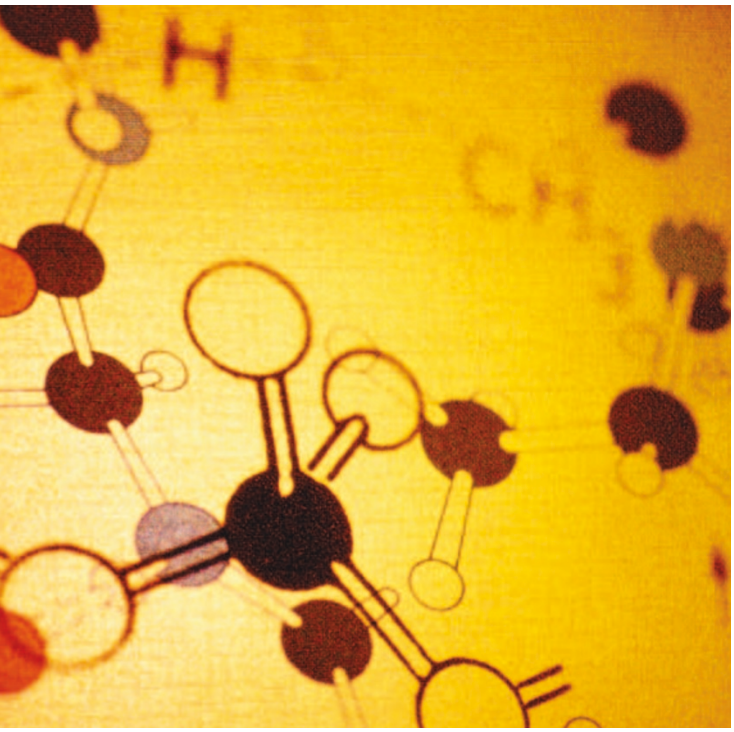
T-CELL SIGNALLING

Methylation modifies T-cell function



The signalling pathways that result in T-cell activation involve post-translational modification of some of the proteins along these pathways. Protein modification by phosphorylation or ubiquitylation has been well documented to be crucial for T-cell activation, but now, in a report published in *The Journal of Experimental Medicine*, protein arginine methylation has been shown to occur following engagement of the co-stimulatory receptor CD28.

Recent technological advances — for example, in mass spectrometry and proteomics — have indicated that methylation of proteins on arginine residues occurs more often than previously thought. So, Blanchet *et al.* set out to investigate whether arginine methylation occurs during T-cell activation. Using an antibody specific for methylated arginine, it was shown that the level of arginine methylation was greater in T cells that were stimulated with both superantigen and fibroblast cells engineered to express the CD28 ligand CD80 (5–3.1-B7 cells) than in T cells that were stimulated with superantigen alone. Furthermore, stimulation with 5–3.1-B7 cells alone increased the level of arginine methylation and the level of protein arginine methyltransferase (PRMT) activity more than stimulation with superantigen alone. Consistent with signalling downstream of CD28 ligation having a crucial role in inducing arginine methylation, 5–3.1-B7-cell stimulation of a T-cell line expressing a mutant CD28 molecule that lacks the ability to transmit intracellular signals after ligation could not induce increased PRMT activity.

Further analysis of known effectors of the CD28-signalling pathway indicated that VAV1 was arginine methylated following CD28 engagement and that this depended on CD28 being able to transmit intracellular signals. Arginine-methylated VAV1 was found exclusively in the nuclear fraction of 5–3.1-B7-cell-stimulated T cells. But, following CD28 co-stimulation, the main functions that

INNATE IMMUNITY

It's not all about TLRs

If you think that Toll-like receptors (TLRs) are all you need to recognize the multitude of bacterial and viral insults, then think again. Shizuo Akira and colleagues now offer an alternative. They show that, instead of TLRs, a recently identified cytoplasmic protein — retinoic-acid-inducible gene I (RIG-I) — is crucial for the detection of RNA viruses by fibroblasts and conventional dendritic cells (DCs).

The first hint that there might be a TLR-independent pathway came from the observation that cells lacking key TLR-signalling molecules — MyD88 (myeloid differentiation primary-response protein 88) and TRIF (Toll/interleukin-1 receptor (TIR)-domain-containing adaptor protein inducing interferon-β) — could still induce expression of type I interferons (IFNs) in response to viral infection. *In vitro* studies led the authors to consider that RIG-I was a good candidate for mediating this response. So, they knocked out the gene encoding RIG-I in mice and measured the ability of cells from these mice to respond to viruses. Because most RIG-I-deficient mice died *in utero*, they first studied mouse embryonic fibroblasts (MEFs). The induction of expression of type I IFNs and IFN-inducible genes in response to RNA viruses (such as Newcastle disease virus and vesicular stomatitis virus) was abrogated in RIG-I-deficient MEFs. Moreover, the absence of the IFN response impaired the ability of these cells to clear the virus.

The transcription factors that are required for induction of expression of type I IFNs and pro-inflammatory cytokines are IFN-regulatory factor 3 (IRF3) and nuclear factor-κB, respectively. The activation of both of these transcription factors was also abrogated in infected, RIG-I-deficient MEFs.

Given that DCs are the main producers of type I IFNs, the authors' attention next turned to DCs. Similar to fibroblasts, RIG-I-deficient conventional DCs showed severely impaired production of type I IFNs after viral infection. But deletion of components of the TLR-signalling pathway did not affect the IFN response of wild-type DCs, confirming the crucial role of the RIG-I pathway in this antiviral response.

In contrast to conventional DCs and fibroblasts, RIG-I-deficient plasmacytoid DCs (pDCs; which were derived *in vitro* from mouse bone-marrow cells, using FLT3 ligand) produced type I IFNs in amounts similar to those produced by wild-type pDCs, after viral infection. Instead of the RIG-I system, pDCs used the TLR system (in particular, TLR7 and TLR9), because induction of expression of antiviral IFNs was severely impaired when pDCs were deficient in these TLRs or their signalling molecules.

So, RIG-I and TLRs can both detect viral components and trigger antiviral responses depending on the cell type.

Lucy Bird

References and links

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have so far been described to depend on VAV1 occur in the cytoplasm, so the identification of arginine-methylated VAV1 in the nucleus indicates that VAV1 might have additional functions in T-cell activation: for example, it might modulate the activity of transcription factors.

Further studies are required to determine which T-cell functions are regulated by arginine methylation downstream of CD28 co-stimulation, although initial experiments reported in this paper indicate that this is one mechanism by which T-cell production of interleukin-2 can be regulated. The authors also suggest that, because arginine methylation is a stable protein modification (no enzymes that can remove this modification have been identified so far), arginine methylation downstream of CD28 co-stimulation might contribute to T-cell-fate differentiation decisions.

Karen Honey

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TUMOUR IMMUNOLOGY

Another way to escape the immune system's watchful eye

It seems that we can learn a thing or two about the immune system from tumours: the genes that they silence might indicate pathways of immune surveillance. Reporting in *Blood*, Marco Colonna and colleagues show that lung cancers frequently silence the tumour-suppressor gene *TSLC1* to escape the cytotoxic activity of natural killer (NK) cells and CD8$^+$ T cells.

***TSLC1* (tumour suppressor in lung cancer 1) encodes a cell-surface protein known as nectin-like 2 (NECL2), which contributes to cell–cell adhesion through homotypic interactions or heterotypic interactions with other nectin or nectin-like proteins. It was thought therefore that the silencing of this gene — a common occurrence in lung cancers — in lung epithelial cells disrupts cell polarity and cell–cell adhesion, favouring neoplastic growth and metastasis. But Colonna and colleagues thought that there might be another reason for its silencing, as they have recently shown that similar proteins, nectin-2 and NECL5, can be recognized by NK cells and activate their cytolytic activity.**

To investigate whether NECL2 is recognized by immune cells, the authors first searched the databases for putative receptors for NECL2, and they came up with a candidate — class-I-restricted T-cell-associated molecule (CRTAM). When cell lines expressing either NECL2 or CRTAM were co-cultured, cell–cell conjugates formed between the NECL2-expressing cells and the CRTAM-expressing cells. These cell–cell interactions could be disrupted by incubation with antibody specific for CRTAM. The interaction between NECL2 and CRTAM was further confirmed by showing that a NECL2–Fc fusion protein could bind the CRTAM-transfected cell line and vice versa.

The authors then showed that CRTAM is expressed by NK cells only after activation: for example, after triggering by PMA (phorbol 12-myristate 13-acetate) and ionomycin or by antibodies specific for NK-cell activating receptors. Similarly, CRTAM expression was detected at the surface of CD8$^+$ T cells only after stimulation through the T-cell receptor. Importantly, CRTAM expression promoted NK-cell lysis of NECL2-expressing tumour cells and increased interferon-γ secretion by CD8$^+$ T cells when co-cultured with a B-cell line expressing high levels of NECL2.

The key question therefore is whether expression of NECL2 by tumour cells allows efficient rejection by NK cells *in vivo*. To test this, mice were injected intraperitoneally with an equal mixture of lymphoma cells that were transfected with either a plasmid encoding NECL2 or a control plasmid and labelled with different amounts of fluorochrome. Before tumour challenge, the mice were stimulated with polyI:C (polyinosinic–polycytidylic acid) to accelerate NK-cell activation. After 48 hours, significantly fewer NECL2-expressing tumour cells than control tumour cells could be recovered from the peritoneal cavity, indicating preferential rejection of these cells. NK cells were crucial for this rejection, because the rejection of NECL2-expressing cells was reduced when mice were depleted of NK cells.

On the basis of these observations, the authors propose that, because NECL2 is normally expressed at epithelial-cell junctions, it might not be readily accessible to the immune system. But, after epithelial cells begin to transform and metastasize, NECL2 could become exposed and induce NK- and T-cell responses. So, NECL2 is another molecular target that allows surveying cytotoxic lymphocytes to distinguish tumour cells from normal cells.

Lucy Bird

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