B CELLS

Ships in the night

The B-cell receptor (BCR) is a multiprotein structure that provides important signalling cues for the development, and activation or inactivation, of B cells. The binding of the BCR to antigen can stimulate two processes: the initiation of signalling events and the internalization of the occupied BCRs.

Surprisingly, little was known about the relationship between signalling and internalization — until now. Reporting in *PLoS Biology*, Hou *et al.* show that BCR signalling and internalization events are mutually exclusive, and that, on binding antigen, the BCR is either phosphorylated and retained at the cell surface, or internalized.

Binding of antigen by the BCR induces the phosphorylation of tyrosines that are within conserved motifs in the cytosolic tails of the two signalling chains of the BCR. The authors determined that the unphosphorylated tyrosines in the cytosolic tail of the main BCR signalling chain, immunoglobulin-α, are required for BCR internalization (also known as endocytosis).

Knowing that the phosphorylation of tyrosine-based motifs in other receptors inhibits internalization, the authors next predicted that, when the BCR is phosphorylated (and therefore can actively signal), it is preferentially retained at the cell surface. They confirmed their prediction using several methods, which included a direct visualization assay in which phosphorylated complexes of BCRs could be seen only on the surface of the cells and not in the cytosol. Interestingly, Hou *et al.* also determined that only a small fraction of surface BCR complexes were actually phosphorylated (and therefore available to initiate signalling) following binding of antigen.

Last, the authors developed a mathematical model that allowed them to compare both a scenario in which receptor internalization and phosphorylation are mutually exclusive events, and a more conventional scenario in which these two processes are competing events. Using this model, Hou *et al.* gained insights into some of the seemingly contradictory observations on the relationships between the internalization and phosphorylation

TUMOUR IMMUNOLOGY

Partners in crime

Only recently has it become apparent why manipulating the antitumour immune response to target established tumours is often unsuccessful — the tumour and the systemic environment is largely immunosuppressive. Thomas Spies and colleagues have identified a new immunosuppressive mechanism that is mediated by a subset of $CD4^+$ T cells and by the shedding of tumour-associated natural killer group 2, member D (NKG2D) ligands.

T-cell responses are governed by a complex interaction between co-stimulatory and inhibitory ligands, and their receptors. NKG2D is a receptor that can function as a co-stimulatory molecule and is expressed by $CD8^+$ T cells but is not thought to be expressed by most $CD4^+$ T cells. NKG2D expression is downregulated by binding to MHC-class-I-like ligands, such as MHC-class-I-related chains A and B (MICA and MICB). These ligands are not involved in antigen presentation but are induced by cellular and genotoxic stress. Some tumours can shed soluble MIC ligands and these are thought to contribute to the suppression of the $CD8^+$ T-cell response.

In agreement with this hypothesis, Spies and colleagues found that $CD8^+$ T cells, isolated from tumours and from the blood of patients with MIC-expressing tumours, had reduced expression of NKG2D. Surprisingly however, they found that an increased proportion of the $CD4^+$ T cells isolated from these patients expressed NKG2D compared with control cells (from healthy individuals and patients with MIC-negative tumours).

NKG2D expression can be induced by a $CD4^+$ T-cell subset following stimulation of the T-cell receptor complex. Co-stimulation of $NKG2D^+CD4^+$ T cells in the presence of MICA resulted in an increased number of $NKG2D^+CD4^+$ T cells, but proliferation of the $NKG2D^-CD4^+$ T cells was inhibited. The authors found that $NKG2D^+CD4^+$ T cells express soluble CD95 ligand (also known as FAS ligand). Soluble CD95L induced activation-induced cell-cycle arrest of $NKG2D^-CD4^+$ T cells, but $NKG2D^+CD4^+$ T cells remained resistant to the soluble CD95L that they produced because they also downregulated expression of CD95 receptor. Moreover, supernatants isolated from $NKG2D^+CD4^+$ T cells induced apoptosis in CD95-sensitive tumour cell lines. Activated $NKG2D^+CD8^+$ T cells were also shown to express active CD95L when

of the BCR. They also showed that when internalization and phosphorylation are mutually exclusive (as was observed experimentally), responses to low-avidity antigens are enhanced — this could have physiological implications for the initial detection of antigens.

The authors suggest that their model might be helpful in explaining some of the disparate observations in the literature regarding BCR spatial regulation, and say that further studies to identify how receptors are selected for internalization are required. The experimental results presented in this paper have certainly provided a useful platform from which we will hopefully be able to decipher the complex dynamics of BCR signalling and internalization.

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ORIGINAL RESEARCH PAPER Hou, P. *et al.* B cell antigen receptor signaling and internalization are mutually exculsive events. *PLoS Biol.* **4**, e200 (2006)

cultured with MIC ligands, and growth of NKG2D$^-$CD8$^+$ T cells was inhibited.

However, unlike CD8$^+$ T cells, prolonged exposure to MIC ligands does not seem to lead to the downregulation of NKG2D by CD4$^+$ T cells. Therefore, the authors conclude that in tumours that express MIC ligands, activation of T cells expressing NKG2D will result in proliferation and the secretion of CD95L that could lead to the elimination of CD95-sensitive tumour cells. However, after prolonged exposure to MIC ligands, CD8$^+$ T cells will downregulate the NKG2D receptor and become susceptible to the growth-suppressive effects of CD95L, which is continually produced by the expanding population of NKG2D$^+$CD4$^+$ T cells.

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ORIGINAL RESEARCH PAPER
Groh, V., Smythe, K., Dai, Z. & Spies, T. Fas ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. *Nature Immunol.* 28 May 2006 (doi:10.1038/ni1350)

AUTOIMMUNITY

Multipronged effects of *FOXP3* mutations

Mutations in the transcription factor forkhead box P3 (*FOXP3*) are responsible for a rare autoimmune disease in young boys known as IPEX (immunodysregulation, polyendocrinopathy and enteropathy, X-linked syndrome). At least in mice, FOXP3 has a crucial role in the development and function of CD4$^+$CD25$^+$ regulatory T (T_{Reg}) cells. Therefore, it has been suggested that a lack of these regulatory cells might contribute to disease in humans, as has been shown in the mouse model of the disease, Scurfy mice. However, Maria Grazia Roncarolo and colleagues now report that the human disease is not necessarily the result of a deficiency of T_{Reg} cells but instead because these cells are dysfunctional. Moreover, they show that effector T cells from patients with IPEX also have a functional defect.

To carry out their studies, four children with IPEX of varying disease severity and with various *FOXP3* mutations were studied, and so correlations could be drawn between different *FOXP3* mutations and T_{Reg}-cell generation and function. The authors were surprised to see that T_{Reg}-cell numbers were normal in most of the patients and were comparable to those of age-matched control donors. Moreover, these T_{Reg} cells had a normal phenotype, including expression of GITR (glucocorticoid-induced tumour-necrosis-factor-receptor-related protein) and CTLA4 (cytotoxic T-lymphocyte antigen 4), and they displayed normal T_{Reg}-cell characteristics, such as anergy and lack of interferon-γ (IFNγ) production after activation.

However, the ability of T_{Reg} cells from the IPEX patients to suppress the proliferation of CD4$^+$CD25$^-$ effector T cells was reduced compared with T_{Reg} cells from normal donors. The degree of defective suppression varied depending on the type of *FOXP3* mutation, the strength of the activation stimuli and the genotype of the target effector T cells. For example, when provided with a 'weak' stimulus (CD3-specific antibody presented by allogeneic antigen-presenting cells), T_{Reg} cells from IPEX patients who express the mutant FOXP3 protein could suppress *in vitro* proliferation of effector T cells from control donors. But when provided with a 'strong' stimulus (beads coated with CD3- and CD28-specific antibody), T_{Reg} cells from these same patients failed to suppress effector T-cell proliferation. Notably, activated T_{Reg} cells from a patient with a *FOXP3* mutation that completely ablated FOXP3 expression were unable to suppress effector T-cell proliferation.

During these studies the authors also noted that, when autologous effector T cells were used as targets for suppression, activated T_{Reg} cells from all four patients were unable to suppress *in vitro* proliferation, independent of the activation conditions used. This led the authors to investigate whether the effector T cells from IPEX patients were defective. Despite their disparate clinical phenotypes, effector T cells from all four patients had a markedly reduced ability to secrete interleukin-2 and IFNγ following T-cell receptor (TCR)-mediated activation compared with cells from normal donors. This defect was not apparent when the cells were activated with phorbol ester and ionomycin, which activate T cells in a TCR-independent manner. This finding indicates that FOXP3 could have a role in regulating the effector T-cell functions that depend on TCR signalling, as well as in regulating T_{Reg}-cell function.

So, these findings indicate that, in contrast to the disease in Scurfy mice, human IPEX is not necessarily due to the absence of T_{Reg} cells but rather to their impaired suppressive function, together with altered effector T-cell function.

Lucy Bird

ORIGINAL RESEARCH PAPER Bacchetta, R. *et al.* Defective regulatory and effector T cell functions in patients with *FOXP3* mutations. *J. Clin. Invest.* **116**, 1713–1722 (2006)