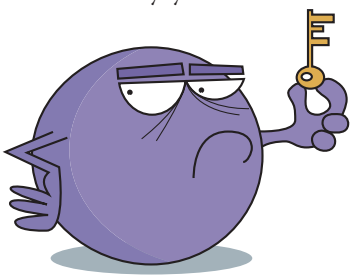
T-CELL SIGNALLING

FYN keeps ITCH under control

E3 ubiquitin ligases mediate the final step of an enzymatic cascade that results in the conjugation of ubiquitin to a protein substrate. New data published in *Molecular Cell* indicate that the function of the E3 ubiquitin ligase itchy (ITCH) is negatively regulated by FYN-mediated tyrosine phosphorylation.

Previous studies indicate that some SRC-family tyrosine kinases are targeted for degradation following ubiquitylation by HECT (homologous to the E6-associated protein carboxy terminus)-type E3 ubiquitin ligases. So, Yang *et al.* set out to investigate whether the SRC-family tyrosine kinase FYN is a target of the HECT-type E3 ubiquitin ligase ITCH in T cells. Surprisingly, ectopic co-expression of ITCH and FYN by a fibroblast-cell line did not result in ITCH-mediated ubiquitylation of FYN but resulted in tyrosine phosphorylation of ITCH. Consistent with this, FYN was shown to phosphorylate ITCH in an *in vitro* kinase assay. And, in wild-type T cells, T-cell receptor (TCR) crosslinking induced rapid tyrosine phosphorylation of ITCH, but this was not observed in FYN-deficient T cells. Further analysis showed that FYN phosphorylates Tyr371, which is found in the third WW domain (a domain that contains two conserved tryptophan residues) of ITCH.



HAEMATOPOIESIS

HSCs find their niche

In adults, haematopoietic stem cells (HSCs) — the primitive progenitors from which all cells of the immune system are derived — are located in the bone marrow, close to the endosteal surfaces of bone. Many cell-surface receptors have been implicated in controlling the localization of HSCs to this endosteal niche. However, a report published in *Nature* now shows that expression of calcium-sensing receptor (CaR) by HSCs is crucial for their lodgement in the endosteal niche.

The endosteum is a site of constant bone modelling and remodelling, and as such it is characterized by high concentrations of calcium ions (Ca^{2+}). CaR is one receptor by which cells sense the extracellular concentration of Ca^{2+}, and because some haematopoietic cells have previously been shown to express CaR, Adams *et al.* set out to investigate whether CaR is important for HSC localization or function in the endosteal niche. CaR was shown to be expressed by lineage $(Lin)^-$ stem-cell antigen 1 $(SCA1)^+KIT^+$ cells (known as LSK cells) isolated from the bone marrow, a population that is enriched in cells with the functional properties of stem cells.

> “expression of CaR by HSCs is required for their lodgement in the endosteal niche”

Consistent with the hypothesis that expression of CaR by LSK cells is important for their localization or function in the endosteal niche, the bone marrow of CaR-deficient mice was shown to contain markedly fewer LSK cells than that of CaR-sufficient littermate control mice. Furthermore, the number of cells able to initiate long-term cultures (an *in vitro* functional assay for HSCs) was also markedly decreased in the bone marrow of CaR-deficient mice. By contrast, the number of LSK cells in the spleen and the blood of CaR-deficient mice was increased. Importantly, the aberrant localization of LSK cells in CaR-deficient mice was not a result of intrinsic HSC abnormalities, because fetal-liver LSK cells from these mice were phenotypically and functionally normal. However, following transfer to lethally irradiated wild-type recipients, CaR-deficient fetal-liver cells failed to accumulate in the bone marrow, indicating a role for CaR in regulating HSC localization in the endosteal niche.

The defect in localization of CaR-deficient HSCs in the endosteal niche

In T cells, the absence of FYN, or the presence of a mutant form of ITCH in which Tyr371 was substituted with phenylalanine, resulted in an increased association of ITCH with its substrate JUNB. Furthermore, TCR-induced ubiquitylation of JUNB was also increased, resulting in increased degradation of the protein.

These data indicate that FYN-mediated phosphorylation of ITCH Tyr371 negatively regulates substrate recruitment by the E3 ubiquitin ligase ITCH. This is in contrast to the positive effect of phosphorylation by the serine/threonine kinase JUN amino-terminal kinase 1 (JNK1) on the E3-ubiquitin-ligase activity of ITCH.

Karen Honey

ORIGINAL RESEARCH PAPER Yang, C. *et al.* Negative regulation of the E3 ubiquitin ligase Itch via Fyn-mediated tyrosine phosphorylation. *Mol. Cell* **21**, 135–141 (2006)
FURTHER READING Liu, Y. -C., Penninger, J. & Karin, M. Immunity by ubiquitylation: a reversible process of modification. *Nature Rev. Immunol.* **5**, 941–952 (2005)

could be a result of aberrant homing to the bone marrow or an inability to lodge in the endosteal niche. CaR-deficient fetal-liver LSK cells were found to express normal amounts of cell-surface molecules involved in homing to the bone marrow, and they were found to enter the bone marrow as efficiently as wild-type fetal-liver cells. By contrast, CaR-deficient fetal-liver cells were markedly impaired in their ability to bind the extracellular-matrix component collagen type I and showed reduced ability to physically associate with the endosteal surface.

These data indicate that expression of CaR by HSCs is required for their lodgement in the endosteal niche. These results provide a mechanism for HSC engraftment in the bone marrow during ontogeny and, as the authors suggest, have important implications for stem-cell transplantation therapies.

Karen Honey

ORIGINAL RESEARCH PAPER Adams, G. B. *et al.* Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature* 28 Dec 2005 (doi:10.1038/nature04247)
FURTHER READING Wilson, A. & Trumpp, A. Bone-marrow haematopoietic-stem-cell niches. *Nature Rev. Immunol.* **6**, 93–106 (2006)

CYTOKINES

TWEAK and TNF: Yin and Yang in innate immunity

The cytokine tumour-necrosis factor (TNF) has a key role in promoting the activation of innate cells and the release of pro-inflammatory cytokines that influence the transition from innate to adaptive immune responses. Now, a study published in *Cell* shows that TWEAK (TNF-related weak inducer of apoptosis) balances TNF activity by repressing the production of pro-inflammatory cytokines and attenuating the transition from innate to adaptive immunity.

To analyse the role of TWEAK in immune responses, Maecker *et al.* generated TWEAK-deficient mice. These mice were healthy and developed normally, but they had more natural killer (NK) cells compared with age-matched wild-type littermate control mice. This was a result of impaired elimination of NK cells by activation-induced cell death, rather than impaired generation of NK cells in the bone marrow.

Using a lipopolysaccharide (LPS) systemic-challenge model, the authors showed that TWEAK-deficient mice were more susceptible to LPS-induced death than were wild-type control mice. Furthermore, *Tweak*$^{-/-}$ NK cells and macrophages produced greater quantities of the pro-inflammatory cytokines interferon-γ (IFNγ) and interleukin-12 (IL-12) but reduced levels of the anti-inflammatory cytokine IL-10, indicating that TWEAK functions to dampen the innate inflammatory responses.

So, how does TWEAK attenuate the production of pro-inflammatory cytokines? First, the authors assessed the effect of TWEAK on the transcription factor STAT1 (signal tranducer and activator of transcription-1), which is important for the production of IFNγ and IL-12 by NK cells and macrophages. *Tweak*$^{-/-}$ mice had increased basal levels of STAT1 phosphorylation and increased LPS-induced STAT1 phosphorylation compared with control mice, as well as reduced induction of SOCS1 (suppressor of cytokine signalling 1). So, attenuation of STAT1 activation through SOCS1 induction is one mechanism by which TWEAK represses the production of IFNγ and IL-12.

Next, the authors looked at the effect of TWEAK on the nuclear factor-κB (NF-κB) pathway, as TNF induces the expression of IFNγ and IL-12 through activation of this pathway. TNF induces transient phosphorylation of the p65 subunit of NF-κB, leading to its association with the p50 subunit and translocation to the nucleus, where the heterodimer transactivates target genes by association with the p300 transcriptional co-activator. By contrast, TWEAK was found to induce prolonged phosphorylation of p65, leading to association not with p300, but with histone deacetylase 1 (HDAC1), which represses target gene transcription. So, HDAC1-mediated transcriptional repression is a second mechanism by which TWEAK modulates pro-inflammatory cytokine production.

To further assess the role of TWEAK in modulating the transition from innate to adaptive immunity, the authors used the C57BL/6 model of rejection of B16 melanoma cells, which is mediated by NK cells and T cells. In contrast to wild-type littermate controls, *Tweak*$^{-/-}$ mice rejected moderately aggressive B16–F10 melanoma cells and had greater numbers of splenic NK cells and CD8^{+} T cells. When *Tweak*$^{-/-}$ mice were injected with more aggressive B16–BL6 melanoma cells, the tumours were infiltrated with 2–8 times more NK cells and T cells than wild-type mice, and together with macrophages, these cells produced more IFNγ and IL-12 upon re-challenge *ex vivo*. Generation of bone-marrow-chimeric mice showed that TWEAK did not act directly on T cells, but rather, it acted indirectly by modulating the T-cell-priming environment.

Together, these results show that TWEAK and TNF act in a 'Yin and Yang' manner in modulating the transition from innate to adaptive immunity.

Elaine Bell

ORIGINAL RESEARCH PAPERS Maecker, H. *et al.* TWEAK attenuates the transition from innate to adaptive immunity. *Cell* **123**, 931–944 (2005)