VIRAL IMMUNITY

New evasion tactic for vaccinia

A recent report in *The Journal of Experimental Medicine* describes a new mechanism by which vaccinia virus (VV) can evade the host immune response — viral protein A46R inhibits Toll-like receptor (TLR) signalling by associating with TIR (Toll/interleukin-1 (IL-1) receptor)-domain-containing adaptor molecules.

Many of the proteins that are encoded by VV, which is the virus used to vaccinate against smallpox, target the host immune system and are therefore important for evasion of the host antiviral response. Previous studies have shown that VV A46R contains a TIR domain and can inhibit activation of nuclear factor-κB (NF-κB) in response to IL-1 but not tumour-necrosis factor. Therefore, Stack *et al.* set out to investigate whether A46R targets the TIR-domain-containing adaptor MyD88 (myeloid differentiation primary-response protein 88), which is important for the transmission of all known IL-1-mediated signals. Indeed, both ectopically expressed A46R and endogenous virally expressed A46R were shown to associate with MyD88.

Many TLR-signalling pathways, including those induced by ligands for TLR1, TLR2, TLR4, TLR5, TLR7 and TLR9, also require MyD88, and A46R was found to impair the NF-κB activation that is induced by stimulation of each of these TLRs. A46R was also shown to have a dose-dependent effect on TLR4-induced activation of NF-κB and to impair TLR4-induced activation of the mitogen-activated protein kinases p38 and extracellular-signal-regulated kinase (ERK). Signals from TLR4 are transmitted not only through MyD88 but also through several other TIR-domain-containing adaptors — MyD88-adaptor-like protein (MAL), TIR-domain-containing adaptor protein inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM) — and A46R was found to associate with each of these adaptors. Its ability to associate with TRIF meant that A46R also inhibited MyD88-independent TLR-signalling pathways, such as activation of interferon-regulatory factor 3 following TLR3 stimulation. Interestingly,

ALLERGY

Immunotherapy: the cat's whiskers

A recent report in *Nature Medicine* describes a new form of allergen-specific immunotherapy for allergy to cats, through targeting the inhibitory receptor FcγRIIb. This approach could be broadly applied for the treatment of any allergy to a defined antigen, and the authors suggest that it might be particularly useful for treating severe food reactions, such as peanut allergy, for which desensitization by repeated injection of small quantities of allergen has been unsuccessful.

One of the main mechanisms of allergy is the release of mediators such as histamine from mast cells and basophils, which results from crosslinking of the surface receptor FcεRI by allergen-specific IgE bound to multivalent allergen. Previous studies have shown that FcεRI signalling leading to mediator release can be inhibited by signalling through FcγRIIb, which binds the constant portion of IgG (Fcγ). Andrew Saxon and colleagues have focused this inhibitory interaction on a specific allergen by creating a chimeric fusion protein (known as GFD) that consists of human Fcγ plus the cat allergen Fel d1. GFD contains the Fcγ binding site for FcγRIIb and also binds Fel d1-specific IgE from the sera of humans who are allergic to cats. In the presence of Fel d1-specific IgE, GFD crosslinks FcγRIIb with FcεRI-bound IgE and should therefore inhibit mediator release in response to the cat allergen.

In vitro, the addition of GFD to cultures of basophils purified from cat-allergic humans (cells which have Fel d1-specific IgE at their surface) inhibited subsequent histamine release in response to Fel d1 in a dose-dependent manner. Similarly, GFD inhibited degranulation of cat-allergen-sensitized cord-blood-derived mast cells. GFD is not an allergen itself, as pre-incubation with GFD did not lead to mediator release from basophils before Fel d1 was added to the culture.

To confirm these results *in vivo*, transgenic mice expressing human FcεRIα were primed intradermally with human serum containing Fel d1-specific IgE, and skin reactivity at sites of GFD injection was measured after intravenous challenge with Fel d1. As the mast cells of these transgenic mice also express mouse FcγRIIb, which can bind human Fcγ, GFD blocked the skin reaction to Fel d1. GFD did not inhibit the specific IgE-induced response to a control antigen, indicating the allergen specificity of this approach. In an active-sensitization protocol, BALB/c mice were sensitized with Fel d1 and treated with GFD or a control; treatment with GFD inhibited the systemic allergic response to challenge with Fel d1, as indicated by a drop in core body temperature.

Therefore, GFD was shown to inhibit allergen-driven, IgE-mediated mediator release both *in vitro* and *in vivo* in an allergen-specific and dose-dependent manner, without itself causing mediator release. This is in contrast to desensitization protocols that use native allergen, which can trigger an allergic reaction, showing the potential of this technique as a new form of antigen-specific immunotherapy.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Zhu, D. *et al.* A chimeric human-cat fusion protein blocks cat-induced allergy. *Nature Med.* **11**, 446–449 (2005)

although A46R could associate with MyD88, MAL, TRIF, TRAM and TLR4, it could not bind all TIR-domain-containing proteins, including TLR3 and sterile α- and armadillo-motif-containing protein (SARM), confirming the specificity of the interactions.

The functional consequence of the inhibitory effects of A46R was highlighted by the observation that mice that were intranasally infected with VV lacking A46R did not become as sick as those infected with wild-type VV or A46R-deficient VV that was re-engineered to express A46R, implying that TLRs have a role in controlling infection with VV. Because variola virus, the causative agent of smallpox, encodes an A46R that differs from VV A46R by only eight amino acids, the authors suggest that viral evasion of TLR-induced immunity might contribute to the virulence of variola virus.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Stack, J. *et al*. Vaccinia virus protein A46R targets multiple Toll-like–interleukin-1 receptor adaptors and contributes to virulence. *J. Exp. Med.* **201**, 1007–1018 (2005)

NATURAL KILLER T CELLS

How NKT cells detect microorganisms

Although natural killer T (NKT) cells that express a semi-invariant T-cell receptor (TCR) α-chain (composed of Vα14–Jα18 in mice and the homologous Vα24–Jα18 in humans) recognize glycolipid antigens presented by CD1d, it is unclear which ligands activate these cells during a microbial infection. Now, two papers published in *Nature* show that both mouse Vα14$^+$ and human Vα24$^+$ NKT cells recognize CD1d-presented glycosphingolipids from Gram-negative bacteria that lack lipopolysaccharide (LPS).

Previous studies have shown that Vα14$^+$ NKT cells are activated during microbial infection; however, it is controversial whether these cells are activated directly, by TCR recognition of CD1d-presented microbial antigens, or indirectly, by other immune cells responding to the pathogen, so the two research groups set out to investigate this issue. Kinjo *et al.* showed that presentation by CD1d of two distinct glycosphingolipid mixtures (GSL-1 and GSL-1′) that were purified from *Sphingomonas* spp., as well as CD1d presentation of GSL-1′sA and GSL-1′sB (which are synthetic versions of individual components of GSL-1′), stimulated cytokine production by both human Vα24$^+$ T-cell lines and mouse Vα14$^+$ NKT cells but not by mouse T cells lacking the semi-invariant Vα14–Jα18 TCR α-chain. In addition, GSL-1′sA-loaded CD1d multimers bound all of the human Vα24$^+$ T cells and a proportion of liver mononuclear cells from wild-type mice, but they did not bind cells from mice that lack Jα18.

Activation of Vα14$^+$ NKT cells in the liver was also observed when wild-type mice were immunized with bone-marrow-derived dendritic cells pulsed with either GSL-1′sA or live *Sphingomonas yanoikuyae* (also known as *Sphingobium yanoikuyae*), and this *in vivo* activation did not depend on Toll-like receptor (TLR) activation of these antigen-presenting cells (APCs) or on APC secretion of interleukin-12. Functionally, *in vivo* Vα14$^+$ NKT-cell activation was associated with bacterial clearance.

In a similar study, Mattner *et al.* showed that heat-killed *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), *Ehrlichia muris* and *Sphingomonas capsulata* (also known as *Novosphingobium capsulatum*) all induced the production of interferon-γ (IFN-γ) by Vα14$^+$ NKT cells. Surprisingly, the response to *S. typhimurium* (an LPS-positive Gram-negative bacterium), but not to the other two bacteria (both of which are LPS-negative Gram-negative bacteria), required TLR signalling by the APCs. In addition, if Vα14$^+$ NKT-cell recognition of the recently identified endogenous glycolipid ligand isoglobotrihexosylceramide (iGb3) was prevented, the response to *S. typhimurium*, but not to the other two bacteria, was reduced, indicating that the endogenous ligand iGb3 activates Vα14$^+$ NKT cells after infection with *S. typhimurium*. By contrast, CD1d presentation of synthetic versions of glycosphingolipids from *Sphingomonas* spp. stimulated IFN-γ production by both mouse Vα14$^+$ NKT cells and human Vα24$^+$ NKT cells, and CD1d tetramers loaded with these compounds bound human Vα24$^+$ T cells and a proportion of mouse Vα14$^+$ NKT cells, indicating that these compounds are recognized directly by the NKT cells. In addition, although mice lacking Vα14$^+$ NKT cells showed impaired bacterial clearance after infection with *S. capsulata* compared with wild-type animals, they also showed reduced lethality after high-dose infection, because they lack the NKT-cell population that produces high levels of cytokines in response to microbial antigens.

These studies provide clear evidence that some microbial antigens can be directly recognized by NKT cells, whereas other microorganisms are sensed indirectly, through recognition of iGb3. The authors of both papers suggest that direct recognition of microbial antigens by NKT cells could be an innate immune mechanism for detecting microorganisms that lack TLR ligands.

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ORIGINAL RESEARCH PAPERS Kinjo, Y. *et al*. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* **434**, 520–525 (2005) | Mattner, J. *et al*. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **434**, 525–529 (2005)