HIGHLIGHTS

HIGHLIGHT ADVISORS

CEZMI AKDIS
SWISS INSTITUTE OF ALLERGY AND ASTHMA RESEARCH, SWITZERLAND

MARCO BAGGIOLINI
UNIVERSITA' DELLA SVIZZERA ITALIANA, SWITZERLAND

BRUCE BEUTLER
SCRIPPS RESEARCH INSTITUTE, USA

ANDREW CHAN
GENENTECH, INC., USA

ANNE COOKE
UNIVERSITY OF CAMBRIDGE, UK

JAMES DI SANTO
PASTEUR INSTITUTE, FRANCE

TASUKU HONJO
KYOTO UNIVERSITY, JAPAN

GARY KORETZKY
UNIVERSITY OF PENNSYLVANIA, USA

CHARLES MACKAY
GARVAN INSTITUTE OF MEDICAL RESEARCH, AUSTRALIA

FIONA POWRIE
UNIVERSITY OF OXFORD, UK

CAETANO REIS E SOUSA
IMPERIAL CANCER RESEARCH FUND, UK

ALAN SHER
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, USA

ANDREAS STRASSER
THE WALTER AND ELIZA HALL INSTITUTE, MELBOURNE, AUSTRALIA

ERIC VIVIER
CENTRE D'IMMUNOLOGIE DE MARSEILLE-LUMINY, FRANCE

SIGNALLING

A handle on the pre-BCR

Signal transduction by the pre-B-cell receptor (BCR) is a crucial checkpoint in B-cell development. However, the fleeting expression of the pre-BCR makes it difficult to investigate. Recently, a study in *Immunity* showed that PAX5-dependent activation of B-cell linker (BLNK) is a pivotal event in pre-BCR signalling. This discovery has led to the development of a new system to study the pre-BCR.

PAX5 is a transcription factor that is essential for B-cell development: without it, lymphoid progenitors cannot commit to the B-cell lineage and development is stalled at the pro-B-cell stage. This block seems to be partly due to a failure to rearrange and express the μ-chain of the pre-BCR. However, a rearranged μ-transgene does not rescue B-cell development in *Pax5*$^{-/-}$ mice. It turns out that there is no pre-BCR signalling in *Pax5*$^{-/-}$ μ-transgenic pro-B cells. So, PAX5 is probably also required for the expression of a crucial signalling component, which Schebesta and colleagues in Vienna set out to identify.

An established system in which PAX5 is fused to the oestrogen receptor (ER) was used to identify new PAX5 target genes in pro-B cells. *Pax5*$^{-/-}$ pro-B cells that expressed the PAX5–ER fusion protein were treated with oestrogen, which resulted in PAX5 activation. PAX5-induced transcripts were enriched by DNA subtraction, and several clones of *BLNK*, which encodes a B-cell adaptor molecule, were isolated. Two PAX5-binding sites were identified in the *BLNK* promoter and this gene was shown to be a direct target for PAX5.

Surprisingly, however, a *BLNK* transgene did not rescue the defect in B-cell development in *Pax5*$^{-/-}$ μ-transgenic mice. Although the reconstitution of BLNK restored pre-BCR signalling functions in pro-B cells — including Ca^{2+} flux, pre-BCR internalization, *c*-Kit downregulation and proliferation — these signals were not sufficient to overcome the block in pro-B to pre-B cell differentiation, indicating that an additional PAX5-driven event is required.

Because they cannot differentiate, BLNK-reconstituted pro-B cells from *Pax5*$^{-/-}$ μ-transgenic mice are locked in a perpetual pre-BCR signalling mode, which neatly circumvents the technical problems that are associated with transient pre-BCR expression. The authors created an inducible pre-BCR signalling system by fusing BLNK to the ER. The treatment of *Pax5*$^{-/-}$ μ-transgenic pro-B cells with a hormone ligand activates the BLNK–ER fusion protein, leading to pre-BCR signal transduction. Microarray analysis of gene expression in this system showed that BLNK-dependent pre-BCR signals control genes that are involved in proliferation, signalling, *V*(*D*)*J* recombination and growth factor responses.

The authors hope that their new pre-BCR signal induction system will open up a more detailed understanding of transcriptional reprogramming at the pre-BCR checkpoint.

Jennifer Bell

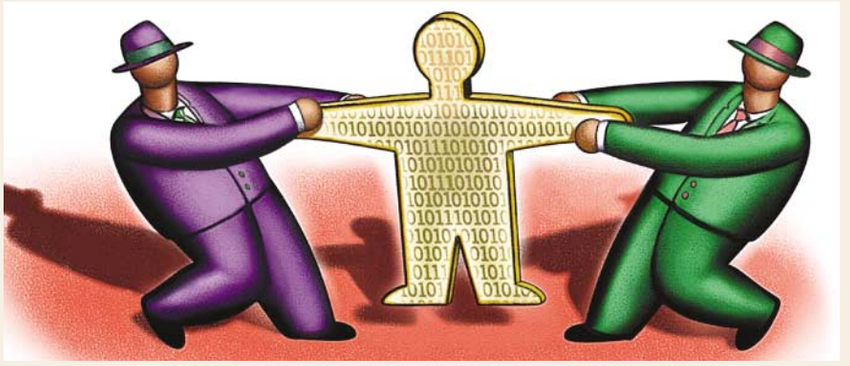
References and links

ORIGINAL RESEARCH PAPER Schebesta, M., Pfeffer, P. L. & Busslinger, M. Control of pre-BCR signaling by PAX5-dependent activation of the *BLNK* gene. *Immunity* **17**, 473–485 (2002)
WEB SITE
Meinrad Busslinger's lab: http://www.imp.univie.ac.at/busslinger/bus_hp.html

© 2002 Nature Publishing Group

HAEMATOPOIESIS

For or against?



The ETS-family transcription factor PU.1 and the GATA-family transcription factor GATA1 have been shown to antagonize each other's function during haematopoiesis. Work from Walsh *et al.* in *Immunity* shows that PU.1 can antagonize the function of GATA2 by blocking its expression, but, surprisingly, these transcription factors can also cooperate to specify mast-cell fate.

Studies with *PU.1*$^{-/-}$ mice have shown that this transcription factor is essential for the generation of myeloid and lymphoid, but not erythroid or megakaryocytic, lineages. Here, the authors establish that PU.1 is required for the survival and differentiation of mast-cell progenitors. Mast cells are absent in *PU.1*$^{-/-}$ mice, and the low number of mast-cell progenitors that are present are blocked at an early stage of development. Retroviral expression of PU.1 in *PU.1*$^{-/-}$ haematopoietic progenitors, however, allows the development of both mast cells and macrophages.

To investigate how PU.1 regulates the mast-cell versus macrophage cell-fate decision, the authors generated a *PU.1*$^{-/-}$ progenitor cell-line that conditionally expressed an activatable form of PU.1. In this setting, active PU.1 resulted in the development of macrophages, but not mast cells. Cells that express the active form of PU.1 lacked *Gata*2 expression, whereas *PU.1*$^{+/-}$ and *PU.1*$^{-/-}$ cells expressed this gene, indicating that PU.1 negatively regulates the expression of *Gata2*.

Further experiments showed that, in the absence of Gata2, PU.1 promotes the differentiation of myeloid progenitors into macrophages — but not into mast cells — and that the re-expression of *Gata2* in these progenitors resulted in the generation of mast cells. The authors propose that, during macrophage differentiation, PU.1 antagonizes *Gata2* expression and function, but that PU.1 and Gata2 work together during mast-cell development.

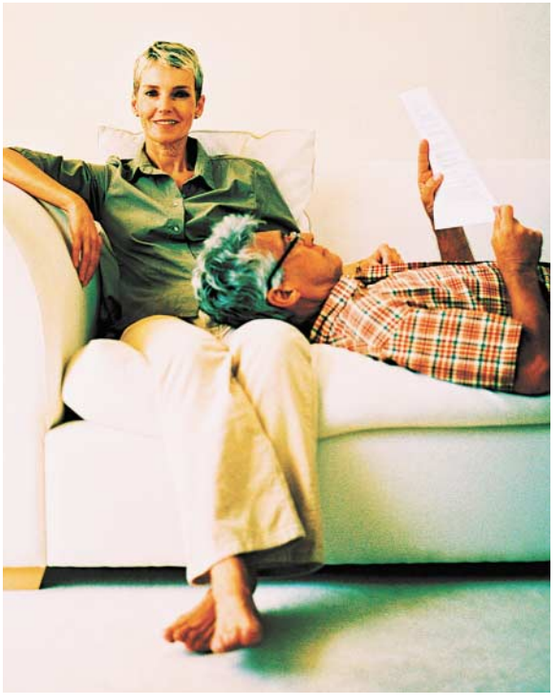
Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Walsh, J. C. *et al.* Cooperative and antagonistic interplay between PU.1 and GATA2 in the specification of myeloid cell fates. *Immunity* **17**, 665–676 (2002)

IMMUNE EVASION

Make yourself at home



If *Legionella pneumophila* — an aquatic bacterium that infects protozoan hosts in freshwater ecosystems — is inhaled by humans, it causes a severe form of pneumonia known as Legionnaires' disease. Once in the lungs, *L. pneumophila* is internalized into the phagosomes of alveolar macrophages. However, rather than being degraded by the macrophage lysosome, this bacterium makes itself at home. It hijacks host vesicle trafficking to make an endoplasmic reticulum (ER)-derived vacuole that supports its replication. So, how does it do it?

New insights are now reported by Kagan and Roy in *Nature Cell Biology*. They began by showing that *L. pneumophila*-containing phagosomes mature into ER-derived vacuoles in a biphasic manner. First, they interact with early secretory vesicles — vesicles travelling from the ER to the ER–Golgi intermediate compartment (ERGIC); then, they acquire markers that are concentrated in the ER. But, how do they get to the ER?

Cholera and Shiga toxins are known to reach the ER using a pathway that takes them through the Golgi, but Kagan and Roy show that *L. pneumophila*-containing phagosomes do not interact with intermediate compartments (the Golgi or ERGIC). Instead, they found that these phagosomes interact directly with transitional ER (tER) sites — dynamic sites where early secretory vesicles exit the ER — and that *L. pneumophila* forms an ER-derived vacuole by subverting vesicular transport from these sites. In addition, they showed that the subversion of early secretory vesicles is required to make a stable vacuole that is kept sequestered from the endocytic pathway.

Therefore, Kagan and Roy have shown that *L. pneumophila* subverts host cellular processes in a new way, and they suggest that understanding the mechanisms that are used by this bacterium to interact with tER sites and to recruit ER-derived vesicles might help us to identify host factors that regulate vesicular transport at these sites.

Rachel Smallridge Associate Editor, Nature Reviews Molecular Cell Biology

References and links

ORIGINAL RESEARCH PAPER Kagan, J. C. & Roy, C. R. *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. *Nature Cell Biol.* **4**, 945–954 (2002)

FURTHER READING Roy, C. R. Exploitation of the endoplasmic reticulum by bacterial pathogens. *Trends Microbiol.* **10**, 418–424 (2002)

WEB SITE

Craig Roy's lab:
http://info.med.yale.edu/micropath/fac_roy.html

© 2002 Nature Publishing Group

AUTOIMMUNITY

A clear suspect

Approximately 0.05% of the population of the Western world suffers from systemic lupus erythematosus (SLE) — a complex autoimmune disease. Although several susceptibility loci for SLE have been identified, the nature of the genes and mutations that underlie this disease has remained unknown. Now, Prokunina *et al.* report in *Nature Genetics* an association between programmed cell death gene 1 (*PDCD1*) and SLE. Importantly, they also propose how a particular sequence variant of *PDCD1* might contribute to the aetiology of this disease.

In a previous study of a Nordic population, the authors identified a susceptibility locus for SLE on chromosome 2. One gene in this region stood out as a potential candidate — *PDCD1*. This is because *PDCD1* encodes an immunoreceptor, also known as PD1, that belongs to the CD28 family and is known to regulate peripheral self-tolerance of T and B cells. Moreover, *Pdcd1*$^{-/-}$ mice suffer from SLE-like symptoms.

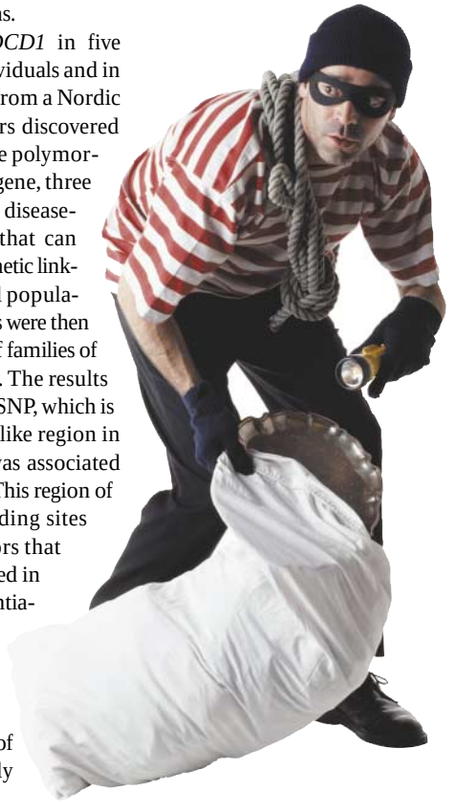
By sequencing *PDCD1* in five healthy, unrelated individuals and in five patients with SLE from a Nordic population, the authors discovered seven single-nucleotide polymorphisms (SNPs) in this gene, three of which constitute a disease-associated haplotype that can account for all of the genetic linkage seen in the original population sample. These SNPs were then genotyped in five sets of families of different ethnic origin. The results were clear — only one SNP, which is found in an enhancer-like region in intron 4 of *PDCD1*, was associated consistently with SLE. This region of intron 4 contains binding sites for transcription factors that are known to be involved in haematopoietic differentiation and in inflammation. In particular, the SNP disrupts a putative binding site for RUNX1, a member of the Runt-related family of transcription factors. Using an electrophoretic mobility-shift assay, the authors confirmed that RUNX1 binds this sequence and that binding is abolished by the sequence change that is associated with the SNP.

The authors propose that binding of RUNX1 to wild-type *PDCD1* modulates its transcription and ensures its correct expression. As PDCD1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), it might be involved in preserving self-tolerance by inhibiting autoreactive cells. It remains to be confirmed whether, in the absence of RUNX1 binding, dysregulation of *PDCD1* leads to loss of self-tolerance and to the chronic lymphocyte hyperactivity that is characteristic of SLE.

Magdalena Skipper, Associate Editor, Nature Reviews Genetics

References and links

ORIGINAL RESEARCH PAPER Prokunina, L. *et al.* A regulatory polymorphism in *PDCD1* is associated with susceptibility to systemic lupus erythematosus in humans. *Nature Genet.* 28 October 2002 (DOI: 10.1038/ng1020)



IN BRIEF

LYMPHOID ARCHITECTURE

Manipulation of lymphoid microenvironments in nonhuman primates by an inhibitor of the lymphotoxin pathway.

Gommerman, J. L. *et al. J. Clin. Invest.* **110**, 1359–1369 (2002)

Continuous lymphotoxin (LT) signals are essential for the maintenance of lymphoid architecture and the network of follicular dendritic cells (FDCs). This reticular network has been implicated in the pathogenesis of HIV and prion diseases, as well as certain lymphomas and autoimmune diseases, so is inhibition of LT a potential therapy? Gommerman *et al.* show that in monkeys treated with human LTβ-receptor–immunoglobulin fusion protein, the FDC networks disappeared within several days and affinity maturation of antibodies was impaired.

APOPTOSIS

TRAIL/Apo-2 ligand induces primary plasma-cell apoptosis.

Ursini-Siegel, J. *et al. J. Immunol.* **169**, 5505–5513 (2002)

At the end of an immune response, most antibody-secreting cells (plasma cells) undergo apoptosis, but the mechanisms that regulate this process are not understood. This study indicates that the death receptor TRAIL might have a specific role in the elimination of plasma cells. Similar to resting and activated B cells, plasma cells were shown to express TRAIL, but only plasma cells were susceptible to *ex vivo* TRAIL-mediated killing.

HIV

In vivo dynamics of T-cell activation, proliferation and death in HIV-1 infection: why are CD4$^+$ but not CD8$^+$ T cells depleted?

Ribeiro, R. M. *et al. Proc. Natl Acad. Sci. USA* **99**, 15572–15577 (2002)

Deuterated glucose has been used to compare the dynamics of T-cell turnover between HIV-infected and -uninfected individuals. Here, Ribeiro *et al.* use a mathematical model, which takes into consideration the fact that only a fraction of T cells are proliferating at any given time, to analyse these data. This study provides insights into the differences between CD4$^+$ and CD8$^+$ T-cell dynamics during HIV-1 infection.

IMMUNOTHERAPY

Gene therapy for Wiskott Aldrich syndrome: rescue of T-cell signaling and amelioration of colitis upon transplantation of retrovirally transduced hematopoietic stem cells in mice.

Klein, C. *et al. Blood* 14 November 2002 (DOI 10.1182/blood-2002-05-1423)

Wiskott-Aldrich syndrome (WAS), an X-linked primary immunodeficiency, is caused by mutations in the *WASP* gene. This study shows that retroviral transduction of *WASP*$^{-/-}$ haematopoietic stem cells with *WASP* can rescue the T-cell signalling defect that is seen in the absence of the protein. These results are encouraging for possible gene-therapy trials for WAS.

© 2002 Nature Publishing Group

HIV

Identity of CAF revealed

Until recently, it was a mystery why some individuals infected with HIV-1 do not become immunodeficient (long-term non-progressors, LTNPs). $CD8^+$ T cells are known to be important, but why are they more effective in some individuals than others? A recent paper in *Nature Immunology* showed that the increased expression of perforin by $CD8^+$ T cells from LTNPs is important for their cytolytic activity (see the Highlight 'Quality control' in our November issue). But, since the first description of CD8 antiviral factor (CAF) — which is secreted by stimulated $CD8^+$ T cells from certain infected individuals — it has been recognized that soluble factors can inhibit virus replication also. Now, Zhang *et al*. report in *Science* the identification of human α-defensins 1, 2 and 3 as one of the main components of CAF.

Previous studies had indicated that β-chemokines (CCL3, CCL4 and CCL5) might account for the antiviral activity of CAF by competing with virus for binding to CCR5, which is used as a co-receptor for virus entry. However, this can only inhibit R5 viruses, and not X4 viruses, which use CXCR4 as a co-receptor. Using protein-chip technology, Zhang *et al.* have shown that the α-defensins carry out much of the anti-HIV activity of supernatants from stimulated $CD8^+$ T cells that is not attributable to β-chemokines.

The authors compared the protein mass spectra of supernatants from stimulated and unstimulated $CD8^+$ T cells of LTNPs, normal progressors and controls. Marked differences between stimulated and unstimulated spectra were observed — specifically, three peaks between 3.3 and 3.5 kD that were present in stimulated cultures from LTNPs and some controls, but not progressors. The three peaks correspond to the molecular masses of human α-defensins 1, 2 and 3, and this result was confirmed by protein sequencing.

INNATE IMMUNITY

Untangling the TLRs

The Toll-like receptor (TLR) family consists of ten germline-encoded microbe sensors that are crucial for host defence. The theory is that each receptor triggers an innate immune response that is appropriate for the class of pathogen that the receptor recognizes. But, differences between TLR signal-transduction pathways that might result in such tailored responses have been hard to find. Now, two studies published in *Nature* show that the adaptor molecule TIRAP (also known as MAL) has a restricted role in a shared TLR2 and TLR4 signal-transduction pathway.

TIRAP is structurally similar to the adaptor protein MYD88, which links the TLRs and interleukin-1 receptors (IL-1Rs) to downstream signalling pathways. MYD88 is essential for the induction of cytokine secretion by all TLR ligands, but the lipopolysaccharide (LPS) receptor TLR4 can stimulate the upregulation of expression of co-stimulatory receptors on dendritic cells (DCs) in the absence of MYD88. Initial *in vitro* studies indicated that TIRAP might function in this MYD88-independent pathway. But, the *Tirap*-knockout mice described by Yamamoto *et al*. and Horng *et al*. show that this is not the case.

Horng and co-workers found that in response to triggering of TLR2 or TLR4, $Tirap^{-/-}$ B cells had impaired proliferative responses and $Tirap^{-/-}$ DCs produced markedly reduced levels of pro-inflammatory cytokines. But, responses to a TLR9 ligand (CpG DNA) were normal in these cells, and injection of the TLR5 ligand flagellin into $Tirap^{-/-}$ mice induced the expression of normal levels of cytokines. Yamamoto *et al*. showed that the production of pro-inflammatory cytokines in response to LPS and various ligands for TLR2 is impaired markedly in $Tirap^{-/-}$ macrophages. Furthermore, $Tirap^{-/-}$ mice were completely resistant to LPS-induced shock. But, the responses of $Tirap^{-/-}$ macrophages to synthetic ligands for TLR7 and TLR3, and CpG DNA, were intact.

In contrast to $Myd88^{-/-}$ mice, both groups showed that IL-1 signalling is not compromised in $Tirap^{-/-}$ mice. So, together, these papers show that TIRAP is dispensable for TLR3, TLR5, TLR7, TLR9 and IL-1R function, but has a specific role in TLR2 and TLR4 signal-transduction pathways.

Similar to MYD88, TIRAP has a crucial role in the activation of nuclear factor-κB and mitogen-activated protein kinases by TLR2 and TLR4. And $Tirap^{-/-}$ mice and $Myd88^{-/-}$ mice have similarly defective responses to LPS, which indicates that TIRAP might be involved in a MYD88-dependent pathway. To test this, Yamamoto and co-workers generated $Myd88^{-/-}Tirap^{-/-}$ mice. The LPS-induced upregulation of expression of co-stimulatory molecules on DCs (a MYD88-independent event) occurred normally in these double-knockout mice, so TIRAP is clearly not part of the MYD88-independent pathway.

Jennifer Bell

References and links

ORIGINAL RESEARCH PAPERS Yamamoto, M. *et al*. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* **420**, 324–329 (2002) | Horng, T. *et al*. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* **420**, 329–333 (2002)

FURTHER READING Medzhitov, R. Toll-like receptors and innate immunity. *Nature Rev. Immunol.* **1**, 135–145 (2001)

WEB SITE

Ruslan Medzhitov's lab: http://info.med.yale.edu/immuno/fac_medzhitov.html

© 2002 Nature Publishing Group

Depletion of these molecules from culture supernatants eliminated activity against X4 viruses and markedly reduced activity against R5 viruses. Residual activity against R5 viruses could be neutralized by the addition of antibodies specific for the β-chemokines. Furthermore, synthetic and purified α-defensins were shown to reduce HIV-1 replication *in vitro*.

So, we are one step closer to understanding the mystery of LTNPs, which can only aid the development of new therapeutics. It remains to be determined how the α-defensins mediate their antiretroviral effects, and whether they are involved directly in non-progression.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Zhang, L. *et al.* Contribution of human α-defensin 1, 2 and 3 to the anti-HIV-1 activity of CD8 antiviral factor. *Science* **298**, 995–999 (2002)

WEB SITE

David Ho's lab: http://www.rockefeller.edu/labheads/ho/ho.html

TOLERANCE

DCs on the beat

Dendritic cells (DCs) have long been recognized as the body's sentinels, but evidence is emerging that they are responsible also for policing the immune system. It has been proposed that DCs are 'on the beat' constantly, ingesting tissue-associated antigens and presenting them in local lymph nodes. In the absence of inflammation, this should result in the elimination of potentially harmful self-reactive T cells. Three papers in a recent issue of *The Journal of Experimental Medicine* provide further support for this model and indicate that CD8α$^+$ DCs might be specialized for this task.

A previous study showed that the presentation of tissue-associated self-antigens by bone-marrow-derived cells in draining lymph nodes results in the deletion of self-reactive T cells. DCs have been implicated in this 'cross-tolerance', but the DC subpopulation(s) that is involved has not been pinpointed. Belz and colleagues generated mice that express a fusion protein containing two MHC class-I-restricted epitopes — an ovalbumin peptide and a herpes simplex virus (HSV) peptide — under the control of the rat insulin promoter. Ovalbumin-specific or HSV-specific TCR-transgenic CD8$^+$ T cells that were injected into these mice were deleted rapidly. By sorting DC populations in the pancreatic lymph nodes, the authors showed that only CD8α$^+$ DCs could stimulate a highly sensitive HSV-specific T-cell hybridoma, indicating that this DC population, which is implicated in cross-presentation, also mediates cross-tolerance.

The second paper addresses the issue of how tolerogenic DCs might acquire antigen in the steady state. An earlier study showed that CD8α$^+$ DCs are highly efficient at taking up and presenting antigens that are associated with dying cells; but, can this lead to tolerance? Liu *et al.* injected mice with transporter for antigen presentation (TAP)-deficient splenocytes that had been loaded with small amounts of ovalbumin protein and osmotically shocked to induce cell death. Because they lack TAP proteins, these cells cannot present their own antigens. The ovalbumin was processed and presented by CD8α$^+$ DCs in the spleen. Transferred ovalbumin-specific TCR-transgenic CD8$^+$ T cells proliferated initially, but were deleted subsequently, and the animals were rendered tolerant to ovalbumin. However, the maturation of DCs induced by a CD40-specific antibody resulted in immunity rather than tolerance. Although these data indicate that dying cells target CD8α$^+$ DCs, it is not known whether the cells that are targeted normally for presentation of self-antigens are living or dead.

The idea that the same CD8α$^+$ DC subpopulation induces both cross-tolerance and cross-presentation is attractive. However, there might be dedicated immunogenic and tolerogenic DC subsets in the CD8α$^+$ DC population. Moreover, studies by other groups have implicated other DC subsets in the induction of tolerance. A paper by Scheinecker and colleagues in the same issue shows that DCs presenting MHC class-II-restricted tissue-specific antigens in the gastric lymph nodes can be CD8$^+$ or CD8$^-$, and CD11b$^+$ or CD11b$^-$. Further studies are required to clarify these issues.

Jennifer Bell

References and links

ORIGINAL RESEARCH PAPERS Belz, G. T. *et al.* The CD8α$^+$ dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. *J. Exp. Med.* **196**, 1099–1104 (2002) | Liu, K. *et al.* Immune tolerance after delivery of dying cells to dendritic cells *in situ*. *J. Exp. Med.* **196**, 1091–1097 (2002) | Scheinecker, C. *et al.* Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med.* **196**, 1079–1090 (2002)

FURTHER READING Mougneau, E., Hugues, S. & Glaichenhaus, N. Antigen presentation by dendritic cells *in vivo*. *J. Exp. Med*. **196**, 1013–1016 (2002)

© 2002 Nature Publishing Group

WEB WATCH

Career choices

- *Science*'s Next Wave: http://nextwave.sciencemag.org/

It's not only cells of the immune system that are faced with decisions, and for those decisions relating to career choice, *Science*'s Next Wave web site could be a good place to start. This career-development resource, which is updated weekly, aims to enable you to find out about the range of options that are open to you at various stages of your career, including research and non-research jobs, whether in academia, industry or elsewhere. Articles provide expert advice from individuals who have followed particular career paths, as well as the latest news from the science job market.

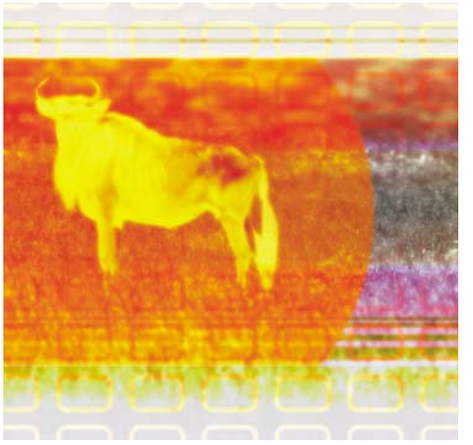
The site is helpfully organized so that recent articles can be found through the country homepages, which bring together news from each region (although homepages for some countries are not available), or through special-focus portals. These portals include the 'Career Development Center', which contains news and feature articles, and a search facility, which allows visitors to search for jobs in academic research or for grants, as well as 'The Grant Doctor', where questions about grants and fellowships are answered. The 'Postdoc Network' includes articles covering many issues that are of importance to postdocs, as well as career-development news, and it is a place where postdocs can raise concerns about their career needs or find the answers to their questions.

Next Wave's Career Resources Library is a useful archive of previously published articles divided into sections, including those specifically for graduate students and for individuals who are interested in career transitions or science policy.

Jenny Buckland

AUTOIMMUNITY

Come and get me!



Few prey specifically try to attract their predator. However, the β cells of the islets of Langerhans in the pancreas do just that, by secreting chemokines to attract islet-specific effector T cells, which then destroy them, according to a paper now published in *Nature Medicine*.

Type 1 diabetes results from the T-cell-mediated autoimmune destruction of insulin-secreting β cells. Early in disease pathogenesis, macrophages and T cells infiltrate the islets and, in combination with pro-inflammatory cytokines, including interleukin-1β, tumour-necrosis factor and interferon-γ, cause insulitis. T cells are not a normal constituent of islets, so what controls the recruitment of these effector cells to these sites?

In this study, Frigerio and colleagues investigated the involvement of chemokines in the recruitment of T cells to the islets. First, they cultured mouse islets and a β-cell line with pro-inflammatory cytokines, and tested whether this resulted in changes in the chemokine expression profiles of these cells. Cytokine treatment caused the upregulated expression of many chemokines, including CXCL9 and CXCL10 transcripts. So, in the presence of pro-inflammatory cytokines, islets and β cells express higher levels of chemokines that are known to attract activated T cells and macrophages.

The authors then used a transgenic mouse model of type 1 diabetes to test the *in vivo* role of chemokines in disease pathogenesis. RIP-GP transgenic mice express the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) under the control of the rat insulin promoter (RIP), which leads to the expression of GP on pancreatic cells. Infection of these mice with LCMV leads to severe insulitis and T-cell-mediated β-cell destruction. After infection, increased levels of pro-inflammatory cytokines, chemokines (including CXCL9 and CXCL10) and chemokine receptors (including CXCR3, the receptor for CXCL9 and CXCL10) were detected in the islets of these mice. Immunohistology of

THYMIC DEVELOPMENT

FoxN1 gene regulation in the thymus

T-cell development and selection in the thymus depend on distinct populations of thymic epithelial cells (TECs). The forkhead transcription factor FoxN1 (also known as Whn), which is lacking in athymic *nude* mice, is required for the growth and differentiation of TECs. However, the signalling pathways that control expression of FoxN1 are not known. In *Nature Immunology*, Balciunaite and colleagues now report that Wnt signalling pathways regulate the expression of FoxN1 and are crucial for TEC development.

Signals from Wnt glycoproteins are transduced through three intracellular pathways, including the Wnt–β-catenin pathway, which is central to many cell-fate decisions during development. Wnt proteins bind cell-surface receptors composed of Frizzled proteins and low-density lipoprotein receptor-related proteins 5 and 6 (Lrp5 and Lrp6), and downstream signalling inhibits the phosphorylation of β-catenin. Dephosphorylated β-catenin interacts with the high-mobility group (HMG) proteins T-cell factor 1 (Tcf1), Tcf3 and Tcf4, and with lymphoid enhancer binding factor 1 (Lef1), enabling these proteins to activate the transcription of downstream target genes.

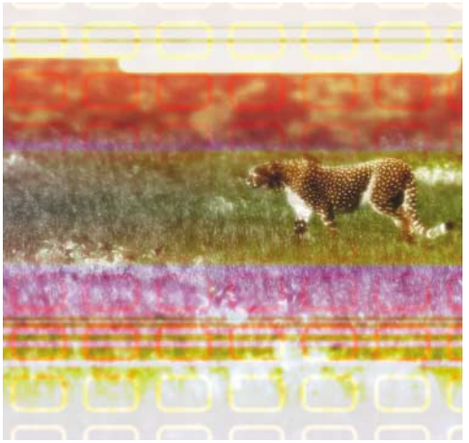
Mice that are deficient in Wnt1 and Wnt4 have reduced thymic cellularity, and following experiments that showed that Wnt glycoproteins are expressed by TECs, the authors investigated whether TECs respond to Wnt signalling. TEC lines were transfected with a reporter construct (known as TOP) that contains the luciferase gene under the control of Tcf and Lef1 proteins. These cells were then co-transfected to overexpress various Wnt proteins. Wnt1 and Wnt4 were shown to activate Tcf- and Lef1-dependent transcription of the TOP construct, which indicates that TECs do respond to Wnt signals.

Next, the authors asked whether *FoxN1* gene transcription is regulated by Wnt signalling. The level of *FoxN1* messenger RNA was increased in TECs cultured with cells that overexpressed Wnt4 or Wnt5. In addition, *FoxN1* transcription was blocked in the presence of soluble Frizzled proteins (which inhibit Wnt signalling). The putative promoter region of *FoxN1* was shown to be responsive to Wnt4 when it was cloned into a promoter-less luciferase construct.

These results show that Wnt signalling pathways control the transcription of *FoxN1* in TECs and that they are implicated in control of the genetic programme of TECs that is required for their development and thymic function.

Jenny Buckland

© 2002 Nature Publishing Group



pancreatic tissue indicated that β cells were the main source of CXCL10 during insulitis.

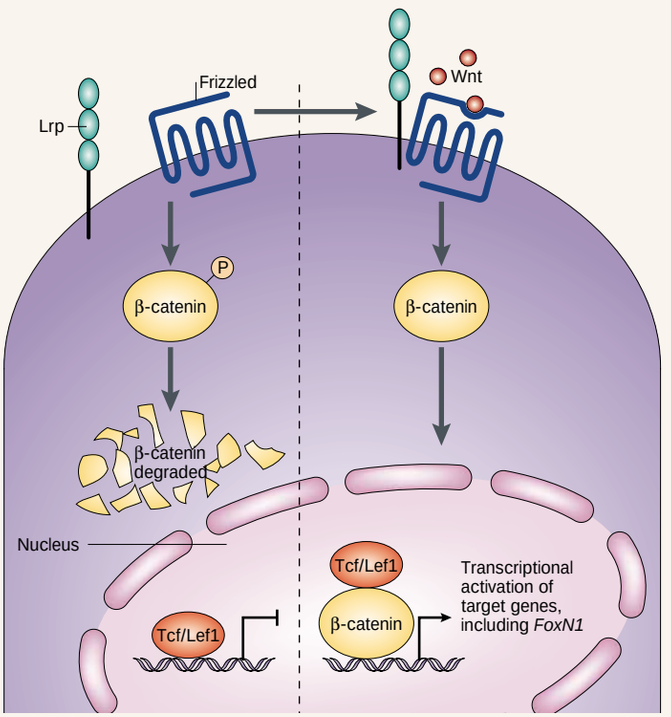
So, chemokines are expressed in islets during insulitis, but are T cells attracted to these chemokines? *In vitro* and *in vivo* studies showed that LCMV-activated T cells are attracted to the chemokines that are present in inflamed islets. To investigate which chemokine receptors are involved, Frigerio *et al.* exposed T cells from LCMV-infected mice to CXCL10 (to desensitize CXCR3 on these cells) before they were cultured with the supernatant from a β-cell line. CXCL10 treatment led to a reduced migratory capacity of T cells towards the supernatants of stimulated β cells, indicating that β-cell chemokines preferentially attract T cells through CXCR3. These observations were confirmed *in vivo* by studies of RIP-GP mice deficient for CXCR3. In the absence of CXCR3, insulitis, diabetes and hyperglycaemia were delayed.

Therefore, in type 1 diabetes, β cells contribute to their own destruction by secreting CXCL9 and CXCL10, which specifically attract $CXCR3^+$ effector T cells to the islets. The authors conclude that CXCR3 might be a new target for therapeutic intervention early in disease.

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Frigerio, S. *et al.* β cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nature Med.* 4 November 2002 (DOI: 10.1038/nm792)



References and links

ORIGINAL RESEARCH PAPER Balciunaite, G. *et al.* Wnt glycoproteins regulate the expression of *FoxN1*, the gene defective in *nude* mice. *Nature Immunol.* 15 October 2002 (DOI: 10.1038/ni850)

FURTHER READING Anderson, G. & Jenkinson, E. J. Lymphostromal interactions in thymic development and function. *Nature Rev. Immunol.* **1**, 31–40 (2002)

TRIAL WATCH

Attack of the clones

Although many studies have shown that tumour-specific T cells can slow tumour growth in mice, there has been little evidence that T-cell-based immunotherapy is effective in human cancer patients. A Phase I clinical trial involving the adoptive transfer of melanoma-specific T-cell clones into patients with therapy-resistant metastatic melanoma has provided new evidence that T cells can be induced to target tumours.

Yee *et al.* isolated cytotoxic T lymphocytes (CTLs) that were specific for two well-defined melanoma/melanocyte antigens, MART1 and gp100, from ten stage-IV melanoma patients. They primed these T cells *in vitro* using peptide-loaded dendritic cells, and then selected those that specifically lysed MART1- or gp100-expressing cells. These CTL clones were expanded in culture, and transferred back into patients in four separate infusions. After the first infusion, the cells were initially observed to have a short survival time (6.7 days), but when interleukin-2 was co-administered with subsequent infusions, the average CTL survival time increased to almost 17 days.

Biopsies taken 3 days post-infusion revealed that the tumour-specific CTLs preferentially localized to the tumour. In one patient, the tumour-antigen-specific CTLs were found to make up 37% of the total tumour-infiltrating CTL population, whereas these cells made up less than 1% of the total CTLs in the peripheral blood. Melanoma-antigen-specific T cells were found to make up 0.5–2.2% of all CTLs, compared with the 0.0–0.3% of tumour-specific CTLs detected in previous studies of patients who received vaccine-based therapies.

The adoptive T-cell therapy resulted in disease stabilization in five of ten patients, and minor or mixed responses in an additional three patients for up to 21 months. The average survival time of patients was 11 months, and some patients survived for as long as 21 months. Although the number of patients in this study is small, this is a large improvement over the median survival time of 4 months for patients with refractory metastatic disease. No serious toxicity was observed in any patients after adoptive therapy.

In an accompanying editorial, Drew Pardoll points out that none of the patients experienced significant tumour regression. This doesn't mean, however, that the transferred CTLs were incapable of antitumour activity. Based on analysis of tumour biopsies, tumour-cell expression of the targeted antigens was lost in three of the five patients examined. This indicates that antigen-expressing tumour cells were eliminated by the CTLs.

These findings support the emerging view that tumour-reactive T cells are present in the peripheral blood of individuals with cancer, and that these can be activated and traffic to metastatic tumour deposits, where they eliminate tumour cells that express target antigen. Further studies to determine the specific signals that regulate T-cell proliferation, as well as ways to increase T-cell activation, localization to tumours and affinity for their antigenic target are necessary to improve this immunotherapeutic approach.

Kristine Novak, Senior Editor, Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Yee, C. *et al.* Adoptive T cell therapy using antigen-specific $CD8^+$ T cell clones for the treatment of patients with metastatic melanoma: *in vivo* persistence, migration, and anti-tumor effect of transferred T cells. *Proc. Natl Acad. Sci. USA* 11 November 2002 (DOI:10.1073/pnas.242600099)

© 2002 Nature Publishing Group