T-CELL DEVELOPMENT

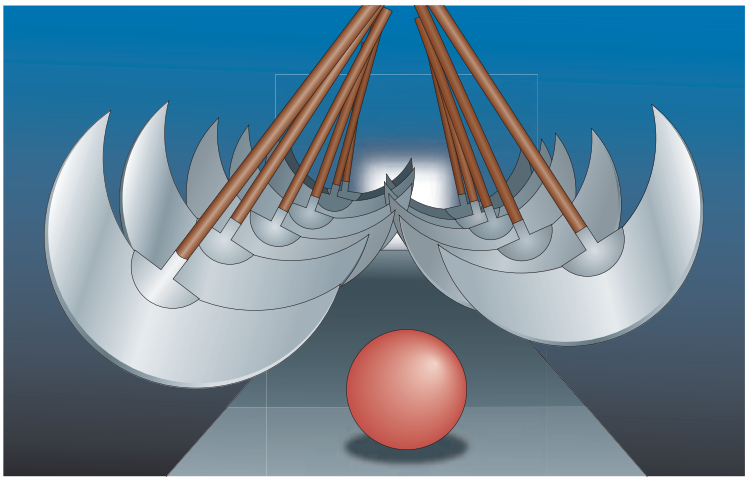
Thymocytes run the 'gauntlet'

The latest findings from Davis and colleagues suggest that positive selection of thymocytes requires continuous low-level stimulation from serial brief encounters with numerous thymic stromal cells. In this way, developing thymocytes are forced to sample many cell surfaces for potentially negatively selecting ligands.

Immature thymocytes are known to be more sensitive to their cognate peptide–MHC ligands than mature T cells, but Ebert *et al*. wanted to know exactly how few complexes would be sufficient for the negative selection of thymocytes. Using a fluorescently labelled peptide to quantify the number of peptide–MHC complexes presented by antigen-presenting cells (APCs) in fetal thymic organ cultures, the authors estimated that less than one peptide per APC was sufficient to cause negative selection of thymocytes in these bulk cultures. Given that this number is surprisingly low, the authors used video fluorescence microscopy to determine more precisely the minimal requirements for negative selection at a single-cell level. As few as two peptide–MHC ligands were found to be sufficient to initiate apoptosis of thymocytes in this assay. In addition, the finding that negative selection still occurred in conditions in which only 1 in 20 APCs were presenting 2 or more negatively selecting peptides suggested that, besides their inherent heightened sensitivity, immature thymocytes need to actively sample numerous APCs before being negatively selected.

However, the need for thymocytes to make numerous interactions with APCs before committing to their fate is at odds with the idea that long-lasting interactions are required for positive selection. To explore this issue, the authors took advantage of their finding that the presence of nuclear factor of activated T cells (NFAT) in the nucleus could be used as an indicator of productive T-cell receptor (TCR) signalling for positive selection. Indeed, the presentation of positively selecting ligands to double positive (DP) thymocytes led to the accumulation of nuclear NFAT, but this seemed to occur without the requirement for a stable interaction with an APC. By contrast, the accumulation of nuclear NFAT that occurred in thymocytes in response to negatively selecting agonist ligands was associated with stable thymocyte–APC contacts, complete with the formation of an immunological synapse (as occurs between mature T cells and APCs in the periphery). The idea that TCR signalling can occur despite the lack of synapse formation was supported by time-lapse imaging data showing that, in the presence of positively selecting ligands, transient but repeated engagements of thymocytes with thymic APCs led to sustained NFAT translocation to the nucleus.

On the basis of these observations, the authors suggest that maturing thymocytes run a 'gauntlet' of repeated engagements with many APCs as they travel through the thymus. This increases their chances of encountering rare negatively selecting ligands that are necessary for stringent central tolerance, while maintaining signals that are necessary for positive selection.



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ORIGINAL RESEARCH PAPER Ebert, P. J. R., Ehrlich, L. I. R. & Davis, M. M. Low ligand requirement for deletion and lack of synapses in positive selection enforce the gauntlet of thymic T cell maturation. *Immunity* **29**, 734–745 (2008)