PERSPECTIVES: IMMUNOLOGY

Dancing the Immunological Two-Step

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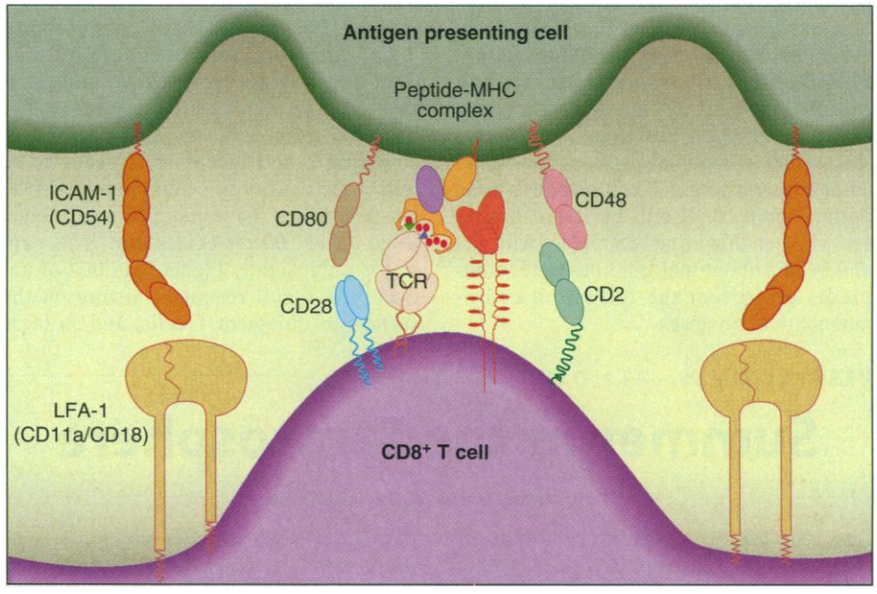
The activation of T cells depends on the interaction of T cell receptors with peptides (the products of viral or bacterial degradation) that are displayed on the surface of antigen presenting cells. T cell receptors scan the surface of antigen presenting cells for specific peptides bound to molecules of the major histocompatibility complex (MHC). Detection of just a few copies of an antigenic peptide triggers robust T cell activation. To succeed in its formidable task, the T cell receptor requires the help of CD4 or CD8 coreceptors and of receptor-ligand pairs, such as LFA-1–ICAM-1, CD2–CD48, and CD28–CD80 (see the figure). On page 221 of this issue, Grakoui *et al.* provide the first dynamic pictures of the multimolecular choreography that takes place as the T cell receptor engages with its peptide-MHC ligand (*1*). The investigators followed the formation of the delicate contacts (called immunological synapses) between the T cell and antigen presenting cell membranes by real-time video imaging.

To mimic the plasma membrane of an antigen presenting cell, the investigators incorporated fluorescently labeled peptide-MHC and ICAM-1 molecules into planar, lipid bilayers on glass supports. The peptide-MHC and ICAM-1 molecules were imbued with a high degree of lateral mobility by substituting their transmembrane segments with glycosyl-phosphatidylinositol. After addition of T cells to the lipid bilayer, there was a time-dependent accumulation of points of contact between the T cell receptors and LFA-1 molecules expressed by T cells and their respective ligands in the bilayer. On the basis of previous work (*2*), the authors assumed that the peptide-MHC and ICAM-1 molecules in the contact areas were bound to T cell receptors and LFA-1 molecules, respectively. The dynamics of T cell receptors and LFA-1 molecules in the T cell membrane could be calculated from the behavior of their fluorescent ligands. Pseudocolor images of the density of peptide-MHC and ICAM-1 molecules in the contact areas at various time points after the T cell–lipid bilayer interaction provide a vivid picture of the formation of immunological synapses.

In the first 30 seconds after initial contact, T cells stop migrating and develop contact points in which peptide-MHC complexes segregate into an outer ring and ICAM-1 molecules coalesce into a central region. The formation of this initial molecular pattern marked the activation of T cell signal transduction pathways, visualized by the release of intracellular calcium ions. During the next 5 minutes, the peripheral peptide-MHC complexes moved into the center of the contact area and the ICAM-1 molecules were relocated away from the center to form a non-overlapping outer ring. This final molecular configuration is strikingly similar to the "supramolecular activation cluster" originally observed by Monks *et al.* with three-dimensional static imaging of the contact points between T cells and antigen presenting cells fixed 30 minutes after formation (*3*). The similarity of the findings from the two studies suggests that contacts between T cells and antigen presenting cells that lead to T cell activation can be accurately simulated with artificial lipid bilayers containing only peptide-MHC complexes and ICAM-1.

Incorporating CD48 and CD80 into the planar bilayer does not measurably enhance the formation of immunological synapses. This is a surprising observation, considering that the CD2–CD48 and CD28–CD80 receptor-ligand pairs are thought to function as molecular rulers. They are believed to keep the T cell and antigen presenting cell membranes at an appropriate distance to allow optimal interaction between the T cell receptor and its peptide-MHC ligand and engaged for enough time to enable a T cell to sample several hundred peptide-MHC complexes (*4*). It is possible, however, that the presumptive role of the CD2–CD48 and CD28–CD80 pairs only becomes effective during interactions between T cell membranes and the more deformable and corrugated surfaces found on professional antigen presenting cells, such as dendritic cells and macrophages.



Getting engaged. Different molecular couples—the T cell receptor (TCR) and peptide-MHC, LFA-1 and ICAM-1, CD2 and CD48, CD28 and CD80—contribute to the formation of an immunological synapse between a T cell and an antigen presenting cell. [Adapted from (*9*)]

The formation of an immunological synapse is completely dependent on the binding of the T cell receptor to its specific peptide-MHC complex. Once assembled, the mature immunological synapse survives for more than an hour. This time frame is compatible with the need to sustain secondary messenger generation for several hours to ensure that a naïve T cell becomes committed to terminal differentiation and clonal expansion. Grakoui *et al.* point out that both the size and density of

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the peptide-MHC clusters that accumulate in the center of the mature synapse are directly proportional to the initial density of peptide-MHC complexes present in the lipid bilayer. Importantly, maximum proliferation of T cells is triggered as soon as a minimum threshold density of peptide-MHC complexes is reached in the mature synapse. The lowest initial density of peptide-MHC complexes in the bilayer that is capable of triggering synapse formation and T cell proliferation compares rather well with the minimum density of specific peptide-MHC complexes that must be displayed on antigen presenting cells to trigger T cell activation.

It is likely that the T cell receptor acts as a molecular processor, translating the different half-lives of its interactions with ligands into distinct biological outcomes (*5*). The Grakoui findings support this view: Short-lived T cell receptor–ligand interactions result in the formation of disorganized contact points that differ from those induced by longer lived interactions. These looser contacts, however, are still capable of triggering less demanding biological responses. Previous studies have shown that suboptimal peptides can weaken or even prevent a T cell response to an optimal peptide. It will be interesting to see whether this effect correlates with the ability of suboptimal peptide-MHC complexes to prevent the formation of immunological synapses.

Analyzing the lipid bilayers by fluorescent photobleaching recovery established that the peptide-MHC complexes in the center of the mature synapse are stable and do not exchange with peptide-MHC complexes localized outside the contact area. Examination of the removal by endocytosis of T cell receptors from the cell surface after stimulation with specific peptide-MHC complexes has prompted the suggestion that a single peptide-MHC complex can engage and sequentially trigger up to 200 specific T cell receptors in less than an hour (*6*). Because the experimental system of Grakoui *et al.* provides only indirect information about the dynamics of ligand-bound T cell receptors, it remains to be determined whether they undergo internalization and are replaced by new receptors. Combining the Grakoui imaging system with T cell receptors tagged with green fluorescent protein should settle this important issue.

It has been argued that ligand-occupied T cell receptors need to congregate into tight arrays to deliver effective intracellular signals (*7, 8*). Once the mature synapse has been formed, however, the density of signaling T cell receptors is expected to mirror the density of central peptide-MHC clusters, which the investigators calculated to be about 100 molecules/μm^2. This density is only slightly higher than that of unoccupied T cell receptors sitting on the surface of quiescent T cells, and so these results do not support the idea of densely packed arrays of signaling T cell receptors.

The findings of Grakoui and colleagues clearly link the biological potency of T cell receptor ligands to their capacity to orchestrate the active assembly of an immunological synapse. The distinctive contact points formed by the T cell surface may polarize the delivery of secondary messenger molecules to a confined region of the cell and protect the T cell receptor core from dephosphorylation by protein tyrosine phosphatases. We do not know whether the stereotyped behavior of immunological synapse formation documented by the elegant real-time imaging of T cells interacting with lipid bilayers applies to the meeting of naïve T cells and professional antigen presenting cells or to the sequential engagement of cytotoxic effector T cells with target cells. Resolution of these questions will have to await a sequel, which, if the current report is anything to judge by, should be well worth watching.

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PERSPECTIVES: ATMOSPHERIC SCIENCE

Summer in the Stratosphere

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Since the discovery in 1985 that an ozone hole develops over the Antarctic in the late winter and early spring, intense research efforts have clarified the roles of atmospheric transport and chemistry in stratospheric ozone changes. The initial focus of the research was on the winter-time conditions, but more recently the stratospheric polar summer has received increased scrutiny.

Enhanced online at www.sciencemag.org/cgi/content/full/285/5425/208

Average ozone concentrations in the polar stratosphere show a pronounced cyclical variation over the course of the year (see the figure at the right). In winter and early spring, ozone builds up at the poles as ozone-rich air is transported from lower latitudes toward the polar regions. But when transport to high latitudes slows and solar illumination increases in late spring and summer, catalytic ozone destruction leads to substantial ozone decreases (~30%). Here we discuss why ozone decreases in the summer polar stratosphere, how this decrease differs from the winter-spring ozone depletion, and how well we understand the underlying chemistry.

Ozone is produced via solar ultraviolet photolysis of oxygen and destroyed through catalytic cycles involving reactive nitrogen (NO_y), halogen (chlorine and bromine), and hydrogen species (HO_x) (*1–3*). Among the NO_y species, NO_x is the major catalyst (see the top panel in the figure on the next page). Stratospheric NO_y and HO_x are

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A cycle of destruction and recovery. Annual cycle of column ozone abundances averaged over 60° to 90°N for 1979–94 (without years affected by volcanic eruptions). Data are from the TOMS satellite (W. Randel, National Center for Atmospheric Research). The mean value (red line) and range of values (green area) are shown. A similar seasonal cycle is revealed by data from high latitudes in the Antarctic region. Losses of ozone in the Arctic and Antarctic regions have lowered column values in both late winter–early spring periods during the last 10 to 15 years (*15*). Averaging the data for the Arctic over 60° to 90°N and not just inside the vortex masks the winter-spring decreases.