SIGNALLING

Rac-king up new roles in haematopoiesis

Rac1 and Rac2 guanosine triphosphatases (GTPases) are crucial signalling regulators in eukaryotic cells, acting downstream of various cellular receptors. Two studies published in *Science* have used conditional gene targeting to clarify the roles of Rac1 and Rac2 in particular aspects of haematopoietic-cell differentiation.

Gu *et al.* looked at the differential activity of Rac1 and Rac2 in haematopoietic stem/progenitor cells (HSC/Ps). As Rac1 deficiency is embryonic lethal, they generated conditional *Rac1*-knockout mice. Deficiency of Rac1 inhibited the ability of HSC/Ps to reconstitute haematopoiesis in an engraftment model (which requires adhesion and proliferation in the bone marrow), and the absence of both Rac1 and Rac2 led to the movement of progenitor cells out of the bone marrow and into the peripheral circulation, as a result of decreased adhesion to fibronectin. Rac1 and Rac2 were also shown to have roles in regulating the cytoskeletal changes that are required for engraftment and mobilization.

$Rac1^{-/-}$, and to a greater extent $Rac1^{-/-}Rac2^{-/-}$, HSC/Ps had reduced proliferation in response to growth factors *in vitro*, correlating with decreased levels of cyclin D1 and phosphorylation of extracellular signal-regulated kinase (ERK), and increased levels of the cyclin-dependent kinase inhibitor KIP1. By contrast, $Rac2^{-/-}$ cells were more susceptible to apoptosis after growth-factor stimulation, associated with reduced Akt activation. Therefore, it seems that Rac1 regulates entry into the cell cycle, whereas Rac2 mainly regulates cell survival.

Walmsley *et al.* looked further downstream at B-cell development by generating, for the first time, mice with a conditional deletion of Rac1 in the B-cell lineage ($Rac1^{B}$ mice). $Rac1^{B}Rac2^{-/-}$ mice had normal numbers of pro-B, pre-B and immature B cells in the bone marrow, but reduced numbers of B cells at later developmental stages in the spleen and lymph nodes. In the absence of both Rac1 and Rac2, B-cell development seems to be blocked at the transitional type 1 B-cell stage in the spleen.

Recent studies have shown activation of Rac1 after B-cell receptor (BCR) stimulation, and signals from the BCR are known to be crucial for B-cell development, so are BCR signalling defects responsible for the observed phenotype of $Rac1^{B}Rac2^{-/-}$ mice? $Rac2^{-/-}$ and $Rac1^{-/+}Rac2^{-/-}$ mature B cells stimulated with an IgM-specific antibody had decreased survival compared with wild-type B cells, which correlated with decreased induction of the anti-apoptotic protein Bcl-X_L. These B cells also proliferated less than wild-type cells. This was shown to be due to decreased induction of the cell-cycle regulator cyclin D2 after stimulation. So, Rac1 and Rac2 transduce BCR signals for B-cell survival and entry into the cell cycle. Finally, in contrast to wild-type cells, BAFF could not increase the survival of $Rac1^{B}Rac2^{-/-}$ immature B cells, and this was probably the result of decreased expression of BAFF receptor (BAFFR). Rac1 and Rac2 were shown to transduce BCR signals leading to the upregulation of expression of BAFFR messenger RNA.

These two studies show that in both B cells and HSC/Ps, Rac1 and Rac2 have essential roles in controlling proliferation and survival. Furthermore, despite their sequence similarity, the two GTPases seem to have partially non-redundant physiological functions. Gu *et al.* confirmed this functional distinction in neutrophils, in which Rac2 seems to be the main GTPase regulating directed migration and superoxide generation, whereas Rac1 regulates neutrophil shape.

Kirsty Minton

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IN BRIEF

NATURAL KILLER CELLS

The mature activating natural killer cell immunologic synapse is formed in distinct stages.

Orange, J. S. *et al. Proc. Natl Acad. Sci. USA* **100**, 14151–14156 (2003)

The interface between a natural killer (NK) cell and its target cell — the activating NK-cell immunological synapse — is highly organized. In this study, Orange *et al.* show that CD2 and CD11b, similar to CD11a, co-localize with filamentous actin in the peripheral supramolecular activation cluster (pSMAC), whereas perforin accumulates in the central SMAC (cSMAC). Polarization of CD2, CD11a and CD11b to the pSMAC was dependent on actin polymerization and Wiskott-Aldrich syndrome protein (WASP), but independent of microtubule function. By contrast, perforin accumulation in the cSMAC was dependent on actin polymerization, WASP and microtubule function. In addition, the rate of cSMAC perforin accumulation was slower than the recruitment of receptors to the pSMAC, indicating that formation of the NK-cell immunological synapse is a highly regulated, sequential process.

MUCOSAL IMMUNOLOGY

Spheniscins: avian β-defensins in preserved stomach contents of the king penguin, *Aptenodytes patagonicus*.

Thouzeau, C. *et al. J. Biol. Chem.* 2 October 2003 (doi:10.1074/jbc.M306839200)

In this paper, Thouzeau and colleagues have identified two new avian antimicrobial peptides in the stomach of the male king penguin. These peptides, known as spheniscin 1 and 2, belong to the β-defensin family and have broad antimicrobial activity against pathogenic bacteria and fungi. The levels of spheniscin 1 and 2 are higher during periods of food storage than when the birds are digesting, and interestingly, a drop in spheniscin levels correlated with a change from food storage to digestion. These antimicrobial peptides act to protect the surface of the bird's gastrointestinal tract from damage or invasion, and might be important for the long-term preservation of stored food, which can be important for chick survival.

LYMPHOCYTE MIGRATION

Restricted entry into lymph nodes

The ectodomain of the adhesion molecule L-selectin — which is crucial for T-cell migration from the bloodstream into peripheral lymph nodes (PLNs) — is proteolytically shed from the cell surface after cross-linking. A new study by Galkina *et al.* has now defined that L-selectin shedding shapes the migration pattern of T cells after antigen activation, but that it is not required for naive T-cell trafficking.

L-selectin mediates tethering and rolling of naive T cells in high endothelial venules (HEVs) prior to their migration to the PLNs. To investigate the importance of L-selectin shedding in this process, Ann Ager and colleagues generated endogenous L-selectin-deficient mice transgenic for either wild-type L-selectin (WT) or uncleavable L-selectin (LΔP) in T cells. The percentage of T cells in the PLNs of these animals was not statistically different, providing preliminary evidence that naive T-cell trafficking is not regulated by L-selectin shedding.

The authors further investigated the importance of L-selectin shedding in T-cell rolling *in vitro*, using a hydrodynamic flow model, and in T-cell trafficking *in vivo* after T-cell transfer to normal or recombinase-activating gene 1 (Rag1)-deficient mice. *In vitro*, the number of rolling LΔP and WT T cells, as well as their rolling velocity and behaviour, was the same. *In vivo,* transferred WT and LΔP naive T cells migrated in equal numbers to the PLNs. Together, these studies indicate clearly that naive T-cell homing to the PLNs is independent of L-selectin shedding.

After activation, T cells downregulate the expression of L-selectin and their migration patterns change. Antigen-specific L-selectin downregulation was shown to be mediated by L-selectin shedding using offspring of

T-CELL SIGNALLING

A new adaptor in the LIME-light

Several transmembrane adaptor proteins found in lipid rafts have been shown to influence the outcome of immunoreceptor signalling in lymphocytes. Two studies in *The Journal of Experimental Medicine* have now defined a new transmembrane adaptor molecule — LIME (LCK-interacting membrane molecule) — which is involved in regulating LCK-transduced signals in T cells.

Using a tyrosine phosphorylation-dependent yeast two-hybrid system, Hur *et al.* identified LIME as a protein that associates with the tyrosine kinase LCK. This association was confirmed in immunoprecipitation studies and was shown to require LCK-mediated phosphorylation of LIME.

In mice, LIME was detected in resting peripheral T cells and its expression levels were upregulated after stimulation with CD3-specific and CD28-specific antibodies. Confocal microscopy showed that LIME was distributed throughout the plasma membrane of resting T cells and that it relocated to the immunological synapse after T-cell receptor (TCR) recognition of antigen. These data indicate that LIME is a component of lipid rafts and this cellular localization was shown to be a result of palmitoylation of the membrane-proximal region of LIME.

Several signalling molecules, including the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and growth factor receptor-bound protein 2 (GRB2), were found to associate with LIME, and LIME-specific signals were shown to induce the phosphorylation of JUN N-terminal kinase (JNK) and extracellular signal-regulated kinase 1 (ERK1)/ERK2. Together, the results presented by Yun and colleagues indicate that through its association with LCK, LIME has a role in several signalling pathways that are activated after TCR stimulation.

In a separate study, Brdičková *et al.* identified mouse LIME by database searching for molecules that contain the domains characteristic of lipid-raft-associated transmembrane adaptor proteins, and then cloned the human homologue. As in mice, LIME protein was shown to be expressed by human peripheral blood T cells, in which it was palmitoylated and present in lipid rafts. However, a decrease in the expression level of LIME was detected after CD3-specific antibody stimulation, and removal of the TCR signal induced LIME re-expression, indicating that TCR signals regulate LIME expression by human peripheral blood T cells.

Stimulation of human peripheral blood T cells with CD4-specific antibodies induced tyrosine phosphorylation of LIME by protein tyrosine kinases, including LCK. Phosphorylation of distinct tyrosine residues mediated the association of LIME with LCK and the negative regulator of SRC kinases, CSK kinase, indicating that LIME phosphorylation can direct LCK and CSK recruitment to lipid rafts.

LIME-associated LCK was phosphorylated at both the negative and positive regulatory tyrosine residues, and overexpression of LIME induced an enhanced calcium response to TCR triggering by CD3-specific antibodies. These observations led Hořejší and colleagues to suggest that after TCR and CD4 co-ligation, LIME might potentiate TCR-mediated signals by binding LCK and so recruiting it to the lipid rafts and blocking its switch to the inactive form.

These two studies have identified a new transmembrane adaptor protein — LIME — that regulates T-cell signalling through the tyrosine kinase LCK; however, further studies will be required to define its role fully.

Karen Honey

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WT and LΔP mice crossed with animals expressing a T-cell receptor specific for an influenza-virus-derived peptide (F5). When transferred to Rag1-deficient mice, activated F5/LΔP T cells were more efficient at entering the PLNs than F5/WT T cells exposed to antigen, indicating that L-selectin shedding prevents the re-entry of activated T cells into the PLNs.

These studies show that the physiological role of L-selectin shedding is to shape the migration pattern of activated T cells and prevent them from re-entering PLNs.

Karen Honey

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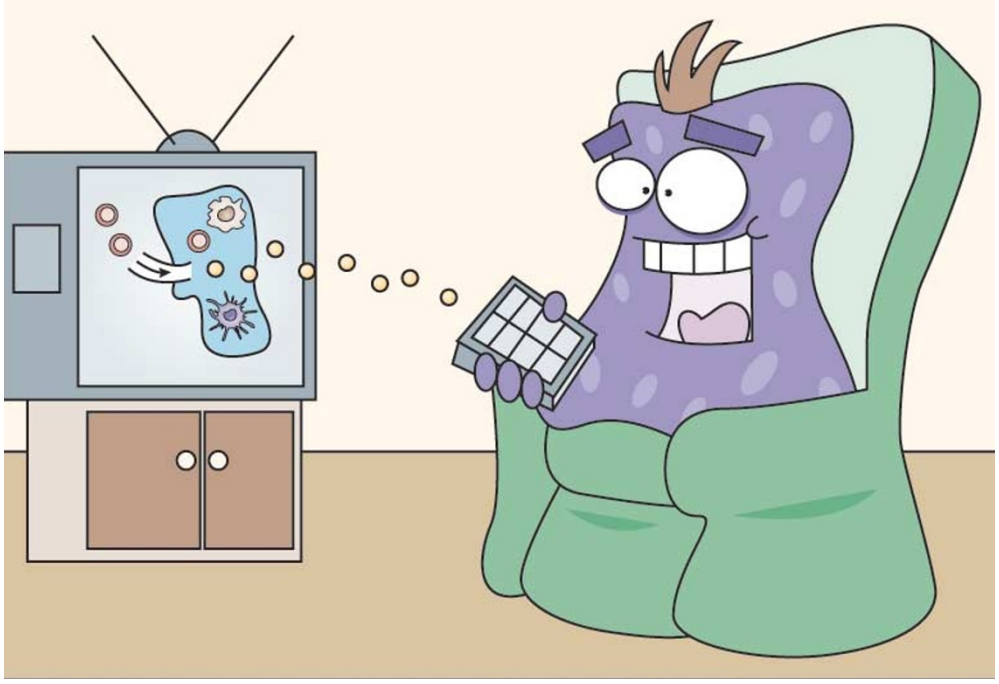

IMMUNE RESPONSES

Mast cells act by remote control

Swollen 'glands' are often one of the first signs that you have picked up a bacterial infection. This lymph-node hypertrophy results from the accumulation of circulating lymphocytes in the draining lymph nodes, which can interact with antigen-presenting cells (APCs) that are loaded with microbial antigen and so initiate an adaptive immune response. Bacterial products at the site of infection are thought to induce the emigration and maturation of APCs, but the signals that control T-cell recruitment to the lymph nodes are unknown. Work now published in *Nature Immunology* shows a central role for mast cells in this process.

Mast cells are important components of the innate immune response against bacteria, through degranulation and the release of inflammatory mediators, including tumour necrosis factor (TNF). Here, McLachlan *et al.* used a mouse model of localized infection to investigate if these cells are involved in controlling bacteria-triggered nodal hypertrophy and so have a role in the induction of adaptive immune defence.

In wild-type mice, injection of bacteria into the footpad resulted in lymph-node hypertrophy within 24 hours. However, lymph-node swelling was markedly reduced in mast-cell-deficient mice (W/W^v mice). Injection of mast cells into the footpads of W/W^v mice before bacterial challenge resulted in a similar level of lymph-node hypertrophy as seen in wild-type mice, indicating an important role for mast cells in this process. The authors confirmed this by using a specific mast-cell activator (48/80), which if injected instead of bacteria into the footpad of mice also resulted in considerable lymph-node hypertrophy.

A closer investigation of the site of infection showed that although the overall number of mast cells had not increased 4 hours after bacterial innoculation, the percentage of degranulated mast cells had. This probably indicates that mast cells act remotely to induce nodal hypertrophy and, instead of leaving the site of infection and migrating to the lymph nodes themselves, they release a product on degranulation that drains into the lymph node and signals for hypertrophy to occur.

Which mast-cell product might be involved? TNF, but not other inflammatory mediators that mast cells release, was shown to have potent hypertrophic effects, and 3 hours after infection (or mast-cell activation with 48/80), the level of TNF in the draining lymph nodes increased markedly. In addition, mast-cell activation resulted in a threefold increase in the numbers of T cells recruited to the lymph nodes.

This study indicates that, through the release of TNF, mast cells provide an essential signal early in infection to trigger, by remote control, the hypertrophy of draining lymph nodes and the initiation of an adaptive immune response.

Jenny Buckland

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WEB SITE

Soman N. Abraham's lab: http://pathology.mc.duke.edu/research/postabraham.html

T-CELL DEVELOPMENT

Directional cues from CXCR4

The CXC-chemokine receptor 4 (CXCR4) provides essential signals that guide thymocytes on their developmental journey through the thymus, according to a recent study in *The Journal of Immunology*.

As thymocytes mature in the post-natal thymus, they migrate from their entry point near the cortico-medullary junction (CMJ), outwards across the cortex towards the capsule, and then back again across the cortex towards the medulla. This migration enables these developing progenitor cells to interact with thymic stromal cells that provide signals that are required for commitment to the T-cell lineage, and for efficient thymocyte differentiation and proliferation.

How is this cortical migration controlled? Petrie and colleagues investigated the role of chemokines in this process by first asking which chemokine receptors were expressed by thymocyte progenitors. Of all of the known chemokine receptors, CXCR4 was the most abundant, being expressed by all thymocyte prognenitors. Further experiments showed that the ligand for this receptor, CXCL12, is produced by cortical stromal cells, indicating that signals through CXCR4–CXCL12 could potentially be involved in guiding progenitors into the cortex.

To investigate this possibility, the authors next measured whether thymocyte progenitors could migrate in response to CXCL12. Transwell migration assays using CXCL12 as a chemoattractant showed that all populations of progenitors that were tested migrated towards CXCL12. To confirm the role of CXCR4 signalling in this directional movement, the *in vivo* migration of CXCR4-deficient progenitor thymocytes was assessed. T-cell numbers were low and thymocyte differentiation was blocked at an early stage in mice reconstituted with bone marrow that lacked CXCR4 expression. Furthermore, thymocytes derived from CXCR4-deficient bone marrow accumulated at the CMJ and did not migrate efficiently into the cortex.

This study highlights the essential and non-redundant role for CXCR4–CXCL12 signalling in controlling the migration of thymocyte progenitors across the cortex — a process that is required for the development of mature T cells in the post-natal thymus.

Jenny Buckland

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WEB SITE

Howard Petrie's lab: http://www.mskcc.org/mskcc/html/11136.cfm

REGULATORY T CELLS

IDO — influencing dendritic-cell options

$CD4^+CD25^+$ regulatory T cells express cytotoxic T lymphocyte-associated antigen 4 (CTLA4) constitutively, and although this has been shown in several settings to be important for regulatory T-cell function, the mechanisms by which CTLA4 induces suppression have remained unclear. Now, a study in *Nature Immunology* shows that CTLA4 can modulate dendritic cells (DCs), initiating the immunosuppressive pathway of tryptophan catabolism.

Interferon-γ (IFN-γ) regulates transcription of the gene encoding indoleamine 2,3-dioxygenase (IDO) — the enzyme that catalyses the initial step of tryptophan degradation. IDO activity has an immunosuppressive effect that has been linked with tolerance induction during pregnancy, transplantation and autoimmunity.

Fallarino *et al.* observed that human Jurkat T cells that were transfected with mouse CTLA4 induced IFN-γ production, upregulation of IDO expression and tryptophan degradation by mouse DCs. They then showed that regulatory T cells also induced IFN-γ production and tryptophan degradation by DCs and that this was inhibited by CTLA4-specific neutralizing antibodies. Activation of regulatory T cells with CD3-specific antibodies enhanced their ability to induce IFN-γ production and tryptophan degradation by DCs and this depended on their increased expression of CTLA4.

Does tryptophan catabolism alter the capacity of DCs to induce immune responses *in vivo*? The ability of $CD8^-$ DCs loaded with a synthetic peptide (NRP-A7) to induce a persistent immune response in mice was eliminated by pre-exposure of the DCs to regulatory T cells activated with CD3-specific antibody. However, the ability of peptide-loaded DCs to initiate an immune response was restored if an inhibitor of IDO was present during DC exposure to the activated regulatory T cells.

These studies identify a CTLA4-dependent mechanism by which $CD4^+CD25^+$ regulatory T cells induce immunosuppression — modulation of DCs to initiate IFN-γ production and, thereby, IDO activity and tryptophan catabolism.

Karen Honey

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B-CELL DEVELOPMENT

Plasma-cell differentiation requires Blimp1

Although the transcriptional repressor B-lymphocyte-induced maturation protein 1 (Blimp1) has been previously shown to induce B-cell differentiation into immunoglobulin-producing plasma cells, until now, it has not been identified as non-redundant and essential for this process.

Mice lacking the gene encoding Blimp1, *Prdm1*, die *in utero*, so to study the dependence of B-cell differentiation and function on Blimp1, Shapiro-Shelef *et al.* generated mice in which *Prdm1* was specifically eliminated in mature B cells, $Prdm1^{flox/flox}CD19^{cre/+}$ mice. B-cell development was normal in these animals but serum immunoglobulin was markedly reduced, even in unimmunized mice.

After immunization with either a thymus-independent (TI) or thymus-dependent (TD) antigen, the immunoglobulin response generated in $Prdm1^{flox/flox}CD19^{cre/+}$ mice was substantially diminished when compared with control animals, as was immunoglobulin secretion after re-challenge with TD antigen. This decrease in immunoglobulin production correlated with a marked reduction in the number of antigen-specific immunoglobulin-secreting cells and consistent with this, few plasma cells or pre-plasma memory B cells could be detected in $Prdm1^{flox/flox}CD19^{cre/+}$ mice after either primary or secondary exposure to TD antigen. So, Blimp1 is required for the development of both plasma cells and pre-plasma memory B cells during both a primary and memory response.

To investigate the mechanisms by which Blimp1 regulates immunoglobulin secretion and plasma-cell differentiation, Shapiro-Shelef *et al.* analysed Blimp1-deficient B cells that were exposed to lipopolysaccharide (LPS) *ex vivo*. In the absence of Blimp1, switching from the membrane-associated form of the immunoglobulin heavy chain (M) to the secreted form (S) was markedly impaired, as was the induction of S messenger RNA. In addition, the transcription factor X-box binding protein 1 (XBP1) was not induced by LPS stimulation of $Prdm1^{-/-}$ B cells, identifying it as a downstream target of Blimp1. However, retrovirus-mediated overexpression of XBP1 by Blimp1-deficient B cells was insufficient to rescue plasma-cell development, indicating that Blimp1 must target many factors to induce plasma-cell differentiation.

These studies using a conditional gene-deletion strategy define Blimp1 as essential for the differentiation of plasma cells and immunoglobulin secretion, and indicate that this transcription factor also has a key role in pre-plasma memory B-cell development.

Karen Honey

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WEB SITE

Kathryn Calame's lab: http://cpmcnet.columbia.edu/dept/gsas/biochem/faculty/calame.html

IN BRIEF

CYTOKINES

IL-23 produced by CNS-resident cells controls T-cell encephalitogenicity during the effector phase of experimental autoimmune encephalomyelitis.

Becher, B., Durell, B. G. & Noelle, R. J. *J. Clin. Invest.* **112**, 1186–1191 (2003)

Interleukin-23 (IL-23) consists of a unique p19 subunit and the p40 subunit of IL-12, and is crucial for the development of experimental autoimmune encephalomyelitis in mice. Becher *et al.* use p40-deficient mice to generate irradiation bone-marrow chimaeras and show that in the absence of p40 expression by irradiation-resistant cells, previously activated antigen-reactive cells infiltrate the central nervous system (CNS), but do not induce encephalitogenicity. In addition, disease induced by immunization is markedly diminished when p40 is absent from irradiation-resistant cells and this is associated with a bias towards the production of T helper 2-type cytokines by infiltrating cells. The authors, therefore, suggest that p40 expression by CNS-resident cells contributes to T-cell polarization and thereby modulates disease pathogenesis.

TRANSPLANTATION

Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor.

Changelian, P. S. *et al*. *Science* **302**, 875–878 (2003)

The development of effective immunosuppressive agents that are targeted specifically to immune cells and do not produce serious side effects remains a pressing need for the prevention and treatment of transplant rejection. This study reports pre-clinical trials of a new immunosuppressive agent (CP-690,550) that prolonged survival in both a mouse model of heart transplantation and a cynomolgus monkey model of kidney transplantation, but that did not result in the dose-limiting side effects that are associated with present therapies. CP-690,550 inhibits Janus kinase 3 (JAK3), which is crucial for signalling through cytokine receptors that use the common γ-chain and, therefore, for development and homeostasis of immune cells. It is the first JAK3 inhibitor to have shown efficacy in non-human primates.

IMMUNOTHERAPY

A critical role for OX40 in T cell-mediated immunopathology during lung viral infection.

Humphreys, I. R. *et al*. *J. Exp. Med*. **198**, 1237–1242 (2003)

Respiratory infections are an important example of how an overactive T-cell response can be more of a hindrance than a help, with T-cell responses resulting in airway occlusion and pathology. Previous T-cell-targeted therapies have affected both bystander and antigen-specific T cells, which might result in dangerous immunosuppression. These authors used an OX40–immunoglobulin fusion protein to target recently activated T cells specifically. OX40 signalling prevents the death of activated T cells, but it is not expressed by naive T cells. Interference with this T-cell survival signal in a mouse influenza model reduced immunopathology without preventing virus clearance.