REGULATORY T CELLS

Perfect partnership

The function of $CD4^+CD25^+$ regulatory T (T_{Reg}) cells is mediated by cooperation between a transcription factor that functions as a lineage-specification factor for T_{Reg} cells and a transcription factor that regulates T-cell activation and anergy, report Anjana Rao and colleagues in *Cell*.

The nuclear factor of activated T cells (NFAT) family of proteins regulates T-cell activation but is also involved in the control of thymocyte development, T-cell differentiation and self-tolerance.

> NFAT is a common regulator in the immune system, where it can switch transcriptional partners

Following its activation, NFAT is dephosphorylated and translocates to the nucleus, where it forms strong, cooperative complexes with activator protein 1 (AP1) to induce the expression of a large number of genes that are central to eliciting an immune response. Another transcription factor, forkhead box P3 (FOXP3), is expressed by T_{Reg} cells and has been identified as a major marker and regulator of the development and function of T_{Reg} cells.

Many of the genes that are targeted by NFAT are also regulated by FOXP3: for example, NFAT activates the interleukin-2 (*IL2*) and *IL4* genes, whereas FOXP3 represses them, and both NFAT and FOXP3 upregulate expression of the T_{Reg}-cell markers cytotoxic T-lymphocyte antigen 4 (CTLA4) and CD25. So by what mechanism does FOXP3 influence the expression of NFAT-dependent genes? Rao and colleagues investigated the molecular interactions between NFAT and FOXP3. They found that FOXP3 carried out its repressive activity by targeting the cooperative NFAT–AP1 complexes specifically, rather than other NFAT configurations. FOXP3 formed a cooperative complex with NFAT on DNA, excluding AP1 without displacing NFAT from the DNA, as had been proposed previously.

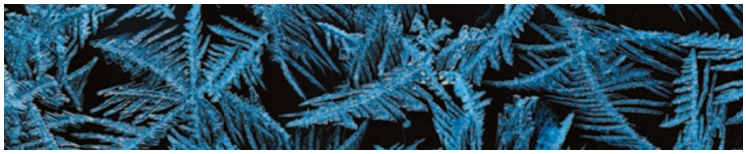
Because the sequences of the forkhead domains of FOXP proteins are highly conserved, the authors could predict the structure of the NFAT–FOXP3 complex based on the crystal structure of an NFAT–FOXP2–DNA complex that they had solved. They then introduced graded, structure-guided mutations into the NFAT-interacting residues of FOXP3 and predicted that these mutations would progressively disrupt the interaction between FOXP3 and NFAT.

To compare the activities of wild-type and mutant FOXP3 proteins, Rao and colleagues expressed both proteins in primary mouse $CD4^+$ T cells. The graded mutations at the NFAT–FOXP3 interface did indeed cause a progressive loss of FOXP3 function, and interfered with the ability of FOXP3 to repress expression of IL-2, to upregulate expression of CTLA4 and CD25, and to confer suppressor function in an *in vivo* mouse model of autoimmune diabetes.

The authors suggest that NFAT is a common regulator in the immune system, where it can switch transcriptional partners from AP1 to FOXP3, thereby converting the effector T-cell activation programme into the T_{Reg}-cell suppressor programme. Because the selective blocking of the interaction of NFAT with AP1, without interfering with the NFAT–FOXP3 interaction, could therefore induce tolerance, these findings might have important therapeutic implications.

Sharon Ahmad

ORIGINAL RESEARCH PAPER Wu, Y. *et al.* FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* **126**, 375–387 (2006)
FURTHER READING Rudensky, A. Y., Gavin, M. & Zheng, Y. FOXP3 and NFAT: partners in tolerance. *Cell* **126**, 253–256 (2006)



INFLAMMATION

Gouty inflammation crystal clear?

The excruciatingly painful inflammatory condition gout is one of the oldest-recorded human afflictions. It is now well known that acute gouty inflammation results from the deposition of monosodium urate (MSU) crystals in the joints of individuals with hyperuricaemia. But exactly how MSU crystals are linked to the inflammatory response has puzzled scientists for centuries. Kenneth Rock and colleagues now reveal that interleukin-1 (IL-1) and signalling through the IL-1 receptor are crucial components of the inflammatory cascade that is triggered by MSU crystals.

A previous observation by these authors led them to propose that MSU crystals might function as danger signals and trigger an innate immune response in a manner similar to microbial molecules — that is, through Toll-like receptors (TLRs). To test this hypothesis, Chen *et al.* injected MSU crystals into the peritoneal cavity of either wild-type mice or mice deficient in various TLRs and then monitored the inflammatory response by quantifying the influx of neutrophils. Surprisingly, none of the eight TLR-deficient mouse strains analysed showed a defect in neutrophil influx. They confirmed these results, and excluded a role for TLR5 and TLR8 (for which gene-knockout mice are unavailable) in the inflammatory response, by showing that cells transfected with genes that encode these TLRs also did not respond to MSU crystals.

Although these studies rendered improbable the hypothesis that MSU crystals exert their function directly through TLRs, the authors examined the role of the Toll/IL-1 receptor (TIR)-domain-containing adaptor proteins that are involved in TLR signalling: MyD88 (myeloid differentiation primary-response protein 88), TIRAP (TIR-domain-containing adaptor protein), TRIF (TIR-domain-containing adaptor protein inducing interferon-β) and TRAM (TRIF-related adaptor molecule). Although a deficiency in TIRAP, TRIF or TRAM had no effect on neutrophil influx, mice deficient in MyD88 showed marked defects in the inflammatory response, including in neutrophil influx and pro-inflammatory cytokine production. So MSU crystals seem to stimulate inflammation through a MyD88-dependent pathway that does not involve TLRs.

In addition to mediating TLR signalling, MyD88 is downstream of the IL-1 and IL-18 receptors. Consistent with a role for the IL-1 receptor, mice lacking this receptor but not the IL-18 receptor showed defective inflammatory responses to MSU crystals (similar to MyD88-deficient mice). In addition, treatment of mice with neutralizing antibody specific for IL-1 also markedly reduced the inflammatory response.

Further studies led the authors to propose that MSU crystals induce cells to produce IL-1. IL-1 then binds non-bone-marrow-derived cells that express the IL-1 receptor, resulting in MyD88-dependent amplification of the pro-inflammatory response — that is, further IL-1 production and neutrophil recruitment.

Although several steps in the inflammatory cascade remain to be defined, elucidation of a central role for IL-1 in gouty inflammation provides a potential new target for the treatment of gout.

Lucy Bird

ORIGINAL RESEARCH PAPER Chen, C.-J. *et al.* MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. *J. Clin. Invest.* **116**, 2262–2271 (2006)
FURTHER READING Martinon, F. & Glimcher, L. H. Gout: new insights into an old disease. *J. Clin. Invest.* **116**, 2073–2075 (2006)

IN BRIEF

DENDRITIC CELLS

NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells.

Savina, A. *et al. Cell* **126**, 205–218 (2006)

A new study shows that the enzyme NADPH oxidase 2 (NOX2), which is an important component of the innate immune response mediated by neutrophils, also has a role in adaptive immunity, through the control of antigen presentation by dendritic cells (DCs). DCs can 'cross-present' exogenous antigens, in the form of proteolytically cleaved peptides, on MHC class I molecules to $CD8^+$ T cells. To ensure recognition by T cells, DCs have developed specialized ways to control the partial degradation of these exogenous antigens. Savina *et al.* provide the first genetic evidence that a novel specific adaptation of the DC endocytic pathway is required for efficient cross-presentation. They show that NOX2-defective DCs have increased phagosomal acidification and antigen degradation, which causes defective antigen cross-presentation. Therefore, NOX2 is a crucial component in the phagocytic pathway of DCs, allowing DCs to process antigens rather than degrading them.

ASTHMA AND ALLERGY

Role of deficient type III interferon-λ production in asthma exacerbations.

Contoli, M. *et al. Nature Med.* 13 Aug 2006 (doi:10.1038/nm1462)

This study looks at the role of the recently discovered type III interferons IFNλ1 and IFNλ2/3 in the susceptibility of individuals with asthma to acute exacerbations associated with rhinovirus infections. The authors show that the production of IFNλ by primary bronchial epithelial cells and bronchoalveolar macrophages in response to rhinovirus replication is significantly lower for patients with asthma than for individuals without asthma. The decreased IFNλ production correlates with increased viral load and severity of symptoms *in vivo*. This indicates that therapies that aim to replace or augment IFNλ production might be a new approach to the treatment or prevention of asthma exacerbations. Further studies are required to determine the mechanism of deficient IFN production in patients with asthma.

T-CELL RESPONSES

Duration of the initial TCR stimulus controls the magnitude but not functionality of the $CD8^+$ T cell response.

Prlic, M. *et al. J. Exp. Med.* 14 Aug 2006 (doi:10.1084/jem.20060928)

The concept of T-cell programming describes how a brief encounter with antigen is sufficient to trigger a cell-autonomous programme that leads to proliferation and differentiation into memory T cells. Many previous studies of T-cell programming have been limited by the need to provide the timed antigen stimulus to T cells *in vitro* before *in vivo* transfer. This study used peptide-pulsed transgenic dendritic cells that were engineered to be susceptible to diphtheria toxin to allow timed antigen exposure entirely *in vivo*. The authors found that the duration of antigen exposure correlated with the magnitude of the primary response but that $CD8^+$ T-cell effector function and memory was independent of antigen timing above a certain minimum exposure time.

In the news

ALLERGY AND PARKINSON'S DISEASE

For the one in three people who are now thought to suffer from some form of allergy, newspaper reports of a possible link between allergic rhinitis and Parkinson's disease make worrying reading. Headlines such as 'Allergies linked to Parkinson's disease' (*Times Online*, 8 August 2006) and 'Does sneezing point to Parkinson's?' (*Daily Mail*, 8 August 2006) arose from a paper published in the 8 August issue of the journal *Neurology* by investigators at the Mayo Clinic College of Medicine, in Rochester, Minnesota (USA).

This case–control study looked at 196 people who developed Parkinson's disease, matched with 196 people of similar age and gender who did not, over a 20-year period. The authors found that those with allergic rhinitis were 2.9-fold more likely to develop Parkinson's disease later in life. According to the lead author, James Bower, people with allergies might be "more likely to mount an immune response in the brain as well … This may release certain chemicals in the brain and inadvertently kill brain cells".

Although the authors emphasized that the study did not prove that allergies can cause Parkinson's disease and showed only that there is an association between the two conditions, others feel that the study is misleading. As reported in the *Guardian Unlimited* (8 August 2006), the Parkinson's Disease Society (UK) has pointed out that, on the scale of clinical trials, 200 cases is a tiny number from which to draw conclusions. Kieran Breen, Director of Research of the society, thinks that reports of a causal link are "the biggest scaremongering thing you can think of." Instead, he says that the study "may demonstrate that allergic rhinitis may be one of the effects of Parkinson's." (*New Scientist*, 8 August 2006).

Kirsty Minton

T CELLS

Competing for dominance

In an ideal world, a single vaccine would be used to immunize many individuals and elicit a broad range of specific T-cell responses to many pathogen- or tumour-derived antigens. But, even though hundreds of potential epitopes might be available in such a vaccine, the T-cell response tends to become rapidly focused on just a few epitopes. This economizing by the immune system is referred to as immunodominance and has hindered approaches using multivalent vaccines that deliver multiple T-cell epitopes simultaneously. Understanding how immunodominance is achieved and how T cells that recognize different epitopes compete has been the subject of intense research but has yielded inconclusive results. Now, Willis, Kappler and Marrack observe that, at a very early stage of the immune response, there is competition between $CD8^+$ T cells specific for different peptide–MHC complexes when these are presented on the same dendritic cell (DC) *in vivo*.

> "$CD8^+$ T-cell immunodominance caused by competition between T-cell populations can occur in the first few hours of a response"

To measure competition between T cells that recognize different peptide–MHC complexes, the authors used T cells from two T-cell-receptor-transgenic mouse strains: P14 mice, in which T cells are specific for H2-D^b and a peptide derived from lymphocytic choriomeningitis virus; and OT-I mice, in which T cells are specific for H2-K^b and a peptide derived from ovalbumin. P14 T cells were first labelled with a fluorescent dye that allowed the authors to follow cell division and were then transferred to wild-type C57BL/6 mice with or without competing OT-I T cells. The next day, DCs expressing both peptide–MHC complexes were also injected into these mice. In the absence of OT-I T cells, a large number of P14 T cells had undergone several rounds of cell division by day 4. By contrast, in the presence of OT-I T cells, fewer P14 T cells had undergone cell division, indicating that there was competition between the T-cell populations for recruitment into the immune response.

SIGNALLING

Conjugating enzyme IDed

Ubiquitin-conjugating enzyme 13 (UBC13) has now been shown to have a key role in the mammalian immune response, in a study published in *Nature Immunology*. Yamamoto *et al.* describe how this enzyme is important for B-cell development and for B-cell and macrophage activation.

Previous studies indicated that UBC13 might be important for the polyubiquitylation of TRAF2 (tumour-necrosis factor (TNF)-receptor-associated factor 2) and TRAF6, an essential step in the transduction of signals from immune receptors to the key transcription factor nuclear factor-κB (NF-κB). To determine the exact mechanism by which UBC13 influences immune signalling, the authors generated mice in which the expression of UBC13 could be conditionally ablated. Using these mice, the authors showed that UBC13 has an important role in the development of marginal-zone B cells and peritoneal $CD5^+$ B1 cells, as well as in the activation, cell-cycle progression and viability of splenic B cells, and in the generation of *in vivo* humoral responses.

To understand the role of UBC13 in response to Toll-like receptor (TLR) activation, UBC13-deficient peritoneal macrophages were stimulated

Despite this reduction in the number of dividing cells in the presence of a competing T-cell population, the activation state of the responding T cells was not affected. Accordingly, the expression of markers of full activation (CD122 and intracellular interferon-γ) was increased in P14 T cells, both in the presence and the absence of competing OT-I T cells. In addition, the authors observed no change in the rate of proliferation of T cells, with or without competition. Together, these data are consistent with competition occurring at a very early step in the immune response, probably owing to competition for a limiting DC-derived factor.

Analysis using bromodeoxyuridine (BrdU) incorporation into DNA as a measure of DNA synthesis showed that competition occurs earlier than the onset of cell division and reduces the expression of early activation markers (CD25 and CD69), which are expressed 18 hours after immunization with the DCs.

So $CD8^+$ T-cell immunodominance caused by competition between T-cell populations can occur in the first few hours of a response. Such competition should therefore be carefully considered by researchers who are designing multivalent vaccines with the aim of generating broad T-cell responses.

Lucy Bird

ORIGINAL RESEARCH PAPER Willis, R. A., Kappler, J. W. & Marrack, P. C. CD8 T cell competition for dendritic cells *in vivo* is an early event in activation. *Proc. Natl Acad. Sci. USA* **103**, 12063–12068 (2006)

with various TLR ligands. Less TNF, interleukin-6 (IL-6) and IL-12p40 was produced by these cells than by UBC13-sufficient cells. Surprisingly, activation of NF-κB was not markedly affected. However, in the absence of UBC13, activation of members of the mitogen-activated protein kinase (MAPK) family was severely impaired in response to various stimuli, including TLR ligands and IL-1β, but not TNF.

To understand at which point in the signal-transduction pathway UBC13 was having an effect, the authors then examined TLR- or IL-1β-induced polyubiquitylation of some of the signalling molecules involved. Polyubiquitylation of TRAF6 was similar in both UBC13-deficient mouse embryonic fibroblasts (MEFs) and UBC13-sufficient MEFs. IL-1β-induced activation of TAK1 (transforming-growth-factor-β-activated kinase 1), which is activated by TRAF6 and is known to activate MAPKs, was slightly affected by the absence of UBC13 in MEFs. However, the authors found that UBC13-dependent polyubiquitylation of IKKγ (inhibitor-of-NF-κB kinase γ) might be involved in the activation of members of the MAPK family. Further studies are now needed to elucidate more fully the involvement of IKKγ in the activation of MAPKs and NF-κB.

So this study shows that UBC13 is involved in the activation of MAPKs, in the generation and activation of B cells, and in the activation of macrophages, indicating that this enzyme has an important role in immune responses.

Olive Leavy

ORIGINAL RESEARCH PAPER Yamamoto, M. *et al.* Key function for the Ubc13 E2 ubiquitin-conjugating enzyme in immune receptor signaling. *Nature Immunol.* 23 July 2006 (doi:10.1038/ni1367)

IN BRIEF

MAST CELLS

Mast cells can enhance resistance to snake and honeybee venoms.

Metz, M. *et al. Science* **313**, 526–530 (2006)

Snake and bee venoms activate mast cells, inducing degranulation and release of biologically active mediators. The idea that such mediators contribute to the tissue injury associated with a bite or sting has been overturned by a new study that shows, instead, that mast-cell activation ameliorates the pathogenic effects of venom. Mice lacking mast cells were tenfold more sensitive than wild-type mice when injected with venom or purified venom toxin. The transfer of mast cells from wild-type mice to the deficient mice restored resistance. Protection was mediated, in part, by proteases capable of digesting venom components. Because mast cells contain cytoplasmic granules that are rich in proteases, they could be active against toxins of diverse origin. So, in contrast to their widely known pathogenic role in allergic disorders, mast cells seem to have a beneficial role in host defence against animal venoms.

IMMUNOTHERAPY

Inhibition of T cell activation and autoimmune diabetes using a B cell surface-linked CTLA-4 agonist.

Fife, B. T. *et al. J. Clin. Invest.* **116**, 2252–2261 (2006)

Cytotoxic T-lymphocyte antigen 4 (CTLA4) has a crucial role in the regulation of T cells and immune tolerance. However, attempts to target CTLA4 directly as a means of suppressing T-cell-mediated autoimmune disorders, such as diabetes, have proved unsuccessful. Agonists of CTLA4 crossreact with other receptors, and CTLA4-specific antibodies administered alone do not stimulate the appropriate signals. In a new approach, the authors developed transgenic mice that have a single-chain, membrane-bound CTLA4-specific antibody expressed by antigen-presenting B cells, which have a crucial role in the non-obese diabetic (NOD) mouse model of diabetes. The presence of these B cells in NOD mice inhibited the spontaneous development of autoimmune diabetes, even in the absence of regulatory T cells. This model system shows that T-cell engagement of a CTLA4 agonist on B cells can block *in vivo* T-cell responses to antigens presented by these B cells, and it opens up a new avenue for immunotherapy.

HIV

Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly.

Saad, J. S. *et al. Proc. Natl Acad. Sci. USA* **103**, 11364–11369 (2006)

Targeting of the retroviral protein Gag (group-specific antigen) to the plasma membrane is essential for the assembly of immature HIV virions and for their budding and release from infected cells. Nuclear-magnetic-resonance studies have now shown that plasma-membrane targeting of Gag is achieved by binding of the matrix (MA) domain of Gag to the membrane lipid phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$). Binding of the MA domain to $PtdIns(4,5)P_2$ leads to a conformational change that exposes a myristyl group in the MA domain, which can then attach to the plasma membrane and stabilize Gag binding. The authors suggest that blocking the MA–$PtdIns(4,5)P_2$ interaction could be a new therapeutic strategy, particularly because the relevant region of the MA domain does not seem to mutate much in HIV-1.

REGULATORY T CELLS

Suspended license to kill

> T_{Reg} cells can selectively attenuate the cytotoxicity of CTLs

A mechanism to explain how $CD4^+CD25^+$ regulatory T (T_{Reg}) cells impair cytotoxic T lymphocyte (CTL) function is reported in *Immunity*. Mempel, Pittet and colleagues used multi-photon intravital microscopy to analyse how CTLs interact with target antigen-presenting cells (APCs) in the presence or absence of activated T_{Reg} cells in the lymph nodes of mice. They found that T_{Reg} cells can selectively interfere with the release of lytic granules by CTLs in a reversible, transforming growth factor-β (TGFβ)-dependent manner.

There is a large body of work highlighting the importance of T_{Reg} cells in the active suppression of the immune system, but there is considerably less evidence about the precise mechanism by which T_{Reg} cells mediate their suppressive effects. T_{Reg} cells have been shown to impair the effector functions of primed CTLs, so the authors investigated several possible mechanisms to explain this impairment, by directly visualizing interactions among CTLs, target APCs and T_{Reg} cells in mouse lymph nodes *in vivo*. They established that suppression of CTL function by T_{Reg} cells can occur exclusively by compromising lytic activity. The clonal expansion, distribution and motility of CTLs, as well as their ability to detect agonist T-cell-receptor ligands on target APCs and to form stable antigen-specific cell–cell conjugates, remained unaffected.

The lytic activity of CTLs occurs mainly through the calcium-dependent release of specialized lytic lysosomal granules after recognition of antigen at the surface of a target cell. So how is this activity compromised by T_{Reg} cells? The authors found that, although the granule content of CTLs and their expression of lytic effector molecules did not change in the presence of T_{Reg} cells, there was delayed lytic-granule release, which impaired the ability of CTLs to induce target-cell death before the cell–cell conjugates dissociate.

Further investigation showed that suppression of CTL function

NATURAL KILLER CELLS

When killers come good

The precise mechanism by which natural killer (NK) cells can promote the induction of tolerance to certain allografts has now been solved. Recent research published in *The Journal of Experimental Medicine* shows, in a skin-transplantation model, that host NK cells kill graft-derived antigen-presenting cells (APCs), thereby preventing these APCs from migrating to lymphoid and non-lymphoid sites in the host, where they directly activate alloreactive T cells.

Transplant rejection involves the priming of alloreactive host T cells in secondary lymphoid organs, mainly by graft-derived APCs. Therefore, the ability of these donor APCs to survive and migrate in the host might be crucial to the induction of the rejection response. NK cells recognize and kill foreign cells displaying MHC class I molecules that are mismatched with those of the host (known as allogeneic cells). Therefore, Yu *et al.* examined the specific role of NK cells in preventing the migration of donor APCs and the activation of alloreactive T cells in a skin-transplantation model.

> the ability of NK cells to recognize and kill allogeneic APCs regulates the activation of alloreactive T cells in skin-transplantation models

The authors examined the migration of donor APCs in two mouse strains: mice deficient in recombination-activating gene (RAG) protein, which are devoid of T and B cells; and mice deficient in both RAG protein and the common cytokine-receptor γ-chain (γ_c), which additionally lack NK cells. Following transplantation of skin allografts from DBA/2 mice, donor cells (including dendritic cells, DCs) were found in the spleens of RAG- and γ_c-deficient mice but were absent from the spleens of RAG-deficient mice. The authors then directly transferred purified DCs from DBA/2 mice to these two mouse strains. They found high numbers of allogeneic DCs in the spleens, livers and lungs of mice deficient in both proteins but not mice deficient in RAG protein alone, indicating that NK cells have a crucial role in preventing the survival and dissemination of donor DCs in host mice.

To determine whether these allogeneic DCs could stimulate the activation of T cells in the absence of NK cells, allogeneic DCs from DBA/2 mice were transferred to both strains of mice, and T cells that were genetically similar to those of the host mice were transferred 2 weeks later. Transferred T cells that were recovered 3 days later from the spleen, liver and lungs of RAG- and γ_c-deficient mice had undergone multiple rounds of cell division and readily produced interferon-γ, whereas T cells that were recovered from RAG-deficient mice had not undergone cell division. These data indicate that, in the absence of NK cells, allogeneic DCs can induce the activation of alloreactive T-cells at multiple sites in host mice.

So the ability of NK cells to recognize and kill allogeneic APCs regulates the activation of alloreactive T cells in skin-transplantation models, highlighting that NK cells are potential therapeutic targets for the induction of tolerance to transplants.

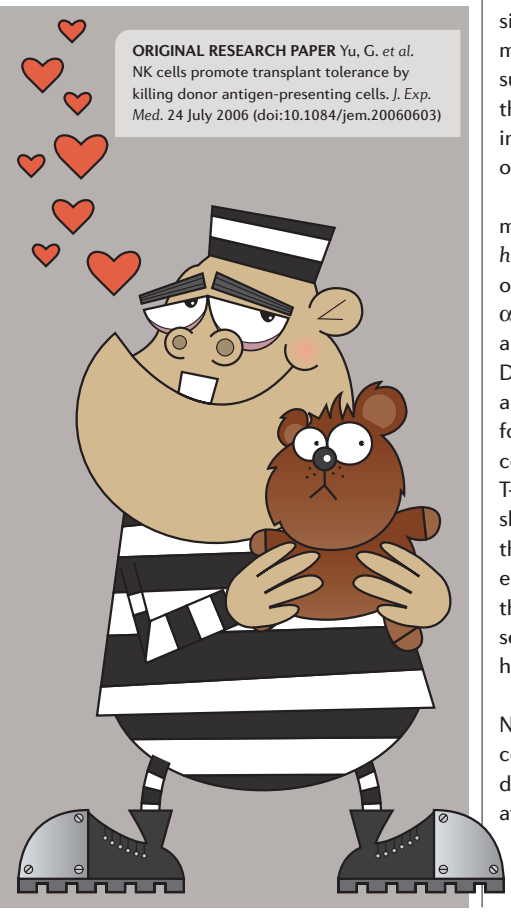
Olive Leavy

by T_{Reg} cells was dependent on TGFβ-induced signalling, although Mempel, Pittet and colleagues conceded that other suppressive signals might also be required. They also found that T_{Reg}-cell-mediated suppression of CTLs was reversible, because sustained CTL suppression required the continuous presence of T_{Reg} cells, although prolonged CTL–T_{Reg}-cell contact was not required.

The authors have shown that T_{Reg} cells can selectively attenuate the cytotoxicity of CTLs, seemingly without affecting their priming or differentiation. These findings, they note, could have implications for the design of therapeutic strategies, particularly for those strategies that rely on the modulation of ongoing immune responses.

Sharon Ahmad

ORIGINAL RESEARCH PAPER Mempel, T. R. *et al.* Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. *Immunity* **25**, 129–141 (2006)



ORIGINAL RESEARCH PAPER Yu, G. *et al.* NK cells promote transplant tolerance by killing donor antigen-presenting cells. *J. Exp. Med.* 24 July 2006 (doi:10.1084/jem.20060603)

T-CELL DEVELOPMENT

The missing link for T cells?

Although there has been much progress in recent years in elucidating the pathway of T-cell development in the thymus, little is known about the link between extrathymic T-cell precursors and intrathymic T-cell progenitors. In this study, Harald von Boehmer and colleagues suggest a role for common lymphoid progenitors (CLPs) as the precursor cells that initially 'seed' the thymus from the blood after bone-marrow transplantation.

The most primitive early T-cell-lineage progenitors (ETPs) in the thymus are found in the double negative 1 (DN1) subset, specifically in the DN1a and DN1b fractions. These ETPs are lineage $(Lin)^-$, stem-cell antigen 1 $(SCA1)^+$ and KIT^{hi} (a phenotype known as LSK) and are therefore similar to bone-marrow LSK cells in terms of their surface markers. Bone-marrow-derived LSK cells with both myeloid and T-cell potential have been identified in the bloodstream, and this finding has led to the hypothesis that bone-marrow LSK cells contain a population of thymus-seeding T-cell precursors. However, migration of these cells into the thymus has not been shown, and whereas ETPs depend on Notch signalling in the thymus for development, bone-marrow LSK cells are not affected by a lack of such signalling. This has called into question the role of bone-marrow LSK cells in thymic immigration and has led to the investigation of alternative possibilities.

Von Boehmer and colleagues used transgenic mice with a human *CD25* reporter gene (denoted *hCD25*) under the control of regulatory elements of the gene encoding the pre-T-cell-receptor α-chain (pTα) to identify T-cell precursors inside and outside the thymus. They showed that DN1a and DN1b fractions in the thymus, which are known to contain ETPs, were both enriched for $hCD25^+$ cells and that the reporter-expressing cells therefore enter the canonical pathway of T-cell development. The authors had previously shown that a CLP subset from the bone marrow that is known as CLP2 cells ($Lin^-KIT^{-/low}B220^+$) expresses hCD25 and can efficiently seed the thymus after intravenous transfer. They therefore set out to analyse the relationship between $hCD25^+$ CLP2 cells and ETPs.

In co-culture with OP9–DL1 cells, which mimic Notch signals found in the thymus, $hCD25^+$ CLP2 cells progressed beyond the DN1 stage of T-cell development (as indicated by expression of CD25) at a similar rate to ETPs, whereas bone-marrow LSK cells had a delay of 2–4 days. This shows that CLP2 cells are more responsive to Notch signals, and therefore to the thymic environment, than

are bone-marrow LSK cells. To explain the finding that ETPs are not phenotypically similar to CLP2 cells in terms of the amount of expression of KIT and B220, the authors showed that short-term differentiation of these cells in OP9–DL1 co-cultures led to rapid changes in the expression of these markers. For example, after 6 days of culture, CLP2 cells had increased the expression of KIT to the same level as ETPs and had completely lost expression of B220 (similar to $B220^-$ ETPs). CLP2 cells were also found to reduce their expression of FLT3 (fms-related tyrosine kinase 3) to a level similar to that of ETPs faster than did LSK cells. The rapid expression of CD25 then progresses the CLP2 cells to a DN2 phenotype.

These results show that KIT and B220 are not stable markers for the differentiation of haematopoietic and lymphoid cells and that the phenotypic difference between CLP2 cells and ETPs does not exclude a role for CLP2 cells in thymic immigration. Furthermore, the rapid acquisition of a DN2 phenotype by CLP2 cells in the thymic environment could explain why it has so far been difficult to detect CLP cells as early immigrants in the steady-state thymus.

Kirsty Minton

ORIGINAL RESEARCH PAPER Krueger, A., Garbe, A. I. & von Boehmer, H. Phenotypic plasticity of T cell progenitors upon exposure to Notch ligands. *J. Exp. Med.* 17 July 2006 (doi:10.1084/jem.20060731)

INNATE IMMUNITY

Pick a CARD

The innate immune system of vertebrates detects pathogenic organisms by recognizing the molecular patterns typical of microbial components. Toll-like receptors (TLRs) constitute a major class of pattern-recognition receptors (PRRs), but other PRRs are also important for the recognition of certain pathogens. However, the way in which these PRRs trigger inflammatory pathways is not well understood. A recent study of the poorly characterized protein caspase-recruitment-domain protein 9 (CARD9) has helped to delineate a novel non-TLR-dependent signalling pathway involved in antifungal innate immunity.

CARD9 is structurally related to CARD–MAGUK (membrane-associated guanylate kinase) protein 1 (CARMA1). CARMA1 mediates nuclear factor-κB (NF-κB) activation through BCL-10 (B-cell lymphoma 10) and MALT1 (mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1) in response to the activation of T- and B-cell receptors. CARD9 can bind BCL-10 but lacks other regions that are typical of CARMA-family members involved in NF-κB activation. Given these characteristics, it was thought that CARD9 might interfere with the interaction between CARMA1 and BCL-10.

> “CARD9 has helped to delineate a novel non-TLR-dependent signalling pathway”

Gross *et al.*, however, found that T- and B-cell function in CARD9-deficient mice resembled that of wild-type mice, and they concluded that CARD9 was probably not involved in receptor signalling through the CARMA1–BCL-10 pathway. When the authors compared the responses of bone-marrow-derived dendritic cells (BMDCs) from CARD9-deficient mice and wild-type mice, they discovered that cytokine production induced by zymosan (a cell-wall component of yeast) or by whole fungal cells of *Candida albicans* was severely impaired in the absence of CARD9. This signalling pathway selectively involved dectin-1, the main mammalian PRR for zymosan, rather than TLR2, which also binds zymosan. Co-expression of CARD9 and BCL-10 showed that these signalling molecules cooperate to induce NF-κB activation. Additional studies indicated that BCL-10 and MALT1 are both required for zymosan-induced activation of BMDCs.

The data indicate that CARD9 is an essential link in a newly defined TLR-independent signalling pathway that links zymosan-activated dectin-1 and BCL-10–MALT1-mediated activation of NF-κB. CARD9 therefore has a role in innate immunity that is analogous to the role of CARMA1 in adaptive immunity.

Edward Wawrzynczak

ORIGINAL RESEARCH PAPER Gross, O. *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* **442**, 651–656 (2006)

ANTIBODIES

Unmasking IgG responses

How is it that steady-state serum IgG has anti-inflammatory effects but antigen-specific IgG promotes a pro-inflammatory response following antigen challenge? New research shows that the presence or absence of sialic acid at the terminus of the core glycan in the Fc region of IgG might be the determining factor in mediating these different immune responses.

IgG is recognized by its receptors (FcγRs) through the Fc region, which consists of the constant domains of both heavy chains. The core glycan that is linked to the asparagine residue at position 297 in the Fc region is essential for the binding of IgG to all FcγRs and mediates the induction of immune responses *in vivo*. It has been proposed that different forms of glycan might have a role in modulating the effector function of IgG *in vivo*, but the exact details had not been determined until now.

The authors found that the presence of sialic acid at the terminus of the core glycan might have a role for the activity of IgG. Intravenous immunoglobulin (IVIG) is a purified IgG product with anti-inflammatory effects that is used at high doses for the treatment of several inflammatory disorders. Kaneko *et al.* showed that desialylation of IVIG, by treatment with neuraminidase to remove the terminal sialic-acid residues, abrogated the anti-inflammatory effects of IVIG in a mouse model of rheumatoid arthritis. They then isolated the sialic-acid-enriched fraction of IVIG and compared the effectiveness of this fraction with that of the unfractionated product, in terms of induction of protection in the rheumatoid-arthritis model. They found that the fraction that had been enriched for sialic acid was tenfold more effective at protecting against disease than the unfractionated product.

By contrast, using an active model of inflammation (the mouse nephrotoxic nephritis model), the authors observed that antigen-specific IgG, which is associated with the pro-inflammatory response, was less sialylated than IgG from pre-immune sera. Therefore, a reduction in sialylation of the core glycan in the Fc region of IgG might unmask the pro-inflammatory effects of IgG, functioning as a switch that shifts IgG from having anti-inflammatory effects in the steady state to having pro-inflammatory effects after antigen challenge.

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ORIGINAL RESEARCH PAPER Kaneko, Y., Nimmerjahn, F. & Ravetch, J. V. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* **313**, 670–673 (2006)