IN THE NEWS

No problem inhaling?
Research presented at the recent American Academy of Allergy, Asthma and Immunology (AAAI) annual meeting indicates that a component in asthma inhalers might cancel out the beneficial anti-inflammatory effects of inhaled steroids (*AAAI*).

Albuterol (Salbutamol) is a β-agonist used in asthma inhalers to promote airway dilation. It is composed of equal amounts of two isomers — one active, R-albuterol, and one inactive, S-albuterol. Studies carried out at the University of Pittsburgh by Dr Bill Ameredes indicated that the anti-inflammatory effects of steroids were enhanced by R-albuterol; however, S-albuterol abolished the anti-inflammatory steroid effects. Dr Ameredes said, "These results indicate that S-albuterol may diminish the beneficial anti-inflammatory effects of steroids… " (*BBC*), and that therefore, we need to consider "the possibility that current combination therapies … may not be realizing their full potential" (*Patient Health International*).

Despite these results, experts are keen to stress that patients should not stop taking their medication. "These findings are related only to Salbutamol used in combination with inhaled steroids, and if you are using this combination then your asthma should be under control and you shouldn't actually need to take your reliever medicine very often, if at all," said Katie Shepard, the National Asthma Campaign's care development manager (*National Asthma Campaign*). Furthermore, albuterol is only designed to relieve asthma symptoms in the short-term, and as Martyn Partridge, Chief Medical Advisor to the National Asthma Campaign, said, "If you're currently using Salbutamol more than three times a week, then you should be on a low-dose inhaled steroid. You'd take that regularly and you'd find that you didn't need the Salbutamol" (*The Guardian*).

THYMOCYTE DEVELOPMENT

Positive about calcineurin

According to new research published in *Immunity*, calcineurin is required for positive but not negative selection of developing T cells in the thymus.

Before T cells emigrate from the thymus, they undergo a selection process, which occurs at the $CD4^+$ $CD8^+$ double-positive (DP) stage of thymocyte development (before the downregulation of either CD4 or CD8 expression). It is thought that only those T cells able to engage in transient interactions with self-MHC molecules are maintained (positive selection) and that those T cells displaying strong reactivity are eliminated (negative selection). But what is the mechanism by which low-intensity interactions result in cell survival and proliferation and high-intensity interactions result in cell death?

Neilson and colleagues set out to explore this by examining the role of calcineurin (which activates Nfatc subunits) in thymic selection. The authors generated mice with a targeted deletion of the regulatory subunit of calcineurin (Cnb1), rendering all isoforms of calcineurin inactive in thymocytes but not non-lymphoid components of the thymus. The thymi of these mice were found to have essentially no single-positive (SP) T cells. This complete failure to progress from the DP to the SP stage indicates that calcineurin is required for positive selection. DP thymocytes were also unable to upregulate the expression of cell-surface markers associated with positive selection, such as the β-chain of the T-cell receptor (TCR-β), CD69 and CD5. Furthermore, positive selection of cells expressing a clonotypic TCR was blocked in the absence of calcineurin.

AUTOIMMUNITY

Breaking barriers

Why are some mouse strains susceptible to autoimmune disease, while others are resistant? Previous studies have suggested that resistance to experimental autoimmune encephalomyelitis (EAE) lies in a T-cell-specific defect. However, Kuchroo and colleagues, reporting in *The Journal of Clinical Investigation*, propose an alternative mechanism involving impaired activation of antigen-presenting cells (APCs) in resistant mouse strains.

SJL mice that express a transgenic T-cell receptor (TCR) specific for the myelin antigen proteolipid protein (PLP) (5B6 TCR-transgenic mice) readily develop spontaneous EAE. However, 5B6 TCR-transgenic mice on the B10.S background rarely develop spontaneous EAE. By comparing these mouse strains, the authors showed that resistance was not due to deletion of autoreactive T cells or to T-cell anergy, as both strains had comparable numbers of PLP-specific T cells, which proliferated in response to PLP peptide presented by artificial APCs *in vitro*. By contrast, analysis of APCs from the two mouse strains showed that those from resistant B10.S mice expressed lower levels of MHC class II and co-stimulatory molecules, and were less efficient at stimulating proliferation of transgenic T cells, indicating that B10.S APCs have a defective activation status.

The authors then went on to show that this defective APC phenotype could be overcome by incubating the cells with oligodeoxynucleotides containing CpG motifs (CpG ODNs), which have been shown to activate APCs through Toll-like receptor 9 (TLR9). Moreover, administration of CpG ODNs, but not non-CpG-containing ODNs, to EAE-resistant mice was sufficient to break T-cell tolerance and induce EAE in these mice. Co-administration of CpG ODNs and the cognate PLP peptide further increased the incidence and severity of disease in the B10.S transgenic mice. This disruption in tolerance was most readily seen when APC activation was mediated through TLR9, because administration of lipopolysaccharide, which is recognized by TLR4, did not have such a marked effect on EAE induction in the resistant strain.

Next, the authors investigated the impact of calcineurin on negative selection. Negative selection of H-Y TCR-transgenic, Cnb1-deficient thymocytes was shown to occur normally in male mice, which express the male-specific H-Y antigen. This lack of requirement for calcineurin was confirmed using several *in vitro* assays. When Cnb1-deficient thymocytes were co-cultured with control thymocytes (from wild-type littermates) in the presence of antibodies specific for both CD3 and CD28, both cell populations displayed similar levels of DP cell death. Similar results were obtained (using a more physiological model) when moth cytochrome c peptide–I-E^k complexes were added to co-cultures of Cnb1-deficient and control thymocytes transgenic for the cognate TCR, 5C.C7. Moreover, Cnb1-deficient thymocytes had the same threshold for negative selection as wild-type thymocytes and expressed similar levels of the pro-apoptotic protein Bim (which is required for negative selection) and the anti-apoptotic protein Bcl-X_L.

Calcineurin and another signal transducer, extracellular-signal-regulated kinase (Erk), are known to be activated by both positively and negatively selecting signals, although neither of these is required for negative selection. So the authors suggest that the lowest molecule which is common to the signalling pathways of both positive and negative selection must be upstream of calcineurin and Erk (and also Bim) and downstream of TCR-proximal signalling molecules, which are clearly required for TCR signalling. They propose a model whereby both low- and high-intensity signals activate the calcineurin and Erk pathways, but high-intensity signals cause negative selection by activating a dominant pathway, which induces apoptosis through Bcl-2 family members (including Bim).

Davina Dadley-Moore

References and links

ORIGINAL RESEARCH PAPER Neilson, J. R. *et al.* Calcineurin B1 is essential for positive but not negative selection during thymic development. *Immunity* **20**, 255–266 (2004)

FURTHER READING Palmer, E. Negative selection — clearing out the bad apples from the T-cell repertoire. *Nature Rev. Immunol.* **3**, 383–391 (2003)

WEB SITE

Jerry Crabtree's lab:
http://crablab.stanford.edu/

So, the authors suggest that activation of APCs through receptors of the innate immune system can tip the balance between peripheral tolerance and autoimmunity in favour of autoimmune disease.

Lucy Bird

References and links

ORIGINAL RESEARCH PAPER Waldner, H., Collins, M. & Kuchroo, V. K. Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. *J. Clin. Invest.* **113**, 990–997 (2004)

WEB SITE

Encyclopedia of Life Sciences:
http://www.els.net/
autoimmune disease: aetiology and pathogenesis

IN BRIEF

AUTOIMMUNITY

Human lupus T cells resist inactivation and escape death by upregulating COX-2.

Zu, L. *et al. Nature Med.* **10**, 411–415 (2004)

This study used microarray analysis to look at why $CD4^+$ T helper (T_H) cells from patients with systemic lupus erythematosus (SLE) are resistant to anergy and activation-induced cell death (AICD). They show that the *PTGS2* gene, which encodes COX2, is upregulated in T_H cells from patients with SLE. COX2 expression protects T_H cells from death by upregulating expression of the anti-apoptotic protein c-FLIP and decreasing signalling through the FAS (CD95) apoptotic pathway. COX2 overexpression did not result in prostaglandin E_2 production by the T_H cells, which indicates that COX2 has a role in transcriptional regulation that does not depend on its enzymatic activity. This could lead to the development of selective inhibitors of the COX2 anti-apoptotic pathway.

LYMPHOPOIESIS

Development of a human adaptive immune system in cord blood cell-transplanted mice.

Traggiai, E. *et al. Science* **304**, 104–107 (2004)

To overcome the limitations of studying development of the human immune system *in vitro*, researchers have long sought to develop an *in vivo* model involving transplantation of human cells into immunodeficient mice. A new system reported in this paper has for the first time led to the formation of a functional adaptive immune response. The authors transplanted $CD34^+$ human cord-blood precursors into the liver of newborn *Rag2*$^{-/-}\gamma c^{-/-}$ mice and analysed them up to 6 months of age. They showed that the development of human T cells, B cells and dendritic cells occurred normally in the xenotransplanted mice and resulted in the formation of organized lymphoid structures. Furthermore, the mice mounted a good immune response to vaccination with tetanus toxoid or infection with Epstein–Barr virus.

AUTOIMMUNITY

Coxsackieviral-mediated diabetes: induction requires antigen-presenting cells and is accompanied by phagocytosis of β cells.

Horwitz, M. S. *et al. Clin. Immunol.* **110**, 134–144 (2004)

This study indicates a crucial role for antigen-presenting cells in initiating the destruction of pancreatic β-cells induced by viral infection. Epidemiological studies have shown a link between infection with coxsackie B virus (CBV) and type 1 diabetes. To investigate the pathogenic process, Sarvetnick and colleagues used a diabetes model involving infection of islet-specific T-cell receptor (TCR)-transgenic mice with CBV strain 4 (CB4). They show that CB4 infects insulin-producing β-cells without causing necrosis. Instead, the stressed islet cells are phagocytosed by resident macrophages, which present islet epitopes to autoreactive T cells. These macrophages could induce diabetes after adoptive transfer to uninfected TCR-transgenic mice. Therefore, viral infection could be the last initiating step in multi-step diabetes development, requiring the pre-existence of autoreactive T cells.