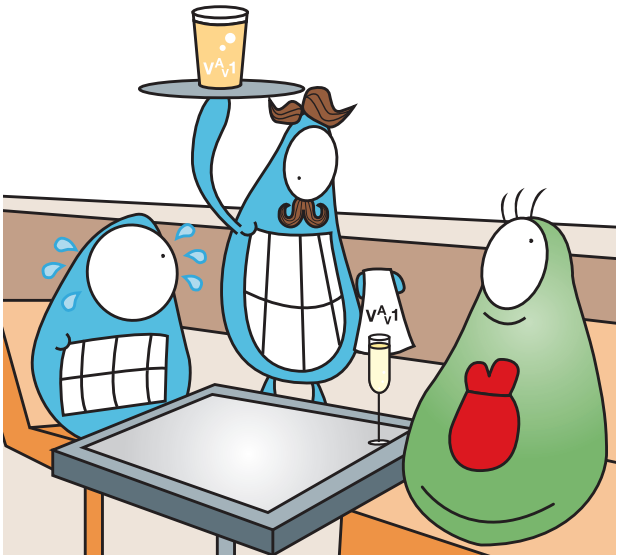
IMMUNE RESPONSES

Helping T cells to relax and interact



T-cell recognition of antigen displayed by an antigen-presenting cell (APC) results in the formation of an area of close membrane contact between the two cells, known as the immunological synapse. Once the immunological synapse has formed, the T-cell stops migrating, changes shape, and forms a tight and long-lasting conjugate with the APC. How is antigen recognition linked to the cytoskeletal changes required for this to occur? A French group has now shown that inactivation of Ezrin-Radixin-Moesin (ERM) proteins through a Vav1–Rac1 pathway leads to relaxation of the T-cell cytoskeleton and favours conjugate formation with APCs.

ERM proteins act as general crosslinkers between the actin network near the cell surface and the plasma membrane, and have previously been shown to be inactivated after antigen recognition by the T-cell receptor (TCR). To investigate this pathway further, the authors focused on Rho GTPases, which are known to be involved in controlling T-cell morphology. Using constitutively activated and dominant-negative mutants of the small GTPases Rac1 and Cdc42, they showed that Rac1, but not Cdc42, is involved in the dephosphorylation and inactivation of ERM proteins downstream of TCR triggering.

The guanine-nucleotide exchange factor Vav1 is involved in controlling actin cytoskeleton reorganization in T cells after TCR ligation, so Faure *et al.* tested whether Vav1 could act to link TCR ligation to ERM-protein inactivation. In contrast to wild-type cells, *Vav1*$^{-/-}$ T cells were resistant to TCR-induced ERM-protein dephosphorylation, indicating that Vav1 is the main exchange factor connecting TCR ligation to Rac1 activation and ERM-protein dephosphorylation.

What effect does this Vav1–Rac1-dependent inactivation of ERM proteins have on T-cell morphology and

T-CELL DEVELOPMENT

Survivin against the odds

Most developing thymocytes are destined to die; so what enables them to overcome the odds, and survive and mature into functional T cells? Reporting in *The Journal of Experimental Medicine*, two groups have identified an important role for survivin — a member of the inhibitor of apoptosis protein (IAP) family — in T-cell development.

T-cell development in the thymus involves a series of distinct stages that can be defined by the expression of cell-surface markers. Early T cells are $CD4^{-}CD8^{-}$ double negative (DN) and can be further subdivided into DN1, DN2, DN3 and DN4 stages based on their expression of CD25 and CD44. Productive rearrangement of the T-cell receptor (TCR) β-chain locus occurs during the DN3 to DN4 transition and leads to expression of the pre-TCR. Only cells that express a functional pre-TCR undergo marked proliferation and differentiate into $CD4^{+}CD8^{+}$ double-positive (DP) cells. However, most DP cells die through negative selection or neglect because their TCRs have too high or too low affinity for peptide–MHC complexes. Those that mature successfully migrate to the periphery as functional $CD4^{+}CD8^{-}$ or $CD4^{-}CD8^{+}$ single-positive T cells.

Survivin is expressed by highly proliferating cells and has previously been implicated in cell-cycle progression. Yet, despite being a member of the IAP family, its role in apoptosis is controversial. Both groups set out to study the role of survivin in the control of proliferation and apoptosis in T-cell development. Because survivin deficiency is embryonic lethal, both groups used a conditional deletion strategy to specifically knockout the gene encoding survivin in developing thymocytes. Zheng Xing *et al.* generated two T-cell-specific survivin-deficient mouse lines with the deletion occurring at different developmental stages. Lck-survivin mice, in which survivin deletion occurs by the DN3 stage, had defective thymocyte development as a result of arrested cell proliferation. However, when survivin was deleted later at the DN4 stage (CD4-survivin mice), the early stages of thymocyte development were normal, but peripheral T cells were immature and markedly reduced in numbers owing to problems with T-cell homeostatic proliferation. Together, these observations indicate an important role for survivin at early and late stages of T-cell development.

Tak Mak and colleagues also found that thymocyte development was blocked at the DN3 to DN4 transition in Lck-survivin mice. They observed increased apoptosis but, in agreement with Xing *et al.*, apoptosis to external stimuli proceeds normally in survivin-deficient cells. In response to proliferative stimuli, the absence of survivin triggered cell-cycle arrest, defective spindle formation and cell death of proliferating thymocytes. Although loss of survivin induced expression of pro-apoptotic p53, neither p53 loss nor overexpression of anti-apoptotic Bcl-2 could restore the development of survivin-deficient DN3 thymocytes, indicating that the protective function of survivin is independent of p53 and Bcl-2. The authors also observed severe defects in chromosomal segregation and cytokinesis in survivin-deficient cells and suggest that the main role of survivin is in controlling mitosis progression, and that cell death was secondary to these defects.

Both papers highlight a key role for survivin in enabling thymocytes to progress from the DN to DP stage and show that survivin does not have a primary role in apoptosis. Further studies aim to understand how survivin-mediated T-cell homeostasis is regulated.

Lucy Bird

References and links

ORIGINAL RESEARCH PAPERS Xing, Z. *et al.* Essential role of survivin, an inhibitor of apoptosis protein, in T cell development, maturation, and homeostasis. *J. Exp. Med.* **1**, 69–80 (2004) | Okada, H. *et al.* Survivin loss in thymocytes triggers p53-mediated growth arrest and p53-independent cell death. *J. Exp. Med.* **3**, 399–410 (2004)

function? Using a dominant-negative ERM molecule to mimic ERM inactivation, the authors showed that in the absence of ERM protein activity, the actin network close to the plasma membrane was disorganized, the rigidity of the T-cell membrane was reduced and T cells showed an enhanced capacity to form conjugates with APCs.

So, through activation of Rac1, Vav1 mediates inactivation of ERM proteins after TCR ligation, leading to morphological changes including relaxation of the cytoskeleton in T cells. This enables them to interact with APCs more efficiently and so generate an effective immune response.

Jenny Buckland

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AUTOIMMUNITY

It's not what you do, it's the way that you do it

An antigen-specific autoreactive T-cell response *per se* does not cause autoimmune disease; rather, it is the quality of the T-cell response that counts. In the first example of its kind in humans, this study shows that non-diabetic individuals do mount responses against islet antigens, but that these responses are regulatory rather than inflammatory.

Previous studies of patients with type 1 diabetes have been hampered by the rarity of islet-specific T cells in peripheral blood, so Mark Peakman and colleagues have refined the cytokine enzyme-linked immunosorbent spot (ELISPOT) technique to develop a new highly sensitive assay for the detection of autoreactive T cells. First, the use of whole antigen preparations can have non-specific inhibitory or stimulatory effects on T cells, so the authors identified naturally processed and presented peptide epitopes (NPPEs) of the islet antigens insulinoma-associated 2 (IA-2) and pro-insulin by direct elution from HLA-DR4 molecules of antigen-pulsed antigen-presenting cells. Second, the sensitivity of the ELISPOT assay was increased by using a multiantigen, multiepitope panel, and by including an initial culture step of peripheral-blood mononuclear cells (PBMCs) in the presence of synthetic NPPEs but in the absence of cytokine-capture antibodies, to allow increased cell–cell interactions and signal amplification.

Using this technique, 72% of patients with type 1 diabetes had PBMCs that produced the pro-inflammatory cytokine interferon-γ (IFN-γ) in response to at least one of the IA-2 and pro-insulin peptides, compared with only 7% of non-diabetic control subjects. However, the PBMCs from non-diabetic controls were not completely unresponsive to the islet antigens. 64% of them made an interleukin-10 (IL-10) response to IA-2 peptides compared with 29% of diabetic patients. Of the diabetic patients that did make an IL-10 response, almost all also made an IFN-γ response, whereas control subjects produced IL-10 in the complete absence of IFN-γ. So, individuals with and without diabetes make qualitatively different responses to the same autoantigens. IL-10 is known to have immunosuppressive functions, so the T cells responding to islet antigens in healthy controls might have a regulatory role. Interestingly, those patients with diabetes who produced IL-10 and IFN-γ in response to IA-2 or pro-insulin tended to be older at diagnosis than patients who produced only IFN-γ, confirming the disease-modifying effect of IL-10.

An assay that can distinguish between tolerant and autoimmune states by analysing peripheral blood will be of great use in monitoring treatment efficacy in immunointervention trials for type 1 diabetes. Given this first description of islet-antigen-specific regulatory T cells in humans, it will also be interesting to see if any treatments can specifically induce these T cells.

Kirsty Minton

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