiency to support a useful response, recent *in situ* imaging studies have concluded that T cell-dendritic cell (DC) interactions in lymph nodes (LNs) are random in nature. We have re-examined this issue using methods that include intravital multiphoton imaging, which allows tracking of single cell behavior over a prolonged time period in live anesthetized animals. Mice immunized with antigen-pulsed DCs were subsequently injected with T cells specific for ovalbumin peptides (CD8+ OT-I and CD4+ OT-II). In the presence of OT-II T cells, OT-I and polyclonal CD8+ T cells aggregated around OT-II-specific peptide-pulsed DCs and showed a 3-5 fold increase in contact frequency compared to interactions with unpulsed DCs (3.5 + 0.11 ($P < 0.001$) and 4.18 + 0.13 ($P < 0.001$), respectively). Investigations into the mechanism underlying this phenomenon reveal that prior to antigen recognition, naive CD8+ T cells upregulate CC-receptor 5 (CCR5) in an inflammatory environment, allowing them to be efficiently attracted under the guidance of the chemokines CCL3 and CCL4 (MIP-1α and β) directly to sites of antigen-specific interaction between CD4+ T cells and DC. Interference with this recruitment eliminates the contribution of CD4+ T cell help to functional CD8+ T cell memory in a vaccine model. These data provide evidence that an orchestrated series of differentiation events drives non-random cell-cell interactions within LNs, optimizing the development of antigen-specific CD8+ T cell immune responses from the few antigen-specific precursors present in the naive repertoire.