Src-family kinase signalling cascades. Confirmation of this was provided by the observation that the Src-family kinase substrates cortactin and paxillin were hyperphosphorylated in Csk-deficient bone-marrow granulocytes cultured *in vitro*. In addition, hyperphosphorylation of the tyrosine kinase Syk — a mediator of the proximal stages of integrin-signalling pathways — was also detected.

These data indicate that Csk negatively regulates granulocyte activation, modulating cellular adhesion and preventing inappropriate granulocyte recruitment. As the phenotype of Csk-GEcre mice is similar to several inflammatory skin conditions of unknown aetiology in humans, the authors suggest that impaired negative regulation of granulocyte activation might have a role in the induction of these inflammatory conditions.

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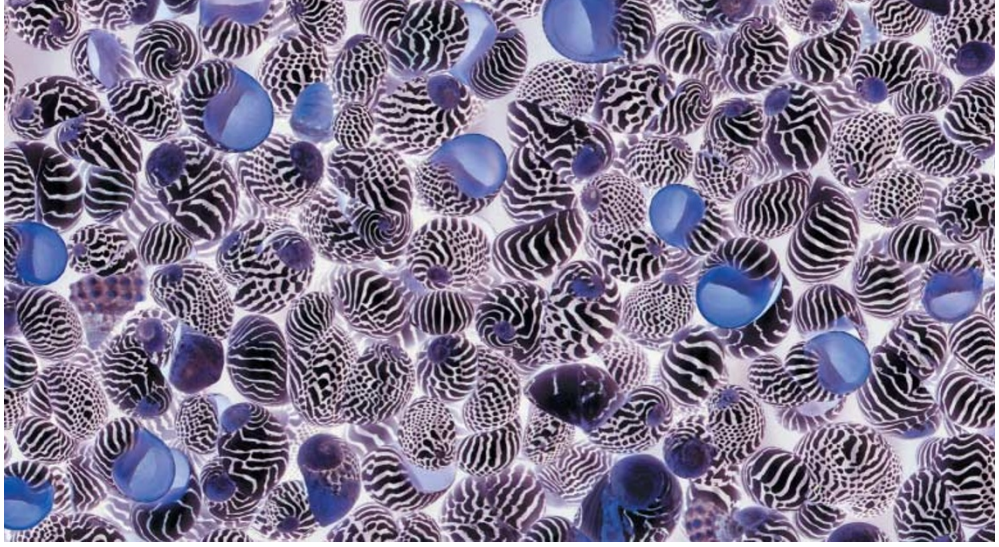
References and links

ORIGINAL RESEARCH PAPER Thomas, R. M. *et al.* C-terminal Src kinase controls acute inflammation and granulocyte adhesion. *Immunity* **20**, 181–191 (2004)



INNATE IMMUNITY

TLR ligands from the natural world

Toll-like receptor 7 (TLR7) and TLR8 are known to be activated by ribonucleoside analogues; however, the identity of their natural ligands had remained undefined until two reports in *Science* characterized single-stranded (ss) RNA as this ligand.

The innate immune response to influenza virus is exemplified by the production of type 1 interferons (IFN-α/β). Although conventional dendritic cells (DCs) can produce high levels of IFN-α in response to double-stranded (ds) influenza RNA — an intermediate of viral genome replication — this response is suppressed during infection by a virus-encoded dsRNA-sequestering protein. By contrast, the production of IFN-α by plasmacytoid DCs (pDCs) is resistant to suppression, indicating that it can occur by a dsRNA-independent mechanism.

Diebold *et al.* set out to identify this influenza-virus recognition pathway in pDCs. Initial analysis showing that IFN-α production by wild-type pDCs was abolished in the presence of chloroquine, combined with the observation that pDCs lacking the TLR-adaptor molecule MYD88 did not produce IFN-α in response to influenza virus, indicated that a TLR molecule known to have endosomal localization was likely to be pivotal in viral recognition. Although TLR3 and TLR9 are known to reside in the endosomal compartment, pDCs derived from mice deficient in these receptors made normal IFN-α responses to influenza virus. By contrast, this response was abolished in the absence of TLR7.

As TLR7 signalling is known to be induced by ribonucleoside analogues, the authors assessed the ability of purified influenza virus genomic ssRNA to induce IFN-α production by pDCs, and showed that viral ssRNA was a ligand for TLR7. Interestingly, ssRNA of non-viral origin, such as poly-uridine (polyU) — but not other nucleic-acid oligomers — and *in vitro*-synthesized RNA encoding green fluorescent protein, induced a TLR7-dependent IFN-α response by pDCs, indicating that ssRNA is the natural ligand for TLR7.

A similar observation was made independently by Heil *et al.*, whose studies were initiated to investigate whether ssRNA might act as a ligand for the TLR9 subfamily of TLRs: TLR7, TLR8 and TLR9. The authors first observed that only nucleosides composed of guanine (G) and U induced human peripheral-blood mononuclear cells (PBMCs) to secrete inflammatory cytokines. However, if the RNA — a synthetic RNA containing the GU-rich sequence in the U5 region of HIV-1 — was complexed in such a way as to facilitate uptake by DCs, IFN-α was also produced. This IFN-α response was restricted to the pDC population. Similarly, GU-rich ssRNA derived from HIV-1 selectively induced mouse pDCs to produce IFN-α.

The involvement of TLRs in this process was confirmed using MYD88-deficient DCs, which failed to respond to ssRNA stimulation. Additionally, analysis of DCs derived from TLR3-, TLR9-, TLR8- and TLR7-deficient mice indicated that, in mice, only TLR7 recognizes GU-rich ssRNA. By contrast, in an *in vitro* culture system, only cells expressing human TLR8, and not TLR7, were activated in response to ssRNA, indicating that there is species-specific recognition of GU-rich ssRNA.

These studies identify ssRNA as the natural ligand for mouse TLR7 and human TLR8, and the authors of both papers suggest that endosomal delivery of ssRNA could provide a novel adjuvant for vaccination and immunotherapy.

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References and links

ORIGINAL RESEARCH PAPERS Diebold, S. S. *et al.* Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* **303**, 1529–1531 (2004) | Heil, F. *et al.* Species-specific recognition of single-stranded RNA via Toll-like receptors 7 and 8. *Science* **303**, 1526–1529 (2004)