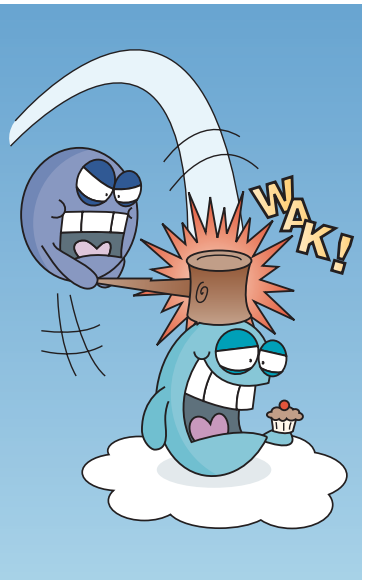
IMMUNOREGULATION

Mediating endotoxin tolerance



Endotoxin tolerance is the situation in which previous exposure to a low level of lipopolysaccharide (LPS) induces a transient period of hyporesponsiveness after subsequent challenge with LPS. LPS-induced inflammatory responses are important for fighting Gram-negative bacterial infections. But if the response gets out of control, endotoxic shock can develop, which can be fatal. So, understanding the ways in which responses to LPS can be regulated could have clinical applications. In a study published in *Immunity*, Gerry Krystal and colleagues describe a regulatory role for the cytosolic phosphatase SHIP (SRC-homology-2-domain-containing inositol-5-phosphatase) in endotoxin tolerance.

LPS binds to Toll-like receptor 4 (TLR4) and stimulates two signalling pathways — a myeloid differentiation primary-response gene 88 (MyD88)-dependent pathway and a MyD88-independent pathway — both of which lead to activation of nuclear factor-κB (NF-κB). Based on studies showing that NF-κB activation is reduced in endotoxin tolerance and that SHIP inhibits the NF-κB pathway in bone-marrow-derived mast cells stimulated with IgE plus antigen, the authors decided to investigate the role of SHIP in endotoxin tolerance.

SHIP-deficient mice were shown to be more susceptible to LPS-induced toxicity than wild-type mice. The SHIP-deficient mice produced considerably increased amounts of pro-inflammatory cytokines and nitric oxide, indicating that SHIP is a negative regulator of LPS-induced production of inflammatory mediators. In contrast to wild-type cells, bone-marrow-derived macrophages and mast cells from SHIP-deficient mice did not show endotoxin tolerance.

So, how does SHIP mediate endotoxin tolerance? Because cell-surface levels of TLR4 were similar for SHIP-deficient and SHIP-sufficient cells stimulated with a tolerizing dose of LPS, TLR4 expression cannot account for the effect. By contrast, SHIP protein expression was markedly upregulated by SHIP-sufficient cells after low-level stimulation with LPS. SHIP-directed antisense oligonucleotides were then used to decrease SHIP expression in SHIP-sufficient cells, confirming that it is the lack of induction of SHIP expression, and not any secondary effects, that accounts for the inability to generate endotoxin tolerance. *In vivo* studies using SHIP-deficient mice also supported a role for SHIP in endotoxin tolerance.

Next, because transforming growth factor-β (TGF-β) has been reported to be an inducer of SHIP expression,

SIGNALLING

MKP5: modifying kinase-regulated pathways

Mitogen-activated protein (MAP) kinases — which are crucial mediators of many signalling pathways that regulate immune responses — are themselves regulated by kinases and phosphatases, such as members of the MAP kinase phosphatase (MKP) family. Specific physiological functions of distinct MKPs have not been defined for the immune system, but a recent study now shows that MKP5 is a regulator of both innate and adaptive immune responses.

JUN amino-terminal kinase (JNK) is a MAP kinase known to be crucial for T-cell function. So, having identified human MKP5 as the mammalian MKP that has the most homology with the *puckered* gene —which encodes a JNK-specific phosphatase in fruit flies — Zhang *et al.* set out to investigate the role of MKP5 in regulating the immune response, through cloning mouse *Mkp5* and generating MKP5-deficient mice.

T helper 1 (T_H1) and T_H2 cells derived from MKP5-deficient mice showed enhanced levels of JNK activity compared with MKP5-sufficient cells, as did macrophages from MKP5-deficient mice treated with lipopolysaccharide (LPS). Consistent with these observations, MKP5-deficient macrophages stimulated with the Toll-like receptor 4 (TLR4) ligand LPS produced more pro-inflammatory cytokines than LPS-treated wild-type macrophages, and stimulation through TLR2 and TLR3 produced similar results, indicating that MKP5 is a negative regulator of innate immunity.

Compared with wild-type $CD4^+$ T cells, MKP5-deficient $CD4^+$ T cells showed reduced proliferation when stimulated with CD3-specific antibodies. By contrast, T_H1 and T_H2 cells derived from MKP5-deficient mice produced considerably more interferon-γ (IFN-γ) and interleukin-4 respectively, and MKP5-deficient $CD8^+$ T cells produced more IFN-γ. These results indicate that MKP5 is required for T-cell proliferation but is a negative regulator of effector T-cell cytokine production. Further evidence of these distinct roles for MKP5 was provided by the observation that T cells from MKP5-deficient mice immunized with antigen and adjuvant showed decreased antigen-specific proliferation but increased antigen-specific cytokine production compared with T cells from similarly treated wild-type mice.

The physiological importance of the different roles of MKP5 in the regulation of T-cell proliferation and effector function was highlighted by the distinct effects of MKP5 deficiency that were observed both using a mouse model of autoimmunity and after infection with lymphocytic choriomeningitis virus (LCMV). When compared with wild-type animals, MKP5-deficient mice showed reduced disease incidence and severity in a model of multiple sclerosis (experimental autoimmune encephalomyelitis), with fewer $CD4^+$ T cells found in the brain, presumably as a result of decreased T-cell proliferation. By contrast, after a second challenge with LCMV, MKP5-deficient mice showed increased mortality compared with wild-type animals, probably as a result of the much greater levels of cytokines produced by the $CD4^+$ and $CD8^+$ T cells of these animals.

These data indicate that MKP5 has a crucial, non-redundant role in the regulation of immune responses, and they add a new layer of complexity to our understanding of signalling pathways in the immune system.

Karen Honey

References and links

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the authors asked whether autocrine production of TGF-β has a role in the LPS-induced increase in SHIP expression. This was found to be the case, because TGF-β increased SHIP expression by SHIP-sufficient cells that were stimulated with LPS. In addition, neutralizing antibodies specific for TGF-β inhibited the increase in SHIP expression, thereby preventing the induction of endotoxin tolerance.

This study considerably advances our understanding of endotoxin tolerance.

Elaine Bell

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FURTHER READING Beutler, B. & Rietschel, E. T. Innate immune sensing and its roots: the story of endotoxin. *Nature Rev. Immunol.* **3**, 169–176 (2003).

WEB SITE
Gerry Krystal's homepage:
http://www.bccrc.ca/tfl/people_gkrystal.html

DENDRITIC CELLS

Guess who

What am I? I am recruited to tumours, where I form new blood vessels to supply nutrients for the growing mass. If your first guess is an endothelial cell or its precursor then think again, because new research published in *Nature Medicine* shows that dendritic cells (DCs) recruited to ovarian tumours, instead of stimulating an immune response, can become blood-vessel cells.

This surprising result arose from the observation that expression of β-defensin 29 (DEFB29) by ID8 mouse ovarian cancer cells markedly accelerated tumour growth when these cells were transplanted subcutaneously in mice, but only when the cells also co-expressed high levels of vascular endothelial growth factor (VEGF; also known as VEGF-A). ID8 VEGF$^+$DEFB29$^+$ tumours contained more blood vessels than ID8 VEGF$^+$ tumours, indicating that DEFB29 functions with VEGF to promote tumour angiogenesis.

Because β-defensins are known to be chemoattractive for DCs, the authors analysed expression of the DC marker CD11c in ID8 VEGF$^+$DEFB29$^+$ tumours. CD11c$^+$ cells were found in capillary-like structures and were shown to have an immature DC phenotype, but they were also found to express endothelial-cell markers, such as CD34 and VE-cadherin. These CD11c$^+$ cells were shown to be responsible for the increased tumour growth by comparing ID8 VEGF$^+$ cells transplanted alone or mixed with CD11c$^+$ cells from ID8 VEGF$^+$DEFB29$^+$ tumours. Further experiments showed that tumour-derived CD11c$^+$ cells can form blood-vessel-like structures *in vitro* and *in vivo*, without proliferation (ruling out the involvement of a stem-cell population), confirming that the CD11c$^+$ cells increase tumour growth through vasculogenesis. Interestingly, tumour-infiltrating CD11c$^+$ cells could present antigen to T cells when removed from the tumour milieu *in vitro*, showing that these cells can function as endothelial-like cells or DCs depending on the environment.

An environment containing high levels of VEGF and DEFB29 favours endothelial specialization, but what are the roles of these two factors? The addition of bone-marrow-derived CD11c$^+$ cells to ID8 VEGF$^+$ tumours resulted in levels of vascularization similar to those of ID8 VEGF$^+$DEFB29$^+$ tumours, confirming that DEFB29 is responsible for the initial recruitment of CD11c$^+$ cells to the tumour. DEFB29 could attract CD11c$^+$ immature DCs *in vitro* and *in vivo* through the chemokine receptor CCR6. However, addition of CD11c$^+$ cells did not increase the growth rate or vascularization of tumours expressing only low levels of VEGF, indicating that VEGF is required for conversion of these immature DCs to an endothelial phenotype. Antibody-mediated blockade of VEGF receptor 2 expressed by DCs prevented upregulation of the endothelial marker CD34 by CD11c$^+$ cells cultured in ID8 VEGF$^+$DEFB29$^+$ tumour-conditioned medium and inhibited the formation of capillary networks *in vivo*.

These findings indicate a new model of tumour angiogenesis in which DEFB29 recruits CD11c$^+$ immature DCs that have the potential to adopt endothelial characteristics in the presence of VEGF. The authors showed that this model is also applicable to human ovarian tumours, which express endogenous β-defensins, and it now needs to be determined whether this model applies to other tumour types. The effects of this angiogenesis pathway on the antitumour immune response also need further study. It seems that by promoting the endothelial specialization of immature DCs, the tumour might not only ensure a sufficient blood supply but also prevent these DCs from initiating an immune response against tumour antigens. In this case, therapeutic targeting of this pathway could both reduce tumour growth and promote immune attack.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Conejo-Garcia, J. R. *et al*. Tumor-infiltrating dendritic cell precursors recruited by a β-defensin contribute to vasculogenesis under the influence of Vegf-A. *Nature Med.* **10**, 950–958 (2004).