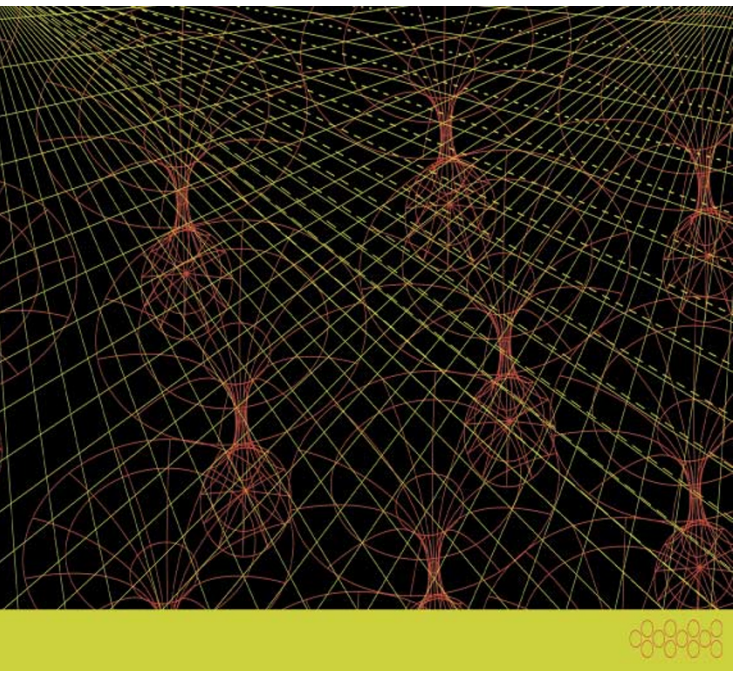
MUCOSAL IMMUNOLOGY

DC networks access all areas



The intestine is a specialized environment in which immune cells must defend the body against pathogens while ignoring beneficial commensal bacteria. Specialized epithelial cells known as microfold (M) cells can sample luminal antigens and pass them to dendritic cells (DCs). Other studies have shown that DCs can directly sample luminal antigens. Now, Hans-Christian Reinecker and colleagues describe a network of myeloid-derived CX_3C-chemokine receptor 1 $(CX_3CR1)^+$ DCs in the lamina propria that can also directly sample luminal antigens.

To study phagocytic cells in the intestine, the authors used mice in which one or both copies of the gene encoding CX_3CR1 — the product of which binds the transmembrane chemokine CX_3C-chemokine ligand 1 (CX_3CL1) expressed by intestinal epithelial and endothelial cells — were replaced with a gene encoding green fluorescent protein (GFP). Heterozygous mice ($Cx_3cr1^{GFP/+}$) express both GFP and the chemokine receptor, whereas homozygous mice ($Cx_3cr1^{GFP/GFP}$) express only GFP. GFP^+ monocytic cells were observed in the lamina propria, mesenteric lymph nodes and Peyer's patches. GFP^+ lamina-propria DCs expressed CD11c and CD11b, as well as MHC class II and the co-stimulatory molecules CD80 and CD86.

The authors used confocal microscopy to look at the distribution of intestinal DCs and their interactions with epithelial cells. In the terminal ileum of $Cx_3cr1^{GFP/+}$ mice, lamina-propria DCs extended dendrites into the intestinal lumen. By contrast, in $Cx_3cr1^{GFP/GFP}$ mice, dendrites could form in the lamina-propria layer but did not extend into the intestinal lumen, indicating that transepithelial dendrite formation is a CX_3CR1-dependent process. Three-dimensional tissue reconstructions based on confocal images showed that the dendrites that extended into the lumen ended in mono- or multi-globular structures. The authors then examined the role of CX_3CR1^+ DCs

DENDRITIC CELLS

TLR3 helps DCs to cross-prime

Presentation of exogenous antigen in the context of MHC class I molecules — cross-presentation — can result in $CD8^+$ T-cell priming (cross-priming) or $CD8^+$ T-cell tolerance (cross-tolerance). New insight into the signals that determine which of these fates is adopted by the $CD8^+$ T cell is provided by a report in *Nature*, which indicates that stimulation of Toll-like receptor 3 (TLR3) promotes cross-priming.

$CD8\alpha^+$ dendritic cells (DCs) are crucial components of the antiviral response in mice, priming virus-specific cytotoxic T lymphocytes (CTLs). They are also the principal mediator of cross-presentation *in vivo*. So, Schulz *et al.* proposed that $CD8\alpha^+$ DCs that are cross-presenting antigens from phagocytosed virally infected cells must receive a signal to favour cross-priming. Given that viral infection is associated with the generation of double-stranded RNA (dsRNA) and that $CD8\alpha^+$ DCs express the dsRNA receptor TLR3, they set out to investigate whether TLR3 might provide this signal.

$CD8\alpha^+$ DCs cultured in the presence of Vero cells (a primate cell line) that had been loaded with synthetic dsRNA (polyinosinic–polycytidylic acid, polyI:C) and exposed to ultraviolet light to induce cell death phagocytosed the polyI:C-loaded Vero cells. This induced increased cell-surface expression of CD40, CD80 and CD86, and increased production of inflammatory cytokines, including interleukin-6. By contrast, mock-treated cells did not induce activation of the $CD8\alpha^+$ DCs. Similar uptake and activation was observed when $CD8\alpha^+$ DCs were cultured in the presence of Vero cells infected with either encephalomyocarditis virus or Semliki Forest virus (SFV). Phagocytosis and phagosomal acidification were essential for the polyI:C-loaded Vero cells to induce $CD8\alpha^+$ DC activation, and further studies showed that TLR3 was also required for activation induced by either infected or polyI:C-loaded Vero cells.

Mice were immunized either with Vero cells infected with SFV that had been genetically modified to produce no progeny and to express ovalbumin (OVA) or with Vero cells loaded with OVA in the presence or absence of polyI:C. Efficient cross-priming of OVA-specific CTLs was observed only in those mice immunized with the virally infected cells or with the Vero cells loaded with OVA in the presence of polyI:C. Further evidence that an intrinsic TLR3 signal induces cross-priming by $CD8\alpha^+$ DCs was provided by the observation that neither TLR3-deficient mice nor lethally irradiated wild-type mice reconstituted with TLR3-deficient bone marrow could cross-prime CTLs after immunization with the virally infected cells or with the Vero cells loaded with OVA in the presence of polyI:C.

This study indicates that TLR3 expression by $CD8\alpha^+$ DCs functions to sense viruses that do not infect DCs and to induce the cross-presentation of cell-associated viral antigens such that $CD8^+$ T-cell cross-priming occurs. TLR3 ligation is unlikely to provide the only signal that elicits cross-priming, and the authors suggest that virally induced type I interferons might function synergistically with TLR3-initiated signals to promote cross-priming.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Schulz, O. *et al*. Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 13 Feb 2005 (doi:10.1038/nature03326).

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in the sampling of the commensal microflora and during infection with the pathogenic bacterium *Salmonella typhimurium*. $Cx_3cr1^{GFP/GFP}$ mice were more susceptible to infection than heterozygous mice, probably as a result of impaired bacterial sampling and lack of transepithelial dendrites.

This study describes a network of lamina-propria DCs that can directly sample luminal antigens using a mechanism distinct from that involving M cells. It remains to be determined which antigen-uptake receptors are expressed at the terminal dendrite structures and whether commensal bacteria can be distinguished from pathogens, but the authors suggest that it might be possible to target these structures to engage intestinal DCs, which could be useful for vaccine development.

Elaine Bell

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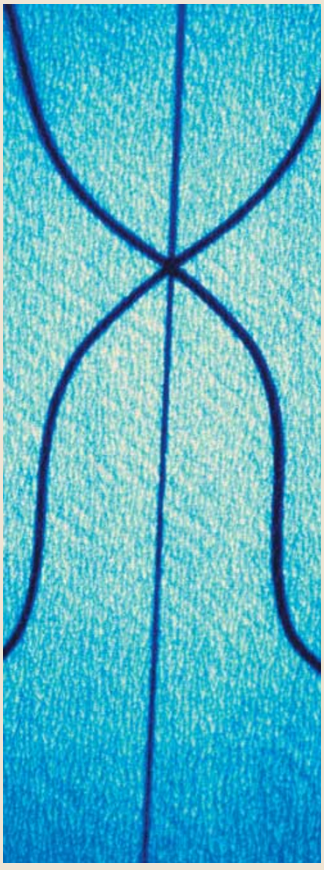
ORIGINAL RESEARCH PAPER Niess, J. H. *et al.* CX_3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258 (2005).



T CELLS

PIM kinases to the rescue

T-cell survival in the presence of the immunosuppressive drug rapamycin depends on PIM kinases, as described in a recent report in *The Journal of Experimental Medicine*. PIM kinases are known to contribute to lymphoid transformation when overexpressed, but until now, their physiological role in the immune response was unclear.

T-cell growth and survival following ligation of cytokine or antigen receptors is mediated, in part, through activation of the effector enzymes AKT and TOR (target of rapamycin) in the phosphatidylinositol 3-kinase pathway. However, alternative pathways of lymphocyte survival are known to exist, because AKT-deficient mice or mice treated with the TOR inhibitor rapamycin mount normal immune responses. In this paper, Craig Thompson and colleagues observed that T cells from mice deficient in PIM1 and PIM2 have increased sensitivity to rapamycin, indicating that PIM kinases might provide this alternative pathway for T-cell survival.

Resting T cells do not express PIM kinases, but PIM kinase expression could be detected in T cells after culturing in the presence of the pro-survival cytokines interleukin-4 (IL-4) and IL-7 or following mitogenic stimulation with CD3- and CD28-specific antibodies, with PIM1 expression being detected 3 hours after stimulation and PIM2 expression detected after 12 hours. Culturing of naive T cells with IL-4 and IL-7 also induced phosphorylation and activation of AKT and the TOR subunit p70 S6 kinase, which as expected, could be inhibited by treatment of the T cells with rapamycin. By contrast, rapamycin treatment did not prevent the induction of PIM protein expression following T-cell stimulation.

In wild-type T cells or T cells deficient in PIM1 only, rapamycin treatment had little effect on cytokine-induced survival; however, treatment of PIM2-deficient T cells reduced their ability to survive in response to IL-4 or IL-7, indicating that PIM2 expression is required to compensate for TOR inhibition by rapamycin. The authors suggested that the mechanism of PIM2-dependent survival is likely to be mediated through regulation of the pro-apoptotic BCL-2-related protein BAD (BCL-2 antagonist of cell death), because phosphorylation of Ser112 of BAD, which contributes to suppression of its pro-apoptotic function, occurred 12 hours after cytokine stimulation, coincident with PIM2 expression.

In addition to having a role in cytokine-induced T-cell survival, the authors show that, in the presence of rapamycin, PIM kinases are required for T-cell blastogenesis, activation and proliferation induced by stimulation with CD3- and CD28-specific antibodies.

Finally, the authors asked whether deficiency in PIM kinases could enhance the immunosuppressive action of rapamycin *in vivo*. T-cell responses were induced in wild-type or PIM-deficient mice using the superantigen toxic-shock syndrome toxin (TSST). Although rapamycin treatment did not affect the activation and clonal expansion of TSST-specific T cells in wild-type mice, PIM-deficient mice had markedly suppressed TSST-induced T-cell responses following rapamycin treatment, consistent with the *in vitro* observations.

So, although this study shows that PIM kinases can rescue T cells from the immunosuppressive effects of rapamycin both *in vitro* and *in vivo*, it does not explain why rapamycin is effective in suppressing transplant rejection in patients who have no known defects in PIM kinases.

Lucy Bird

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ORIGINAL RESEARCH PAPER Fox, C. J., Hammerman, P. S. & Thompson, C. B. The Pim kinases control rapamycin-resistant T cell survival and activation. *J. Exp. Med.* **201**, 259–266 (2005).

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