IN THE NEWS

Diabetes cure?

A 61-year-old man has become the first person in the UK to be cured of type 1 diabetes, thanks to a ground-breaking cell-transplant procedure. Richard Lane of Bromley in Kent, who has been dependent on multiple daily injections of insulin and has suffered frequent hypoglycaemic attacks for 30 years, received three islet-cell transplants from donors who had died. These were injected into his liver, and he now no longer needs insulin injections. He told *The Guardian* newspaper: "I haven't felt better in myself for 30 years. I have to pinch myself to ensure I am not dreaming".

Surgeon James Shapiro, in Edmonton, Canada, pioneered the technique and was the first to achieve insulin independence, in 2001. Previously, two other patients in the UK have undergone the procedure, but both still require small doses of insulin. In light of the recent success, Annwen Jones, Chief Executive Officer of Juvenile Diabetes Research Foundation International, said that "Great improvements have been made since the first procedure of this type in 2001 and we are delighted that we now have the expertise to achieve insulin independence in the UK." (*BBC News*).

However, there remain several drawbacks. First, patients who receive islet-cell transplants spend the rest of their lives on immunosuppressive drugs to prevent rejection, and second, there is a severe shortage of donor pancreases from which to extract islet cells. Acknowledging these shortcomings, Professor Stephanie Amiel, a consultant on diabetes at King's College London said that "more research needs to be done to perfect the islet isolation procedures and the drugs we use to prevent rejection of the islets and recurrence of the diabetes" (*The Guardian*).

Lucy Bird

TECHNIQUE

A new approach to studying tolerance

Antigen-receptor-transgenic mice are commonly used to study lymphocyte responses because they increase the frequency of antigen-specific cells to easily detectable levels. However, such systems are designed to distort lymphocyte development and generate quasi-monoclonal immune systems. These non-physiological changes mean that results from receptor-transgenic mice require verification in normal, polyclonal immune systems. Nemazee and colleagues have developed a new approach using single-chain antibodies that allows them to do just that, by creating a superantigen that binds a high frequency of polyclonal B-cell receptors (BCRs).

They generated a single-chain antibody consisting of an Fv domain specific for the constant region of mouse Igκ light chain, hinge regions and the Fc portion from rat IgG1, and transmembrane and cytoplasmic regions from H–2K^b. Cells transfected with the chimeric gene expressed a stable cell-surface protein with the desired specificity for mouse Igκ. Transgenic mice were then created that have uniform and ubiquitous expression of this protein under control of the ubiquitin C promoter. This 'macro-self' antigen can bind the BCRs of a high proportion of polyclonal B cells (those that are Igκ$^+$) without requiring skewing of the lymphocyte repertoire.

Almost all of the B cells in these transgenic mice expressed Igλ light chains rather than Igκ, indicating that the antigen can induce B-cell tolerance. One potential mechanism for such tolerance is receptor editing involving secondary light-chain

T-CELL RESPONSES

Too fat to respond

During the lifetime of a 'successful' T cell, it must migrate in the following way: out of the thymus, where it develops; into the peripheral blood, where it circulates through lymph nodes searching for its cognate antigen for activation; and eventually, into the target organ, where it mediates its effector functions. These migration patterns are carefully controlled by the expression of specific chemokines and adhesion molecules. The lipid mediator sphingosine 1-phosphate (S1P) was recently recognized to be a new type of chemotactic signal for T cells, acting through sphingosine 1-phosphate receptor 1 ($S1P_1$), which is expressed by developing T cells, to promote egress from the thymus. Hongbo Chi and Richard Flavell now show that the regulation of $S1P_1$ signals is also important for the effector responses of mature T cells.

The role of $S1P_1$ in mature T-cell responses has been difficult to address so far because $S1P_1$-deficient mice lack peripheral T cells, owing to a block in egress from the thymus. On the basis of the observation that $S1P_1$ is highly expressed by naive T cells but is downregulated after T-cell activation, the authors created transgenic mice that constitutively express $S1P_1$ under control of the human *CD2* promoter in all T cells to analyse the biological relevance of this downregulation of $S1P_1$.

The transgenic mice had slightly fewer T cells in peripheral lymph nodes, but they had 70% more splenocytes than wild-type mice and an increased number of CD4$^+$ and CD8$^+$ T cells in the peripheral blood. As expected, the transgenic T cells underwent significantly greater chemotaxis in response to S1P *in vitro* than did wild-type T cells. Adoptive transfer of CD4$^+$ wild-type or transgenic T cells was then used to show that $S1P_1$ also regulates mature T-cell migration *in vivo* and can account for the increased distribution to the blood. Five hours after transfer to wild-type mice, there were 60% more transgenic CD4$^+$ T cells than transferred wild-type T cells in the blood of recipients. Given that the concentration of S1P is higher in the blood than in other tissues, it is probable that this results from increased egress of the transgenic T cells into the blood, rather than from reduced entry to secondary lymphoid organs.

Chi and Flavell then analysed the effects of altered T-cell migration owing to enforced $S1P_1$ expression in three models: contact hypersensitivity, autoimmunity and immunization of T-cell-receptor-transgenic mice with cognate antigen. In all cases, the *S1p1*-transgenic mice had a

rearrangements, but this has not been shown *in vivo* in non-receptor-transgenic mice. Using various approaches, the authors showed that, in their system, tolerance is the result of developmental arrest followed by receptor editing concomitant with upregulated expression of recombination-activating genes (*Rag1* and *Rag2*). It was not associated with clonal deletion of Igκ$^+$ B cells or proliferation of Igλ$^+$ cells.

This technique could, in theory, be used for any type of antigen receptor for which there is a specific monoclonal antibody. The authors also suggest that an adoptive-transfer approach, using macro-self-transgenic mice as hosts for the adoptive transfer of normal or mutant bone marrow, could speed up the screening of mutant mice for immune-tolerance phenotypes, which currently requires time-consuming and expensive crossing with receptor-transgenic mice.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Ait-Azzouzene, D. *et al*. An immunoglobulin Cκ-reactive single chain antibody fusion protein induces tolerance through receptor editing in a normal polyclonal immune system. *J. Exp. Med.* **201**, 817–828 (2005)

delayed or reduced T-cell-mediated immune response. For example, in the autoimmunity model, wild-type mice immunized with myelin oligodendrocyte glycoprotein rapidly developed experimental allergic encephalomyelitis, whereas in transgenic mice, there was a significant delay before symptoms began.

The authors conclude that, as they saw no intrinsic defects in proliferation or cell death of the transgenic T cells, the defective immune responses probably result from insufficient retention of circulating T cells in the lymph nodes, where they might meet cognate antigen. Downregulation of $S1P_1$ is therefore required to maximize T-cell priming, and the next step will be to identify the factors that regulate this *in vivo*.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Chi, H. & Flavell, R. A. Regulation of T cell trafficking and primary immune responses by sphingosine 1-phosphate receptor 1. *J. Immunol.* **174**, 2485–2488 (2005)

IN BRIEF

B-CELL DEVELOPMENT

Basal immunoglobulin signaling actively maintains developmental stage in immature B cells.

Tze, L. E. *et al*. *PLoS Biol.* **3**, e82 (2005).

This study shows that basal signalling through the B-cell receptor (BCR) of immature B cells is crucial to suppress expression of the recombination-activating genes (*RAG1* and *RAG2*) and to prevent 'back-differentiation' to the pro-B-cell stage. Therefore, such basal signalling is important to maintain allelic exclusion of the immunoglobulin light chains (which rearrange at this stage of development) and ensure self-tolerance. When basal IgM signalling in immature B cells was inhibited, microarray analysis and flow cytometry showed the upregulation of genes and proteins that are selectively expressed by pro-B cells. The requirement for basal signalling to maintain B-cell development could be an important quality-control mechanism to test for a functional BCR.

ANTIBODY RESPONSES

MutSα binds to and promotes synapsis of transcriptionally activated immunoglobulin switch regions.

Larson, E. D. *et al*. *Curr. Biol.* **15**, 470–474 (2005).

Class-switch recombination (CSR) — the process by which a new immunoglobulin constant region is joined to the rearranged heavy-chain variable (VDJ) region — requires activation-induced cytidine deaminase (AID) and the mismatch-repair heterodimer MutSα (MSH2–MSH6). This study clarifies the role of MutSα by showing that it specifically binds to regions of G4 DNA (four DNA strands associated through bonds between guanines) in transcribed switch regions that are produced during CSR and to the U•G mismatches that are created by AID. Binding of MutSα promoted interactions between the G-rich loops, thereby leading to switch-region synapsis.

NATURAL KILLER CELLS

A subset of natural killer cells achieve self-tolerance without expressing inhibitory receptors specific for self MHC molecules.

Fernandez, N. C. *et al*. *Blood* 22 Feb 2005 (doi:10.1182/blood-2004-08-3156).

Natural killer (NK) cells are thought to express at least one inhibitory receptor specific for a self-MHC class I molecule, and this is thought to maintain NK-cell self-tolerance. However, Fernandez *et al.* detected a population of NK cells that lack expression of all known inhibitory receptors specific for self-MHC class I molecules. These NK cells were hyporesponsive *in vitro* when cultured with either cells lacking cell-surface expression of MHC class I molecules or tumour cells expressing ligands for NK-cell activating receptors. Similar hyporesponsiveness was observed *in vivo*, as these NK cells were inefficient at mediating rejection of bone marrow lacking cell-surface expression of MHC class I molecules, indicating that, for some NK cells, self-tolerance is not a result of inhibitory-receptor interaction with self-MHC class I molecules but of hyporesponsiveness to self.