

**“Imaging of Specific MHC/Peptide Complexes and Other Molecules
at the Immunological Synapse”**

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Using antigen presenting cells (APC) expressing MHC class II molecules fused to antigenic peptide and green fluorescent protein, we can visualize the distribution of peptide-loaded MHC molecules during antigen recognition by CD4 T cells by video microscopy of living cells. Antigen recognition occurs within seconds of contact with the APC, as shown by a sharp increase in intracellular calcium in the T cell, following which the T cell spreads out on the APC surface. Over several minutes, MHC molecules accumulate in the contact zone to form an immunological synapse. Accumulation of MHC molecules in the synapse is peptide-specific under our conditions, and depends in part on costimulation by B7 and ICAM-1. Cells stop crawling during synapse formation, but occasionally start moving again. When they do, the accumulated MHC molecules in the synapse move with them over the APC surface. When T cells detach spontaneously from the APC, they capture MHC/peptide complexes directly from the synapse. The captured MHC/peptide complexes are retained for a time as a compact cluster on the T cell surface. Anergic T cells also form synapses, but different cytoplasmic molecules accumulate at synapses formed by anergic cells. In particular, c-Cbl, a negative regulator of T cell antigen receptor signaling, accumulates at anergic synapses but not those formed by responsive T cells. (Supported by NIH grants AI29544 and AI50823 and a grant from the Oregon Health Sciences Foundation. S.A.W. was supported by training grant EYO7123 from the National Eye Institute.)

Reference from my laboratory:

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