ANTIGEN PRESENTATION

γδ T cells turn professional

Human γδ T cells can now join dendritic cells (DCs) in the professional antigen-presenting cell (APC) club — a paper recently published in *Science* reports that human γδ T cells that have been activated *in vitro* display typical cell-surface markers of APCs and can stimulate the proliferation and differentiation of αβ T cells.

γδ T cells differ from αβ T cells in many ways. Human γδ T cells can recognize small non-peptidic antigens that are derived from microorganisms or necrotic host cells and that do not require antigen processing, and this recognition does not depend on classical MHC class I or class II molecules. In addition, their effector functions include both innate and adaptive immune functions (that is, secretion of chemokines and cytokines, as well as the ability to provide B-cell help and to develop memory function).

In human peripheral blood, most γδ T cells express the Vγ2Vδ2$^+$ T-cell receptor (TCR) and are referred to as Vδ2$^+$ T cells. Following TCR triggering, these cells transiently upregulate the lymph-node homing receptor CC-chemokine receptor 7 (CCR7) and can be observed in the lymph nodes that drain mucosal tissues. In this study, tonsillar γδ T cells were shown to express CD69, a cell-surface marker that is associated with *in vitro*-stimulated cells, as well as MHC class II molecules and a range of co-stimulatory and adhesion molecules. Similarly, peripheral-blood Vδ2$^+$ T cells stimulated with isopentenyl pyrophosphate (IPP), the prototypical ligand for these cells, expressed various cell-surface markers that are associated with professional APCs, and these markers were almost identical to those expressed by monocyte-derived DCs stimulated with lipopolysaccharide (LPS).

The ability to migrate to lymph nodes and the expression of inducible markers of APCs prompted the authors to examine a potential role for Vδ2$^+$ T cells in antigen presentation to αβ T cells. First, they measured the ability of IPP-stimulated Vδ2$^+$ T cells to stimulate CD4$^+$ αβ T cells in a mixed lymphocyte reaction and in primary responses to superantigen. The proliferation of CD4$^+$ T cells and their differentiation into T-helper cells occurred to a similar extent to that elicited by LPS-matured DCs.

Next, the ability of Vδ2$^+$ T cells to process antigen for presentation to αβ T cells was examined. The authors used two model antigens — tetanus toxoid (TT) and the complex mixture of proteins in *Mycobacterium tuberculosis* protein derivative (PPD) — and they observed proliferation of CD4$^+$ and CD8$^+$ αβ T cells in response to activated Vδ2$^+$ T cells presenting either TT or PPD. Presentation was dependent on intracellular processing, because blockade of protein degradation and peptide loading onto MHC class II molecules, using chloroquine, was shown to inhibit proliferation.

These results show a new role for γδ T cells, in the initiation of adaptive immune responses. Although it will be important to examine the physiological relevance of this function, it seems that Vδ2$^+$ T cells could have a role as APCs in initiating immune responses in inflammatory and/or infectious situations.

Elaine Bell

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CYTOKINES

WSX1 exposed to positive and negative influences

A recent report in *The Journal of Immunology* indicates that expression of the ligand-specific component of the interleukin-27 (IL-27) receptor, WSX1, is differentially regulated after activation of distinct lymphoid-cell populations — WSX1 expression is increased after T-cell activation but decreased after natural killer (NK)-cell or natural killer T (NKT)-cell activation.

Initial reports indicated that the level of mRNA encoding WSX1 was decreased after activation of naive $CD4^+$ T cells and that WSX1-deficient mice were more susceptible to infection with intracellular pathogens. Therefore, it was proposed that IL-27 is an important factor for the activation of naive $CD4^+$ T cells to mediate type 1 immunity. However, subsequent studies showed that IL-27 was an inhibitor of effector T cells: WSX1-deficient mice infected with *Toxoplasma gondii* generated an appropriate immune response but succumbed to an inflammatory disease.

So Villarino *et al.* set out to study the level of expression of WSX1 on the lymphoid-cell populations that are known to have a role in controlling infection with *T. gondii*. A significantly lower proportion of NK cells and NKT cells (which are activated during the acute phase of infection) expressed high levels of WSX1 in mice infected with *T. gondii* than in uninfected animals. Furthermore, those cells still expressing high levels of WSX1 retained an unactivated phenotype, indicating that downregulation of WSX1 correlates with cellular activation. By contrast, the proportion of $CD4^+$ and $CD8^+$ T cells (which are required for resistance to infection with *T. gondii*) expressing high levels of WSX1 was increased after infection. Furthermore, the $WSX1^{hi}CD4^+$ T cells had an effector phenotype, indicating that WSX1 upregulation correlates with T-cell activation.

Consistent with a role for T-cell activation as a regulator of WSX1 expression, T-cell-receptor crosslinking on $CD4^+$ T cells *in vitro* resulted in increased cell-surface expression of WSX1, although this increase was only transient. Interestingly, upregulation of WSX1 required that the cells entered the cell cycle, but continued cell division was associated with the decrease in WSX1 expression levels. Additional signals that negatively regulate WSX1 expression levels were shown to come from IL-2.

This study indicates that activation of distinct lymphoid cells results in differential regulation of WSX1 expression levels and that both positive and negative signals can regulate this process, both in distinct cell types and within a responding population of cells.

Karen Honey

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FURTHER READING Hunter, C. A. *et al.* New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nature Rev. Immunol.* **5**, 521–531 (2005)

IN BRIEF

STRUCTURE

Signaling conformations of the tall cytokine receptor gp130 when in complex with IL-6 and IL-6 receptor.

Skiniotis, G. *et al. Nature Struct. Mol. Biol.* 15 May 2005 (doi:10.1038/nsmb941)

Structural analysis of the cytokine-binding domains of gp130 (glycoprotein 130), interleukin-6 (IL-6) and the α-chain of the IL-6 receptor (IL-6Rα) indicated that this trimolecular complex dimerizes for signalling to occur. However, the membrane-proximal domains of gp130 were predicted to extend away from each other, rather than enter the membrane in close proximity as would be expected for signalling to occur. Skiniotis *et al.* visualized the conformation of the extracellular portion of gp130 (the cytokine-binding and membrane-proximal domains) complexed with IL-6 and IL-6Rα and observed that the gp130 molecules are bent such that the membrane-proximal domains interact close to the membrane. The authors suggest that this enables activation of intracellular signalling and that bending of the gp130 molecules might occur as a conformational transition when ligand binds.

DENDRITIC CELLS

Nectin-like protein 2 defines a subset of T-cell zone dendritic cells and is a ligand for class-I-restricted T-cell-associated molecule.

Galibert, L. *et al. J. Biol. Chem.* **280**, 21955–21964 (2005)

Galibert *et al.* set out to identify markers of dendritic cell (DC) subsets that are conserved across species. A single-chain antibody fragment (scFv) that specifically labels human $BDCA3^+$ DCs was isolated using a whole-cell-panning phage-display approach. When expressed as an Fc fusion protein, this scFv also bound mouse splenic $CD11c^+CD11b^-CD8\alpha^+$ DCs. The scFv target was shown to be nectin-like protein 2 (NECL2), and its ligand was identified as class-I-restricted T-cell-associated molecule (CRTAM), which is expressed by activated $CD8^+$ T cells. CRTAM–NECL2 interactions induced increased expression of interleukin-22 mRNA by the activated $CD8^+$ T cells, leading the authors to suggest that this conserved interaction probably contributes to DC–T-cell crosstalk.

LYMPHOCYTE SIGNALLING

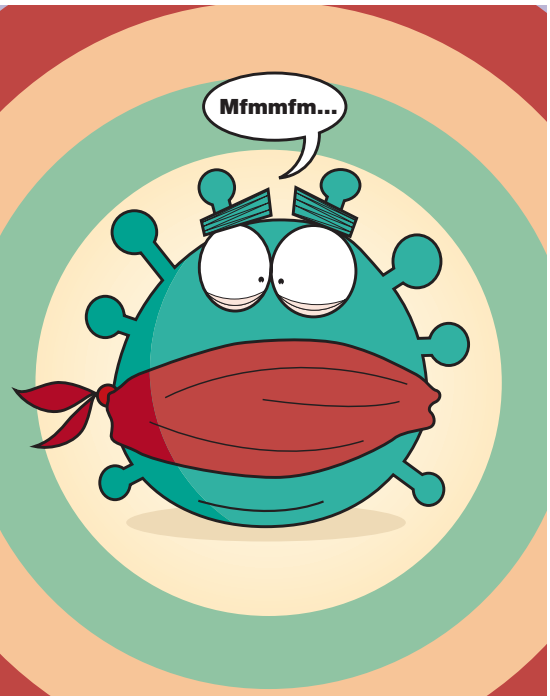
Cytokine-driven cell cycling is mediated through Cdc25A.

Khaled, A. R. *et al. J. Cell Biol.* 31 May 2005 (doi:10.1083/jcb.200409099)

Lymphocytes are known to require cytokine-mediated signals, such as those provided by interleukin-7 (IL-7) or IL-3, for both survival and proliferation, but until now, little has been known about the cytokine-driven proliferation pathway. This study shows that cytokine signals are required to prevent p38 mitogen-activated protein kinase (MAPK)-mediated phosphorylation and degradation of the phosphatase CDC25A (cell-division cycle 25A). CDC25A, in turn, dephosphorylates cyclin-dependent kinases such as CDK2, which promotes association with cyclin E, phosphorylation of the cell-cycle inhibitor retinoblastoma-susceptibility protein and entry to the cell cycle. Cytokines, therefore, seem to protect lymphocytes from a stress response that involves activation of the stress kinase p38 MAPK by cytokine withdrawal.

HIV

Silencing HIV the natural way



A recent paper in *Immunity* describes the first natural example of RNA silencing in mammalian host–virus interactions. This evolutionarily ancient form of nucleic-acid-based immunity has previously been found in plants and invertebrates. But the demonstration that human cells can physiologically silence HIV-1 RNA highlights the universal importance of this system.

RNA silencing (also known as RNA interference, RNAi) in invertebrates is based on the cleavage of double-stranded RNA precursors by the RNase Dicer to form short interfering RNAs (siRNAs) of ~21 nucleotides; siRNAs then induce the degradation of complementary target RNAs, leading to post-transcriptional gene silencing. Silencing of viral genes is a natural antiviral response in plants and insects, using siRNA derived from the viral genome by the host processing machinery.

To examine the relevance of this response in immunity to HIV-1, the authors looked for sequences in the viral genome that could form the RNA-duplex precursors necessary for cleavage by host Dicer. They found one sequence in HIV-1, in the *env* gene (which encodes the envelope surface glycoprotein), that formed a 19-base-pair duplex, and this sequence was functionally recognized by Dicer *in vitro*. Furthermore, the Dicer cleavage product — viral siRNA (vsiRNA) — was detected in HIV-infected T cells but not in mock-infected cells.

To test whether the vsiRNA could mediate RNAi, they used constructs of its target RNA sequence fused to enhanced green fluorescent protein (EGFP) such that cleavage of the target RNA would prevent transcription of the downstream EGFP-encoding RNA. The addition of vsiRNA precursors led to a dose-dependent silencing of EGFP expression, and these precursors could also silence expression of the cognate target HIV-1 *env* mRNA.

Because HIV-1 successfully replicates in human cells to high levels of cell-free RNA, it must be able to defend itself against this host-exploited RNAi. Synthetic siRNA precursors specific for the HIV-1 transactivation response element (TAR) were ineffective at suppressing EGFP expression from a TAR–EGFP transcript when the HIV-1 transcriptional transactivator (Tat) was included in the assay, and the suppressive function of Tat was also shown for other non-TAR sequences. This indicates that Tat is a general suppressor of RNA silencing (SRS). This

AUTOIMMUNITY

Homing in on the target

Although multiple pancreatic islet-cell molecules are targets of autoimmune responses in human type 1 diabetes and in mouse models, conclusive evidence of the autoantigens that are important for initiation of the disease has been difficult to obtain. Now, two articles in *Nature* show that insulin is likely to be a crucial autoantigen in the development of type 1 diabetes in both the mouse model and human patients.

Previously, it has been shown that most CD4$^+$ T cells infiltrating the pancreas in non-obese diabetic (NOD) mice recognize insulin, in particular peptide 9–23 of the insulin B chain. So, to test the role of this response in the development of disease, Nakayama *et al.* generated NOD mice in which both insulin genes (*Ins1* and *Ins2*) were deleted and replaced with a transgene encoding mutant pro-insulin. The mutant pro-insulin contained a single amino-acid change at position 16 of the insulin B chain, which preserves the metabolic activity of insulin but prevents recognition by the infiltrating T cells. None of the *Ins1*$^{-/-}$*Ins2*$^{-/-}$ NOD mice expressing the modified insulin showed signs of an immune response to the islet cells, and they did not develop diabetes (although autoimmune reactions were still evident in the salivary glands). By contrast, the presence of either insulin gene in these mice was sufficient to restore diabetes, confirming a crucial role for both insulin genes as targets of organ-specific autoimmunity.

Unlike mouse studies, the study of human autoimmune diseases is often hindered by the scarcity and lability of the relevant target tissues or draining lymph nodes. But in the second study, Kent *et al.* obtained viable pancreatic draining lymph nodes from three individuals with type 1 diabetes (two with long-term disease and one with recent-onset disease) and three control individuals. From these lymph-node samples, the authors generated single T-cell clones in a non-biased manner and analysed their T-cell receptor (TCR) repertoire and their antigen specificity. T-cell clones isolated from pancreatic lymph nodes of control individuals expressed heterogeneous TCR repertoires, indicative of polyclonal expansion. By contrast, more than half of the T-cell clones from the long-term diabetic individuals expressed identical Vβ chains, and of these clones, half had the same TCR α-chain, implying antigen-driven expansion of a common progenitor cell. The clones from the long-term diabetic individuals, but not those from control individuals, proliferated specifically in response to peptide 1–15 of insulin A in a dose-dependent manner. This response was restricted by the MHC class II allele HLA-DRB1*0401, which is known to confer genetic susceptibility to diabetes.

To rule out the possibility that the insulin-specific T-cell responses could have arisen from long-term use of daily insulin injections (to control blood glucose levels), Kent *et al.* showed that insulin-reactive T cells were not found in the spleen of one of the long-term diabetic individuals, and CD4$^+$ T-cell clones from pancreatic draining lymph nodes of

was not a consequence of the transcription of a downstream effector, as a point mutant of Tat without transcriptional activity could still function as an SRS. Instead, Tat was shown *in vitro* to target the host RNAi mechanism upstream of vsiRNA generation, at the level of Dicer.

Another Tat mutant was transcriptionally proficient but lacked SRS activity; recombinant HIV containing this mutated form of Tat replicated less efficiently than HIV containing wild-type Tat. However, the difference was only slight, indicating that HIV infection is controlled by a range of host factors, one of which is the balance between host-exploited RNAi and viral SRS. When this balance was disrupted by the addition of oligonucleotides that inhibit vsiRNA to HIV-1-transfected cells, there was a dose-dependent increase in viral replication.

The authors suggest that the finding of only one duplex sequence in the HIV-1 genome indicates that there is a strong selective pressure exerted by host RNAi against such sequences and that the remaining sequence must have a crucial non-mutatable function for HIV-1, requiring the maintenance of a viral SRS.

Kirsty Minton

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an individual with type 2 diabetes did not recognize any pro-insulin peptide.

Knowing which autoantigens trigger autoimmunity is still out of reach for many researchers studying autoimmune disease, but the evidence from both of these papers that insulin has a crucial role will no doubt increase our chances of developing antigen-specific tolerization therapy for diabetic patients.

Lucy Bird

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TUMOUR IMMUNOLOGY

B cells lead the way in tumour progression

Many studies have reported a link between chronic inflammatory diseases and cancer development. However, it has not been clear how the recruitment of inflammatory innate immune cells is initiated or sustained at sites of tumour development. Lisa Coussens and colleagues now report an important role for B cells in promoting innate-immune-cell inflammation in a mouse model of epithelial carcinogenesis, which is in contrast to the view that adaptive immune cells are involved in 'surveillance' against developing neoplasms.

K14-HPV16 **mice express oncogenes from human papillomavirus type 16 under the control of the human keratin 14 promoter/enhancer, leading to multi-stage epithelial carcinogenesis. The pre-malignant stage is characterized by infiltration of innate immune cells such as granulocytes and mast cells into the skin. However, when *K14-HPV16* mice were crossed with recombination-activating-gene-1-deficient mice (*K14-HPV16/Rag1*$^{-/-}$ mice), which lack T and B cells, this infiltration was significantly reduced. Decreased infiltration of the skin was associated with decreased activity of matrix metalloproteinase 9 (MMP9) compared with *K14-HPV16* mice. MMP9 is secreted by leukocytes and has a role in cancer development through its effects on tissue remodelling and release of the angiogenic growth factor vascular endothelial growth factor (VEGF) from the extracellular matrix. Correspondingly, *K14-HPV16/Rag1*$^{-/-}$ mice had reduced levels of VEGF in skin lysates compared with *K14-HPV16* mice, as well as decreased markers of angiogenesis.**

The lack of typical pre-malignant inflammatory characteristics in *K14-HPV16/Rag1*$^{-/-}$ mice was associated with decreased progression to epithelial carcinoma. Only 6.4% of *K14-HPV16/Rag1*$^{-/-}$ mice developed full-blown carcinomas compared with 47% of *K14-HPV16* mice. Therefore, the lack of T and B cells of the adaptive immune system inhibits pre-malignant inflammation and tumour progression in this model.

To examine the specific role of B cells in this process, the authors looked at antibody deposition. B cells do not infiltrate pre-malignant skin, but they might exert their effects through the systemic production of antibodies specific for antigens in the skin. Deposits of IgG and IgM could be detected in the skin of *K14-HPV16* mice by 1 month of age, and these increased up to 6 months of age in association with the development of chronic inflammation. The adoptive transfer of B cells from *K14-HPV16* mice, which would contain primed and/or memory B cells of the desired specificity, to *K14-HPV16/Rag1*$^{-/-}$ mice confirmed the important role of B cells by restoring leukocyte infiltration of the skin and other downstream characteristics such as angiogenesis. The transfer of serum from *K14-HPV16* mice had a similar effect, which is in line with the postulated systemic actions of B cells through the production of antibodies and/or soluble mediators such as cytokines.

The authors conclude that B cells are a crucial part of tumour progression in this model through the promotion of chronic inflammation in the pre-malignant state. Therefore, therapies that aim to stimulate B-cell responses, such as vaccination, should be used with caution in cancer-prone patients or patients with pre-malignant disease.

Kirsty Minton

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GENE REGULATION

Cytokine loci co-localized and poised

How are cytokine loci arranged in the nucleus to ensure coordinated and reciprocal expression of cytokine genes by T helper 1 (T_H1) versus T_H2 cells if they are present on different chromosomes? In a recent study in *Nature*, Richard Flavell and colleagues address this conundrum and provide the first direct evidence that cytokine loci on different chromosomes that are alternatively expressed by T_H-cell subsets are brought together in the nucleus, locating them in nuclear sites that are conducive to rapid expression in response to immune stimuli.

Expression of the T_H1-cytokine gene interferon-γ (*Ifng*) is regulated by elements near it on chromosome 10, whereas expression of the T_H2-cytokine genes interleukin-4 (*Il-4*), *Il-5* and *Il-13* on chromosome 11 are regulated by a locus control region (LCR) on the same chromosome, which controls expression of the entire T_H2-gene complex. But, using a recently developed chromosome-conformation capture technique, the authors could detect interchromosomal interactions between the promoter region of *Ifng* and the T_H2 LCR in naive T_H cells, which seems to add a further dimension to the regulation of these genes. After stimulation of naive cells to induce differentiation into T_H1 or T_H2 cells, the chromosomes seemed to move apart. Concomitant with loss of the interchromosomal interactions, in T_H1 cells, intrachromosomal interactions between *Ifng* and its downstream regulatory element were favoured, indicating that the interactions are dynamic and cell-subset specific. These results were confirmed using fluorescence *in situ* hybridization (FISH).

The authors proposed that the purpose of such dynamic chromosome interactions was to position genetic loci in subnuclear compartments that poise and prepare these loci for rapid expression after stimulation. Consistent with this, deletion of an LCR regulatory element (RHS7), which disrupted interchromosomal associations, delayed the rapid induction of *Ifng* expression after stimulation under T_H1-cell-promoting conditions. Similarly, under T_H2-cell-promoting conditions, cells that lacked RHS7 expressed less IL-5 at early time points.

So, the T_H2 LCR seems to regulate not only the transcription of the T_H2-cytokine genes but also the expression of *Ifng* through mediating interchromosomal associations. Whether this phenomenon will be a common feature of other coordinately regulated genes awaits future research.

Lucy Bird

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MHC MOLECULES

Driving diversity

One explanation for the remarkable diversity of the MHC — which is the most polymorphic gene cluster in the human genome — is that genetic diversity is the result of pathogen-driven selection processes. Although there is some evidence indicating that this is the case, until now, there has been little direct evidence to support this idea.

If diversity is the result of pathogen-driven selection, then one prediction would be that, in geographical regions of high pathogen diversity, humans should have increased diversity of HLA genes in relation to their average genomic diversity. To test this, the authors used 3 types of information: data on the genetic diversity of HLA class I molecules from 61 human populations; estimates of pathogen richness (that is, the total number of intracellular pathogens that are known to be present in each country); and the geographical distance of each population from East Africa (to control for the effect of past colonization history).

The authors found that human populations located further from East Africa had lower genetic variability and that human colonization history accounted for 17–39% of the diversity of HLA genes. The remaining diversity was significantly correlated with pathogen richness: that is, a population exposed to a more diverse range of pathogens has greater HLA diversity than one exposed to fewer pathogens. The results showed that pathogens (mainly viruses) exerted the greatest pressure on genes in the HLA-B locus; interestingly, it has recently been shown that HLA-B genes could have a larger role in containing viral infections than HLA-A genes.

These results show that human colonization history has been important in shaping the diversity of the HLA locus, but pathogen-driven selection has also had a role.

Elaine Bell

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LYMPHOCYTE DEVELOPMENT

Signalling identity

Developing thymocytes can differentiate into either αβ or γδ T cells, but how do they decide which path to take? Two papers in *Immunity* provide evidence that the key determinant in the αβ/γδ lineage-fate decision is the strength of the signal transmitted by the T-cell receptor (TCR).

Thymocytes at the $CD4^-CD8^-$ (double negative, DN) stage of development can express either the γδ-TCR or the pre-TCR (which consists of a complex of the TCR β-chain and the invariant pre-TCR α-chain). Although it is clear that the TCR is important in αβ/γδ lineage commitment, its exact role in this process remains controversial. Recent data show that both the αβ-TCR and the γδ-TCR can promote cross lineage development, which is inconsistent with models stating that the specific TCR isoform (pre-TCR or γδ-TCR) only directs development or promotes survival along its respective lineage. This, together with speculation that the pre-TCR provides a weaker signal than the γδ-TCR, led both groups to ask whether TCR signal strength is an important factor.

To manipulate TCR signalling potential, both groups used γδ-TCR-transgenic mice crossed with mice that are deficient in various molecules involved in either ligand recognition or TCR signal transduction. In γδ-TCR-transgenic mice, thymocyte development results not only in γδ T-lineage cells, which remain DN, but also in αβ T-lineage cells, which can be detected as $CD4^+CD8^+$ (double positive, DP) cells. Haks *et al.* used mice transgenic for the KN6 γδ-TCR crossed onto the recombination-activating gene (RAG)-deficient background to prevent expression of other TCR isoforms (the pre-TCR or αβ-TCR). In these mice, almost all thymocytes expressed the γδ-TCR. They then showed that backcrossing these mice onto the β_2-microglobulin-deficient background (which lacks the KN6 ligand) or the tyrosine kinase LCK-deficient background markedly reduced the number of mature γδ T cells but allowed the generation of a substantial population of DP thymocytes with the hallmarks of αβ T-lineage cells. This indicates that interfering with ligand binding or limiting downstream signalling enables a γδ-TCR to efficiently promote the development of αβ, rather than γδ, T-lineage cells.

In the model used by Hayes *et al.*, reducing γδ-TCR cell-surface expression resulted in a significant increase in the number of DP thymocytes and a corresponding decrease in $\gamma\delta\text{-TCR}^+$ thymocyte numbers. Because TCR signalling is coupled to ITAMs (immunoreceptor tyrosine-based activation motifs) in the TCR-associated CD3 ζ-chain, genetic reconstitution of γδ-TCR-transgenic, CD3ζ-deficient mice with transgenes encoding CD3ζ molecules that had between zero and three ITAMs enabled the authors to show that attenuation of the γδ-TCR signal favoured αβ T-cell development. Conversely, increasing γδ-TCR cell-surface expression or removing a negative regulator of signalling, CD5, favoured γδ T-cell development. So, in these experiments, reducing the signal strength resulted in more αβ T-lineage cells at the expense of γδ T-lineage cells, whereas increasing the signal strength had the converse effect.

Importantly, both groups showed, in non-transgenic mice, that $\gamma\delta\text{-TCR}^+$ thymocytes have higher levels of activated intracellular-signalling molecules than do pre-TCR^+ thymocytes. Taken together, these data are consistent with a model in which $\gamma\delta\text{-TCR}^+$ thymocytes that receive a 'strong' signal develop into γδ T-lineage cells, whereas $\gamma\delta\text{-TCR}^+$ or pre-TCR^+ thymocytes that receive a 'weak' signal develop into αβ T-lineage cells.

Davina Dadley-Moore

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IN BRIEF

INFLAMMATION

Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury.

Thiel, M. *et al. PLoS Biol.* **3**, e174 (2005)

This study looks at the clinical implications of a recently described hypoxia-driven anti-inflammatory mechanism for patients with acute respiratory distress syndrome (ARDS) who are treated by mechanical ventilation with high oxygen concentrations. In several models, accumulation of adenosine in hypoxic conditions induces a physiological tissue-protective pathway through adenosine receptors (A2ARs). The authors confirm that oxygenation can exacerbate inflammatory lung damage by blocking the A2AR pathway in several *in vivo* mouse models of lung disease. Lung inflammation could be prevented by the addition of an A2AR agonist. They suggest that ventilation of patients with ARDS can cause an iatrogenic exacerbation of lung inflammation that could be treated with A2AR agonists or other anti-inflammatory drugs.

IMMUNE REGULATION

Negative feed back regulation of T helper type 1 (T_H1)/T_H2 cytokine balance via dendritic cell and natural killer T cell interactions.

Minami, K. *et al. Blood* 10 May 2005 (doi:10.1182/blood-2004-12-4738)

This study describes how interactions between dendritic cells (DCs) and natural killer T (NKT) cells can regulate the T helper 1 (T_H1)/T_H2-cytokine balance. DCs can stimulate NKT cells by CD1d-restricted presentation of α-galactosylceramide (α-GalCer), but the NKT-cell response seems to be influenced by extracellular stimuli received by the DC. Indeed, pretreatment of α-GalCer-loaded DCs with T_H1 or T_H2 cytokines *in vitro* led to increased NKT-cell production of T_H2 or T_H1 cytokines, respectively, indicating that the NKT cells might provide a negative-feedback mechanism to maintain the cytokine balance. α-GalCer has been shown to have antitumour effects that require NKT-cell production of interferon-γ. So, by pretreating tumour-bearing mice with a T_H2 cytokine, interleukin-4, the authors could enhance the α-GalCer-induced antitumour response and reduce tumour metastases.

AUTOIMMUNITY

Initiation and exacerbation of autoimmune demyelination of the central nervous system via virus-induced molecular mimicry: implications for the pathogenesis of multiple sclerosis.

Croxford, J. L. *et al. J. Virol.* **79**, 8581–8590 (2005)

Viral infections are suspected of triggering various autoimmune diseases, but proving this has proved controversial. This study shows that infection with a non-pathogenic variant of Theiler's murine encephalomyelitis virus (TMEV) expressing a *Haemophilus influenzae*-encoded peptide that mimics a self-myelin epitope induces and exacerbates disease in a mouse model of multiple sclerosis. The disease is mediated by cross-activation of autoreactive T cells and was shown to depend on processing of the mimic from the native *H. influenzae* protein and on virus-activated innate immune signals.

IN THE NEWS

Monkey vaccines

A vaccine has been developed that protects monkeys from Ebola and Marburg viruses, as reported in *Nature Medicine*. The breakthrough comes at a time when both viruses are on the rampage in Africa and are considered to be potential agents of bioterrorism.

Ebola and Marburg are closely related viruses that cause haemorrhagic fever — massive internal and external bleeding — and are lethal in up to 90% of infected monkeys and humans.

The authors of the recent report generated a replication-competent vaccine based on attenuated vesicular stomatitis virus (VSV) vectors expressing a glycoprotein from Ebola or Marburg virus. A single intramuscular injection of monkeys induced protective immune responses — both cellular and humoral — against lethal challenges with either virus.

As the authors report, the use of VSV vectors is "particularly attractive because they can be mucosally administered … [and] … VSV infections in humans occur fairly rarely" (*Nature Medicine*). Moreover, the live vaccine replicates in the recipient for a short time, generating a rapid and strong immune response. Although these are important features, the authors admit that there are still "questions regarding the safety of live attenuated vectors", owing to their potential ability to recombine with other viruses (*New Scientist*).

Because monkeys suffer almost identical disease to humans, Steven Jones, the primary author of the study, said that "If we can protect them [monkeys] using this vaccine … then this gives us a good deal of confidence that this will work in humans." (*Reuters*). However, "it will be some time before we can use these vaccines in the field, but it is satisfying to know we are getting closer", said one of Jones's co-authors, Heinz Feldmann (*The Guardian*).

Lucy Bird

IMMUNOLOGICAL SYNAPSES

Visualizing T-cell fate: tolerance or priming

Whether the characteristics of a dendritic cell (DC)–CD4$^+$ T-cell interaction determine whether the CD4$^+$ T cell is primed or tolerized remains an open question. However, new insight into this is provided by two groups who have shown *in situ* that there are only subtle differences in the behaviour of CD4$^+$ T cells after antigen encounter under conditions that result in priming or tolerization.

Previous two-photon microscopy studies that visualized T cells interacting with antigen-loaded DCs *in situ* indicated that, under priming conditions, antigen-specific T cells form stable contacts with DCs, whereas under tolerizing conditions, these stable contacts do not form. This has led to the hypothesis that the stability of the DC–T-cell interaction determines whether T cells are tolerized or primed. Because this hypothesis remains controversial, both groups set out to visually compare CD4$^+$ T-cell priming and tolerance induction *in situ*.

To deliver antigen to DCs *in vivo*, Shakhar *et al.* used a fusion protein consisting of a DC-specific antibody fused to the ovalbumin (OVA) peptide that is recognized by T cells expressing the OT-II T-cell receptor (TCR). Antigen was delivered alone or in combination with a CD40-specific antibody to create tolerizing or priming conditions, respectively. T cells expressing both the OT-II TCR and enhanced green fluorescent protein (EGFP) were transferred to the immunized animals, and the behaviour of the cells in the lymph nodes was tracked intravitally. Enhanced cyan fluorescent protein (ECFP)-expressing, antigen non-specific CD4$^+$ T cells were co-transferred with the EGFP$^+$OT-II TCR$^+$ T cells to distinguish antigen-specific and non-specific behaviour. During the first 6 hours of imaging, under both tolerizing and priming conditions, EGFP$^+$OT-II TCR$^+$ T cells moved more slowly than the antigen non-specific ECFP$^+$CD4$^+$ T cells. In addition, a large proportion of the EGFP$^+$OT-II TCR$^+$ T cells were immobile, spending more time arrested than the control ECFP$^+$CD4$^+$ T cells. The only differences between tolerizing and priming conditions were observed between 6 and 12 hours, when the EGFP$^+$OT-II TCR$^+$ T cells regained speed more quickly under tolerizing conditions. Subsequently, between 12 and 18 hours, there was again little difference in the speed of the cells and in the amount of time spent arrested between EGFP$^+$OT-II TCR$^+$ T cells and control ECFP$^+$CD4$^+$ T cells under either tolerizing or priming conditions.

By contrast, Zinselmeyer *et al.* induced priming or tolerance by oral administration of OVA in the presence or absence of cholera-toxin adjuvant, and they imaged mucosal and systemic lymph nodes both *ex vivo* and intravitally. In these studies, mice had previously been administered CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester)-labelled CD4$^+$ T cells expressing the OVA-peptide-specific DO11.10 TCR. Similar to the observations of Shakhar *et al.*, under both tolerizing and priming conditions, DO11.10 TCR$^+$ cells slowed down, formed clusters and appeared to stop. Further analysis indicated that the only differences were subtle: there were fewer T cells in each cluster under tolerizing conditions than under priming conditions, although the proportion of T cells entering clusters was greater under tolerizing conditions (particularly 20 hours after antigen feeding); also, at 20 hours after antigen feeding, the size of the clusters was greater under priming conditions.

These reports indicate that there are no marked differences in the initial phases of DC–CD4$^+$ T-cell interactions that lead to the distinct outcomes of tolerance and priming. Indeed, stable interactions of antigen-specific T cells with DCs (which are thought to be the *in vivo* counterparts of immunological synapses) were correlated with activation and proliferation but not with tolerance or priming. However, further work will be required to determine whether the subtle differences that were observed in these studies are responsible for determining tolerance versus priming, as suggested by Zinselmeyer *et al.*, or whether events at time points later than those studied in these reports control this commitment step, as suggested by Shakhar *et al.*

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References and links

ORIGINAL RESEARCH PAPERS Shakhar, G. *et al*. Stable T cell–dendritic cell interactions precede the development of both tolerance and immunity *in vivo*. *Nature Immunol.* **6**, 707–714 (2005) | Zinselmeyer, B. H. *et al*. *In situ* characterization of CD4$^+$ T cell behavior in mucosal and systemic lymphoid tissues during the induction of oral priming and tolerance. *J. Exp. Med.* **201**, 1815–1823 (2005)