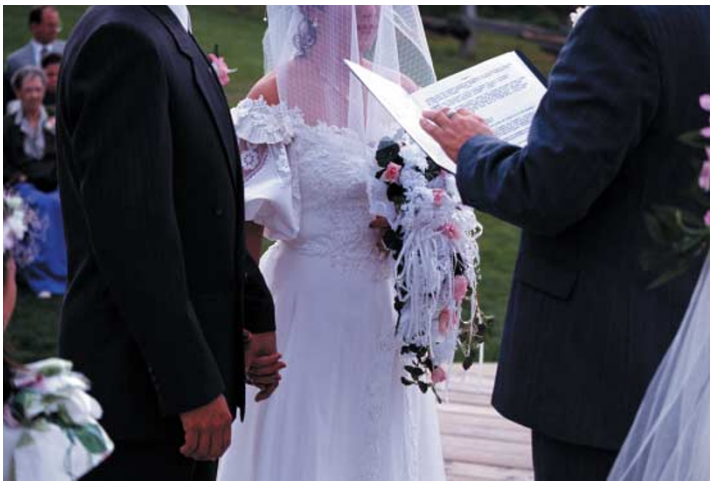
T-CELL DEVELOPMENT

Committing to the CD4 lineage



The molecular mechanisms that regulate one of the key decisions in T-cell development — the CD4/CD8 lineage choice — have been difficult to identify. Now, in a study published in *Nature*, the transcription factor T-helper-inducing POZ/Krüppel-like factor (Th-POK; also known as cKROX and ZFP76) is characterized as the central regulator of this decision.

Positive selection of a thymocyte occurs if its T-cell receptor (TCR) interacts with a self-peptide–MHC complex. The CD4/CD8 lineage choice occurs concomitantly with this, and it correlates with TCR engagement of self-peptide–MHC class II or class I, respectively. Previous studies of mice with a spontaneous recessive mutation, the helper deficient (HD) mutation, identified a locus that is required for CD4 lineage commitment, because in these mice, MHC-class-II-restricted thymocytes are redirected to the CD8 lineage. So, He *et al.* set out to characterize the molecular defects in HD mice. Initial studies showed that the redirection of MHC-class-II-restricted thymocytes to the CD8 lineage was not the result of either a defect in CD4 expression or inappropriate upregulation of CD8 expression. Similarly, no defects in signalling downstream of the TCR were detected, indicating that the HD mutation affects only T-cell lineage commitment.

Genetic mapping and a bacterial-artificial-chromosome complementation approach were used to identify *Th-pok* as the candidate HD gene. Consistent with this, in HD mice, cDNA encoding Th-POK was shown to have a single nucleotide substitution that resulted in an amino-acid substitution at a position predicted to mediate DNA binding.

In the thymus of wild-type mice, mRNA encoding Th-POK was specifically expressed by CD4 single positive (SP) thymocytes and by MHC-class-II-restricted $CD4^+CD8^{low}$ thymocytes. Overexpression of wild-type Th-POK by bone-marrow cells from HD mice led to the TCR-dependent generation of $CD4^+$ SP thymocytes and an absence of $CD8^+$ SP thymocytes. Further evidence of a crucial role for Th-POK in lineage commitment was provided by the observation that the $CD8^+$ SP thymocyte population found in transgenic mice expressing an MHC-class-I-restricted TCR was absent if the T cells of these mice were engineered to express Th-POK. Instead, these mice had $CD4^+$ SP thymocytes that expressed *Gata3* mRNA (a marker of CD4 lineage commitment) but not perforin mRNA (a marker of CD8 lineage commitment).

This study shows that Th-POK is a crucial regulator of T-cell lineage commitment: during positive selection its expression leads to CD4 lineage commitment, and only in its absence can a cell become committed to the CD8 lineage. Future studies to identify the factors controlled by Th-POK will provide insight into the molecular pathways that determine the CD4/CD8 lineage choice.

Karen Honey

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IMMUNE REGULATION

NKT-cell defects in XLP

X-linked lymphoproliferative disease (XLP) is a human immunodeficiency syndrome caused by mutations in the gene encoding the adaptor molecule SAP (signalling lymphocytic activation molecule (SLAM)-associated protein). SAP-deficient humans and mice have normal numbers of T cells, B cells and natural killer (NK) cells, but their T cells and NK cells have functional defects that lead to impaired immune responses. Now, Kim Nichols and colleagues show that SAP deficiency blocks the development of natural killer T (NKT) cells and that this seems to be a contributory factor in XLP.

SAP is known to recruit the SRC-family kinase FYN to receptors of the SLAM family. As FYN is known to be required for the development of NKT cells, the authors speculated that NKT-cell defects might contribute to XLP. Reverse-transcriptase PCR analysis showed that SAP is expressed by NKT cells from wild-type mice. When splenocytes from SAP-deficient mice were treated with the NKT-cell-specific agonist α-galactosylceramide, they failed to produce detectable levels of interferon-γ and interleukin-4. To determine whether the NKT-cell defect is quantitative or qualitative, cells from SAP-deficient mice were analysed by flow cytometry: significantly fewer NKT cells were found in SAP-deficient mice than in wild-type mice, indicating that the defect is quantitative.

The authors next asked whether the defect is intrinsic to SAP-deficient haematopoietic cells or non-haematopoietic cells. In experiments using bone-marrow chimeras, NKT cells could develop only from wild-type bone-marrow cells, so the defect is haematopoietic-cell autonomous. When 17 patients with XLP were examined, the authors observed a 97% reduction in NKT-cell numbers in peripheral blood compared with individuals who do not have XLP. Genetic analysis of a female carrier of XLP revealed that X-chromosome inactivation was skewed in NKT cells but not in T or B cells, supporting the idea that SAP is required for NKT-cell development but is dispensable for T- and B-cell development.

This study shows that SAP is crucial for the development of NKT cells in humans and mice and that lack of NKT cells might contribute to XLP. It is not clear which SLAM-family member is associated with SAP in NKT cells, and the details of the signalling pathway have yet to be worked out.

Elaine Bell

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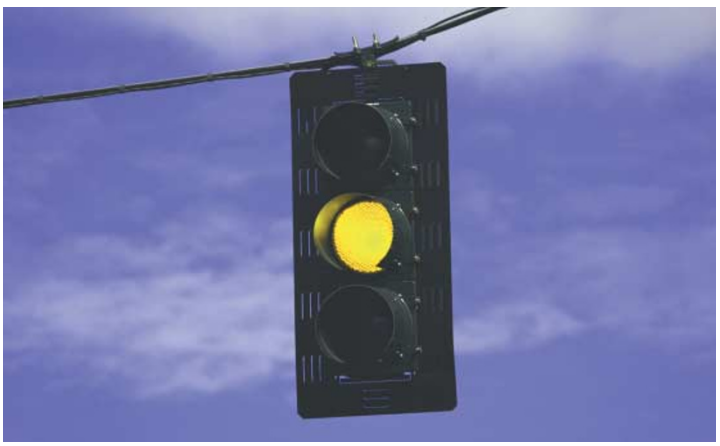
FURTHER READING Nichols, K. E. *et al*. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol. Rev.* **203**, 180–199 (2005)



IN BRIEF

INNATE IMMUNITY

Dual role of α-defensin-1 in anti-HIV-1 innate immunity.

Chang, T. L. *et al*. *J. Clin. Invest.* **115**, 765–773 (2005).

α-Defensin-1 — an antimicrobial peptide that is produced by neutrophils — has been shown to inhibit HIV-1 replication. In this study, Chang *et al.* found that α-defensin-1 directly inactivates HIV-1 virions but that this inactivation is abrogated in the presence of serum or increased numbers of virus particles. Instead, in the presence of serum, α-defensin-1 mediates its antiviral effects on the target cells. Interestingly, protein kinase C (PKC) phosphorylation was reduced in $CD4^+$ T cells treated with α-defensin-1, and HIV-1 replication was blocked at similar stages in $CD4^+$ T cells treated with either α-defensin-1 or an inhibitor of PKC-α and PKC-β. α-Defensin-1, therefore, mediates its cellular effects, at least in part, through inhibition of PKC-signalling pathways.

VIRAL IMMUNITY

Inverse correlation between IL-7 receptor expression and CD8 T cell exhaustion during persistent antigen stimulation.

Lang, K. S. *et al*. *Eur. J. Immunol.* **35**, 738–745 (2005).

Infection with lymphocytic choriomeningitis virus is characterized by persistence of the virus and is often associated with exhaustion of $CD8^+$ T cells. In this study, the authors show that persistent viral antigen suppressed the expression of the interleukin-7 receptor α-chain (IL-7Rα) by antigen-specific T cells. Because IL-7 is associated with the survival of memory T cells, prolonged downregulation of its receptor correlated with diminished T-cell responses in chronically infected mice. By contrast, the presence of short-lived antigen resulted in only transient suppression of IL-7Rα expression and did not cause T-cell exhaustion, indicating that antigen longevity can determine T-cell fate.

T-CELL RESPONSES

The role of herpesvirus entry mediator as a negative regulator of T cell-mediated responses.

Wang, Y. *et al*. *J. Clin. Invest.* **115**, 711–717 (2005).

Herpesvirus entry mediator (HVEM) has previously been described as a T-cell co-stimulatory receptor, but to the surprise of these researchers, HVEM-deficient T cells, when compared with wild-type T cells, showed increased proliferative responses after stimulation with CD3-specific antibody or exposure to concanavalin A (conA) *in vitro*. Furthermore, they showed that administration of conA to HVEM-deficient mice resulted in increased morbidity and mortality, owing to the development of severe T-cell-mediated hepatitis. And compared with wild-type splenocytes, conA-treated HVEM-deficient splenocytes produced higher levels of several cytokines. Finally, HVEM-deficient mice showed increased susceptibility to experimental allergic encephalomyelitis. Together, these results indicate that HVEM can function as a negative regulator of T-cell responses.

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

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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

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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

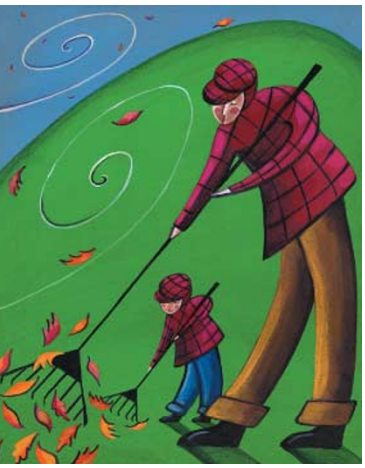
A subset of natural killer cells achieve self-tolerance without expressing inhibitory receptors specific for self MHC molecules.

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.

INFLAMMATION

D6 clears away inflammatory chemokines



Although much is known about the initiation of pro-inflammatory responses, relatively little is known about how these responses are resolved. In a recent report in *Nature Immunology*, Thomas Jamieson *et al.* show that the chemokine receptor D6 is involved in clearing β-chemokines from inflamed skin and therefore in resolving inflammation.

D6 is a seven-membrane-spanning receptor that binds pro-inflammatory members of the β-chemokine family, such as CC-chemokine ligand 2 (CCL2; also known as MCP1) and CCL3 (also known as MIP1α), but it does not bind constitutively expressed β-chemokines, such as CCL19 and CCL21. However, unlike other chemokine receptors, D6 does not seem to signal after ligand binding but, instead, internalizes its ligands and targets them for degradation, indicating that D6 might function as a decoy receptor. So, to test the *in vivo* functions of D6, the authors generated D6-deficient mice and analysed their responses in models of cutaneous inflammation. At 8 and 18 hours after the induction of inflammation in the skin by the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), the concentrations of CCL2 and CCL3 were similar in the skin of both D6-deficient and wild-type mice. However, by 24 hours, the concentrations of these chemokines were significantly higher in the D6-deficient mice, which is consistent with a role for D6 in the post-inflammatory clearance of β-chemokines from the skin. By contrast, levels of the non-D6-binding chemokine CXC-chemokine ligand 2 (CXCL2; also known as MIP2) did not differ between wild-type and D6-deficient mice at any time point.

The authors next asked whether the increased concentrations of residual pro-inflammatory β-chemokines had pathological consequences. Indeed, although inflammation in wild-type mice induced by 3 applications of TPA resolved after 4 days, D6-deficient mice developed skin pathology that was characterized by epidermal hyperproliferation and inflammatory-cell infiltration — similar to the pathology of human psoriasis. They showed that this process was dependent on tumour-necrosis factor (TNF), as TNF-specific antibodies completely abrogated the

ANTIGEN PRESENTATION

A new route to cross-presentation

MHC class I molecules usually present peptides that are derived from endogenous antigens. But they can also present peptides that are derived from exogenous antigens, through a process known as cross-presentation. Various mechanisms have been proposed to explain how this occurs. Now, Joost Neijssen and colleagues propose a novel mechanism — which they have named gap-junction-mediated immunological coupling (GMIC) — to add to the list.

Gap junctions are channels that form between adjacent cells. Each cell contributes a hemichannel of six connexin molecules to the functional gap junction. These channels allow the passive exchange of ions, nutrients and signalling components between cells. Haematopoietic cells express connexin-43, so the authors set out to investigate the role of gap junctions in peptide transfer between cells and whether this could contribute to cross-presentation.

First, the authors looked at whether peptides can be transferred between cells through gap junctions. The human squamous-cell carcinoma line A431, which does not form gap junctions, was stably transfected with connexin-43, resulting in the formation of gap junctions. Non-degradable, fluorescently labelled peptides were introduced into these cells, and peptide transfer was analysed by confocal microscopy. This showed that such transfer was possible and could be blocked using gap-junction inhibitors. The rate of peptide transfer was also determined and was shown to be inversely proportional to the size of the peptide: that is, the transfer rate was decreased for longer peptides.

Because the cytoplasm contains several cytosolic peptidases, the authors next looked at the impact of these peptidases on peptide transfer between cells. Using quenched peptides that become fluorescent after degradation, they found that cytosolic peptidases can limit, but not prevent, the spread of peptides from cell to cell.

So, does this peptide transfer have immunological relevance? To test this, the authors expressed an influenza-derived peptide in connexin-43-transfected A431 cells, which do not express HLA-A2, and they cultured these cells with *HLA-A2*-transfected A431 cells. When HLA-A2-restricted T cells specific for the influenza-derived peptide were added to the culture, a T-cell response was detected, showing that peptide transfer had occurred through gap junctions. Next, the authors carried out similar experiments using primary human monocytes. Connexin-43-transfected A431 cells were infected with influenza, and after 16 hours, viral propagation was inhibited. These infected cells were then cultured with primary human monocytes that had been stimulated to express connexin-43 by exposure to interferon-γ and tumour-necrosis factor. The infected cells were loaded with a dye such that monocytes that had obtained peptides through gap junctions were detectable. Only dye-containing monocytes were found to stimulate a cytotoxic T-lymphocyte response.

These findings indicate a novel mechanism for cross-presentation that does not require release of intracellular antigens from cells. Interestingly, Langerhans cells and intestinal dendritic cells have many gap-junction contacts with surrounding cells, and monocytes can form gap junctions in response to 'danger' signals. In addition, gap junctions are inactivated in many tumour cells, indicating that this is another mechanism by which tumour cells might avoid detection. Apoptotic bodies can be cross-presented to $CD8^+$ T cells, but in situations in which cells are prevented from undergoing apoptosis, cross-presentation could be achieved by GMIC.

Elaine Bell

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psoriasiform pathology. Further analysis of the inflammatory infiltrate indicated that T cells and mast cells accumulated at high levels in the inflamed skin of D6-deficient mice but not in that of wild-type mice. Infiltration of T cells into the epidermis was partially responsible for the subsequent accumulation of dermal mast cells, which contribute to development of the pathology through their release of granule products.

So, D6 functions *in vivo* as a decoy receptor, clearing away residual β-chemokines in inflamed skin, thereby helping to avoid aberrant recruitment of inflammatory cells and subsequent pathology.

Lucy Bird

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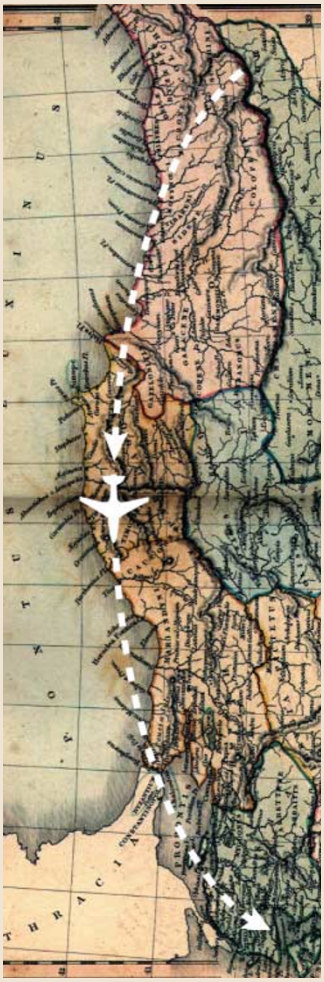

THYMIC DEVELOPMENT

TRAF6 distributes tolerance

Medullary thymic epithelial cells (mTECs) are important for the negative selection of thymocytes. However, the molecular regulation of mTEC differentiation and organization within the thymus is not well understood. New insight into this process has now been provided by a study published in *Science*, which shows that tumour-necrosis-factor receptor (TNFR)-associated factor 6 (TRAF6) is required for the differentiation and organization of mTECs and for the induction of T-cell self-tolerance.

TRAF6 is a cytoplasmic adaptor protein that transduces signals from several members of the TNFR superfamily, leading to the activation of transcription factors such as activator protein 1 (AP1) and nuclear factor-κB (NF-κB). Previous studies from Jun-ichiro Inoue's group have shown that the thymi of TRAF6-deficient mice are atrophied, leading the researchers to hypothesize that TRAF6 is important for thymic organogenesis. In this new study, the medulla was shown to be smaller in thymi from TRAF6-deficient mice than in thymi from wild-type animals, and the corticomedullary junction was ill defined. mTECs were present in the *Traf6*$^{-/-}$ thymi, but they were dispersed and not clustered in the medulla; they also failed to bind *Ulex europaeus* agglutinin-1 (UEA-1), a lectin that binds mature mTECs.

In addition to impaired maturation, TRAF6-deficient mTECs also expressed reduced levels of *Aire* mRNA, which encodes a protein that induces expression of tissue-specific antigens (TSAs) such that developing thymocytes are tolerized to these TSAs in the thymus. Consistent with this, TRAF6-deficient mice developed inflammation in several organs, including the lungs, liver and pancreas. Such inflammation in TRAF6-deficient mice was probably not only a result of decreased expression by mTECs of mRNA encoding TSAs but also a result of the reduced numbers of $CD4^+CD25^+$ regulatory T (T_{Reg}) cells. Similar inflammatory infiltrates were observed when TRAF6-deficient thymi depleted of haematopoietic cells were transplanted into nude mice, indicating that an intrinsic non-haematopoietic-cell defect was responsible for the autoimmune phenotype of TRAF6-deficient mice.

The phenotype of TRAF6-deficient mice was similar to that observed in mice lacking the NF-κB-family member REL-B. So, the authors examined fetal thymic stroma isolated from *Traf6*$^{-/-}$ mice and found decreased levels of REL-B in these cells compared with wild-type fetal thymic stroma. Furthermore, when TRAF6 was complemented in TRAF6-deficient mTEC lines, REL-B levels increased. Additional support for the idea that TRAF6 induces *Rel-b* transcription is provided by the presence of two NF-κB- and putative AP1-binding sites (transcription factors that are known to be activated downstream of TRAF6 signalling) in the *Rel-b* promoter.

This study identifies TRAF6 as a crucial molecular component of the signalling pathway that regulates mTEC differentiation and organization in the thymus. TRAF6 is also important in the development of self-tolerance, and future studies will focus on how TRAF6-induced REL-B regulates expression of *Aire* and development of T_{Reg} cells. Interestingly, the phenotype of TRAF6-deficient mice is also similar to that of mice lacking NF-κB-inducing kinase (NIK), which regulates the proteasomal processing of the NF-κB-family member p100 to generate the REL-B-binding partner p52. Understanding the relationship between NIK- and TRAF6-regulated activation of NF-κB-family members will help to define the molecular control of thymic organogenesis and central tolerance.

Karen Honey

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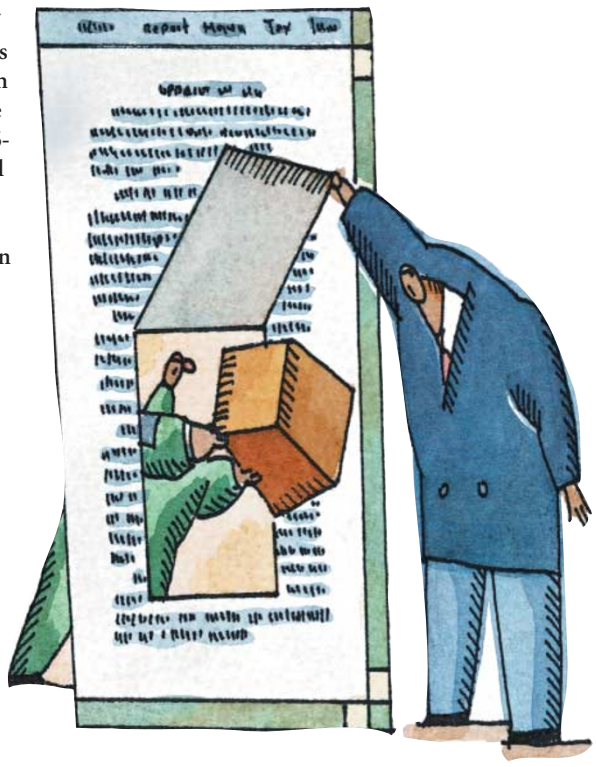

T-CELL RESPONSES

Function and partner found for orphan SIRP-β2

Until now, the expression, specificity and function of the recently identified member of the signal-regulatory protein (SIRP) family, SIRP-β2, was unknown. Reporting in *Blood*, Marco Colonna and colleagues now show that SIRP-β2 has an important role in T-cell adhesion to antigen-presenting cells (APCs), through binding CD47, and that this co-stimulates T-cell proliferation.

So far, three members of the SIRP family of transmembrane glycoproteins have been identified: SIRP-α, SIRP-β1 and SIRP-β2. SIRP-α is an inhibitory receptor that modulates macrophage and dendritic-cell function after it binds CD47 and undergoes phosphorylation of its cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). By contrast, SIRP-β1 lacks ITIMs but associates with the adaptor protein DAP12, through a lysine residue in its transmembrane domain, to mediate activating signals. SIRP-β2, however, lacks both ITIMs and the transmembrane lysine residue, so the authors proposed that it might be involved in cell–cell adhesion rather than in promoting inhibition or activation.

First, the authors showed that, unlike SIRP-α and SIRP-β1 (which are mainly expressed by myeloid cells), SIRP-β2 is expressed by T cells and activated natural killer cells. But similar to SIRP-α, SIRP-β2 binds CD47, although the affinity of this interaction is lower for SIRP-β2 than for SIRP-α. Expression of SIRP-β2 allowed T cells to adhere to cells expressing CD47. Moreover, the SIRP-β2–CD47 interaction between T cells and APCs promoted antigen-specific T-cell proliferation and co-stimulated T-cell activation to a similar extent to ligation of the co-stimulatory molecule CD28.

These results considerably extend our current knowledge of this new SIRP-family

member, and they implicate SIRP-β2 as an important molecule in the regulation of T-cell responses.

Lucy Bird

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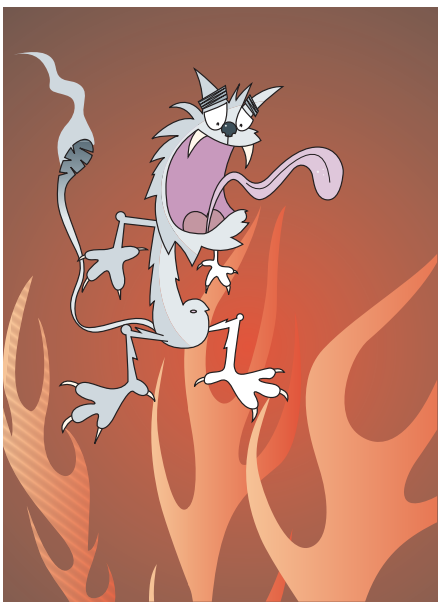
FURTHER READING Brooke, G., Holbrook, J. D., Brown, M. H. & Barclay, A. N. Human lymphocytes interact directly with CD47 through a novel member of the signal regulatory protein (SIRP) family. *J. Immunol.* **173**, 2562–2570 (2004) | Oldenborg, P.-A. CD47 and SIRPs: new openings. *Blood* **105**, 2245–2246 (2005)

AUTOIMMUNITY

Out of the frying pan into the fire

Whereas the pathogenesis of some autoimmune diseases, such as rheumatoid arthritis and psoriasis, is associated with tumour-necrosis factor (TNF), other diseases such as systemic lupus erythematosus (SLE) and type 1 diabetes are thought to involve interferon-α (IFN-α). Jacques Banchereau and colleagues have studied the reciprocal relationship between these two cytokines in autoimmune disease to explain the finding that treatment of rheumatoid arthritis with TNF antagonists, although an effective therapy for many patients, can induce features of SLE, such as an increased titre of nuclear-specific antibodies.



Peripheral-blood mononuclear cells (PBMCs) isolated from paediatric patients with systemic-onset juvenile arthritis and treated with TNF-specific antibodies were shown to express a set of genes that are known to be upregulated by IFN-α. Because inhibition of TNF is associated with increased transcription of IFN-α-regulated genes, TNF might usually function to downregulate IFN-α responses. Indeed, adding TNF to PBMCs that were isolated from healthy donors and cultured with influenza virus inhibited the production of IFN-α. This was shown to be the result of targeting immature plasmacytoid dendritic cells (pDCs), which are one of the main sources of IFN-α *in vivo*. pDCs were cultured *in vitro* from $CD34^+$ haematopoietic stem cells (HSCs) and then exposed to influenza virus; addition of TNF to the culture inhibited IFN-α production by up to 40%, by stimulating pDC maturation. Conversely, pretreatment of pDCs *in vitro* with TNF-specific antibody resulted in threefold higher levels of IFN-α when the pDCs were re-exposed to influenza virus, by inhibiting virus-induced pDC maturation. Finally, TNF was also shown to block the generation of pDCs, but not myeloid DCs, from HSCs.

These results led the authors to conclude that endogenous TNF controls the production of IFN-α by immature pDCs by inhibiting the generation of these cells and by stimulating their maturation. Therefore, decreased levels of TNF in patients treated with TNF antagonists will result in increased IFN-α levels. This is thought to lead to SLE-like symptoms through stimulating the maturation of myeloid DCs, which can then activate, rather than tolerize, autoreactive T and B cells. This cross-regulation between TNF and IFN-α is supported by the fact that patients with SLE have increased levels of circulating soluble TNF receptors, which correlate with disease activity.

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References and links

ORIGINAL RESEARCH PAPER Palucka, A. K., Blanck, J.-P., Bennett, L., Pascual, V. & Banchereau, J. Cross-regulation of TNF and IFN-α in autoimmune diseases. *Proc. Natl Acad. Sci. USA* **102**, 3372–3377 (2005)