Milestone 16

Imaging how T cells hit the bullseye

The formation of the specialized junction between a T cell and antigen-presenting cell (APC) occurs via a multistep process that involves multiple receptor–ligand interactions. Successful coordination of this process is essential for the optimal activation of T cells and the engagement of effector functions including proliferation, cytokine secretion and cytolysis. A major breakthrough in our understanding of junction formation came from Colin Monks, Abraham 'Avi' Kupfer and colleagues in 1998 when they presented 3D images of the $CD4^+$ T cell–APC interaction that revealed a clear segregation of the contact area into different concentric zones that had not been visible from the previous 2D images.

Michael Norcross proposed functional similarities between the T cell–APC junction and neuronal synapses and suggested a hypothesis for the processes that formed the 'lymphocytic synapse'. William 'Bill' Paul and Robert Seder coined the term 'immunological synapse' in 1994, which was subsequently widely adopted to cover synapse formation by various immune cells, and in 2001 was shown for both B cells (Batista et al.) and cytotoxic T lymphocytes (Stinchcombe et al.). Importantly, Gillian Griffiths and colleagues additionally revealed an important role for the T cell immunological synapse in the focused secretion of lytic granules and in intracellular signalling.

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In 1998 Monks et al. used serial optical sections of paraformaldehyde-fixed cells to reconstruct a 3D image of a single antigen-specific T cell interacting with its cognate APC. As had been reported previously, the cytoskeletal protein talin and the T cell receptor (TCR) signalling molecule PKCθ both cluster at the region of cell contact. Surprisingly, 3D imagery revealed that talin and PKCθ were actually present in distinct, non-overlapping domains within this contact region. PKCθ and the TCR–CD3 complex were enriched in a small central contact area, which they referred to as the central supramolecular activation cluster (c-SMAC), which was surrounded by a peripheral domain (p-SMAC) region that was enriched for talin and the adhesion molecule LFA1. Importantly, formation of these domains was a regulated process that was dependent on the recognition of cognate antigen by the TCR.

Inspired by these static images of the 'bullseye'-like structure of the synapse, work by Michael Dustin and colleagues shed further light on the mechanisms and initiation of synapse formation. Grakoui et al. (1999) introduced the use of supported lipid bilayers that contain freely diffusing peptide–MHC and ICAM1 (the ligand for LFA1) as an artificial APC system, which allowed them to image the interaction with live T cells. They showed that the formation of the bullseye pattern was a dynamic process that required active transport of the TCR from the outermost ring of a nascent synapse to the c-SMAC of the mature synapse that had been described by Monks et al. previously. Further work from Dustin and colleagues in 1998 described a role for the T cell adhesion molecule CD2 in facilitating protein segregation into p-SMAC and c-SMAC domains, and linking to the cytoskeletal polarization that was required for full T cell activation and effector function. T cells that were plated on lipid bilayers that contained CD58 (the ligand for human CD2) and ICAM1 formed broad contact zones, with CD2 clustering at the centre of the contact area and LFA1 being excluded from sites of CD2 engagement. They identified a novel SH3 domain-containing adaptor protein, named CD2AP, that was required for the formation of the central receptor cluster and T cell polarization that was dependent on TCR engagement. Later studies focused on the signalling downstream of TCR ligation in the synapse, including Bunnell et al. in 2002 who showed that TCR signalling microclusters form within seconds of TCR engagement and can assemble, disassemble and change composition multiple times in advance of TCR transport into the mature c-SMAC.

These and other studies together revealed the distinctive appearance of an active and ordered process in the formation of the T cell immunological synapse and establish its critical importance in the optimal activation and engagement of the T cell response.

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Milestone study

Monks, C. R. F. et al. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82–86 (1998)

Further reading

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