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

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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.

Karen Honey

References and links

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