➡ **AUTOIMMUNITY**

Reversing diabetes

Type 1 diabetes is one of the most prevalent autoimmune diseases worldwide. It results from the destruction of pancreatic islets (including the β cells, which produce insulin) by autoreactive $CD4^+$ and $CD8^+$ T cells. At present, individuals with type 1 diabetes are treated with insulin, but this does not prevent the development of severe complications. So, researchers have long sought a cure for this disease. Many animal studies have described interventions that are effective at preventing the development of diabetes. Now, Matthias von Herrath and colleagues build on recent studies and report substantial progress towards a treatment that reverses type 1 diabetes after the onset of disease.

The authors exploited findings from two previous sets of studies. First, it has been shown that intranasal or oral immunization of mice with islet autoantigens, such as insulin or glutamic-acid decarboxylase 65 (GAD65), can elicit antigen-specific $CD4^+CD25^+$ regulatory T (T_{Reg}) cells,

and this prevents, but does not reverse, the development of type 1 diabetes. Second, a non-Fc-receptor-binding CD3-specific monoclonal antibody (an $F(ab')_2$) has been shown to provide long-term, but not permanent, protection when administered to mice with recent-onset diabetes. This protection possibly results from a reduction in the number of autoreactive T cells and an increase in the number of T_{Reg} cells. So, the authors proposed that combining these interventions would increase efficacy: the CD3-specific monoclonal antibody should create an immunomodulatory milieu that allows clonal expansion of the antigen-specific T_{Reg} cells elicited by the co-administered islet antigen.

They examined the efficacy of immunization with the CD3-specific monoclonal antibody together with various islet antigens or antigen-derived peptides using various routes of delivery, in two mouse models of type 1 diabetes. Intranasal delivery of a pro-insulin-derived peptide had the strongest synergy with the

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➡ **B CELLS**

Spread yourself thin

It is easy to imagine how a B cell can bind and internalize soluble antigens through B-cell receptors (BCRs) and be activated depending on the affinity of the interaction. But it is less clear how B cells acquire and sense the affinity of membrane-associated antigens, which are known to be highly effective in driving B-cell activation. By elegant imaging and computer modelling, Facundo Batista and colleagues show that, for optimal B-cell activation, the B cell must spread across antigen-bearing membranes and then contract, thereby collecting the antigen–BCR complexes into a central cluster. How effectively this process occurs depends on the strength of the antigen–BCR interaction.

In a similar manner to the intimate interaction between T cells and antigen-presenting cells (APCs), when a B cell recognizes antigens tethered on a cell surface, a cluster of
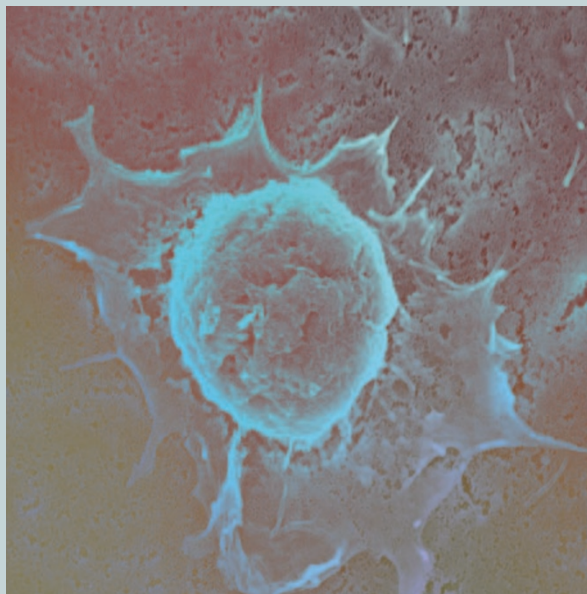
A scanning electron microscopy image of a B cell spreading on the surface of a cell displaying its cognate antigen. Image courtesy of S. Fleire, London Research Institute, UK.

BCRs and their ligands forms at the contact site. At the same time, other membrane proteins are recruited and reorganized to form an immunological synapse.

To follow the early morphological changes of B cells during activation, the authors studied, by electron microscopy, the interaction of B cells that expressed a transgenic BCR specific for hen-egg lysozyme (HEL) with target cells that expressed a membrane-tethered form of the HEL antigen. The authors observed that on recognition of antigen, the B cell rapidly (2–4 min after contact) spreads across the target cell membrane before gradually contracting. This cellular response was dependent on recognition of the specific antigen, signal transduction through the BCR and actin polymerization. Importantly, the antigen–BCR complexes were initially gathered into microclusters before being concentrated in a central cluster, from where the B cell could efficiently acquire the antigen.

Using a set of mutant HEL proteins that have a range of affinities for the transgenic BCR, the authors next showed that the level of the B-cell

CD3-specific monoclonal antibody and was shown to cause long-term reversal of recent-onset diabetes in both mouse models. This reversal was associated with increased numbers of peptide-specific T_{Reg} cells, which were shown to produce various regulatory cytokines *in vitro* and to have a bystander effect *in vivo* that results in the suppression of heterologous autoreactive T-cell responses in the pancreas.

The combination of a systemic immunomodulator and an inducer of autoantigen-specific T_{Reg} cells has the dual advantage of reversing recent-onset type 1 diabetes and of reducing the risk of side-effects (given that the T_{Reg} cells only suppress immune responses in a site-specific manner). This combination therapy, therefore, might be a useful clinical strategy.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Bresson, D. *et al.* Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs. *J. Clin. Invest.* **116**, 1371–1381 (2006)

response was influenced by the density of the antigen and the affinity of the antigen for the BCR. Moreover, the amount of antigen that was taken up by the B cell and the subsequent ability of the B cell to present the antigen to T cells were proportional to the total amount of antigen accumulated in the central cluster.

On the basis of these observations the authors generated a computer model that reproduced the empirical data. The model showed that this antigen-collection process allows the B cells to discriminate between ligands of different affinity. So, high-affinity interactions favour further B-cell spreading and exposure to membrane-bound ligands before contraction and collection. By contrast, low-affinity interactions result in inefficient spreading and low levels of antigen collection and therefore reduced B-cell activation.

Lucy Bird

ORIGINAL RESEARCH PAPER Fleire, S. J. *et al.* B cell ligand discrimination through a spreading and contraction response. *Science* **312**, 738–741 (2006)
FURTHER READING Harnett, M. M. B cells spread and gather. *Science* **312**, 709–710 (2006)

⤷ INNATE IMMUNITY

Finding flagellin

Bacterial flagellin is a known ligand for Toll-like receptor 5 (TLR5). However, several recent papers have now revealed that in addition to the TLR5 pathway, which responds to extracellular flagellin, host macrophages can respond to cytosolic flagellin through members of the NOD-like receptor (NLR) family.

The recognition of pathogen-associated molecular patterns by host TLRs is a key component of innate immunity, and much has been learned about TLRs and their signalling pathways over the past decade. More recently, attention has turned to the role of non-TLR pattern-recognition receptors in innate immunity, including the cytoplasmic NLR family. Details of the NLR-signalling pathways are beginning to emerge, and NLRs are known to be involved in secretion of the pro-inflammatory cytokine interleukin-1β (IL-1β) by macrophages. IL-1β is produced initially as a zymogen that is activated for secretion by caspase-1.

In *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) infection, the NLR protein ICE-protease activating factor (IPAF; also known as CARD12 and CLAN) was known to be involved in caspase-1 activation and IL-1β secretion, but until now, the *S. typhimurium* ligand for

IPAF was unknown. Two independent groups led by Gabriel Núñez and Alan Aderem investigated the nature of the innate immune response to *S. typhimurium* infection. Both groups confirmed that IPAF was required for IL-1β production and caspase-1 activation by macrophages. Additionally, they both found that *S. typhimurium* mutants that either lack or have mutated flagella did not stimulate caspase-1 activation or IL-1β secretion, suggesting that flagellin is the *S. typhimurium* ligand for IPAF.

As flagellin is also a known ligand for TLR5, the involvement of TLRs was examined. Both groups found that *S. typhimurium* could stimulate caspase-1 activation and IL-1β secretion by TLR5-deficient macrophages and by wild-type macrophages, and in addition, Franchi *et al.* found normal levels of caspase-1 activation and IL-1β secretion by tolerant macrophages that are refractory to TLR stimulation. Taken together, these results suggest that macrophages sense flagellin through a TLR5-independent pathway that relies on the cytoplasmic sensor IPAF.

Further confirmation that IPAF senses flagellin in the cytosol independently of TLR5 comes from the fact that both groups also demonstrated that purified flagellin delivered to the cytosol triggered caspase-1 activation in wild-type but not IPAF-deficient macrophages. The mechanism by which flagellin accesses the cytosol during infection remains to be completely elucidated. However, genetic evidence presented by Miao *et al.* suggests that it is transferred directly into the eukaryotic cytoplasm by the virulence-associated type III secretion system.

These results are echoed by results published recently in two independent papers, one in *PLoS Pathogens* and one in *The Journal of Experimental Medicine*, which indicate that an NLR is involved in cytosolic sensing of *Legionella pneumophila* flagellin through a TLR5-independent, caspase-1-dependent pathway.

Sheilagh Molloy, Senior Editor, Nature Reviews Microbiology

ORIGINAL RESEARCH PAPERS Franchi, L. *et al.* Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1β in *Salmonella*-infected macrophages. *Nature Immunol.* **7**, 576–582 (2006) | Miao, E. A. *et al.* Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. *Nature Immunol.* **7**, 569–575 (2006) | Ren, T. *et al.* Flagellin-deficient *Legionella* mutants evade caspase 1 and Naip5-mediated macrophage immunity. *PLoS Pathog.* **2**, e18 (2006) | Molofsky, A. B. *et al.* Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* **203**, 1093–1104 (2006)