LYMPHOCYTE MIGRATION

T cells and DCs get it together

Naive T cells must interact with dendritic cells (DCs) — the professional antigen-presenting cells of the immune system — to become activated. The dynamics of T-cell–DC interactions have been examined in several studies using excised lymph nodes, in which lymph and blood flow were not maintained, and the results have been variable. In a new study published in *Nature*, Ulrich von Andrian's group used two-photon microscopy to study T-cell–DC interactions *in vivo* in the intact popliteal lymph nodes (PLNs) of anaesthetized mice, in which blood and lymph flow were maintained, and show that T-cell migration occurs in three distinct phases.

Mice were injected with fluorescently labelled DCs into the footpad, and by the following day, DCs had accumulated in the PLNs. Lipopolysaccharide was co-administered with DCs to induce the expression of CC-chemokine receptor 7 (CCR7) by the DCs, which allows them to home to lymph nodes. Eighteen hours after DC injection, T-cell-receptor-transgenic $CD8^+$ T cells were injected intravenously. Two hours after injection, the transgenic T cells had migrated to the PLNs and constituted 1–2% of T cells.

Where do the injected DCs localize in the PLNs? The authors found that many of the DCs localized around high endothelial venules (HEVs) — a site that allows the DCs to interact with T cells as they enter the node.

Analysis of T-cell dynamics revealed that T cells interact with DCs in three distinct phases. In phase one, covering the first eight hours after T-cell entry to the PLNs, T cells formed brief contacts with DCs that lasted for less than 30 minutes. These interactions resulted in increased expression of early activation markers by the T cells. Phase two covered the 8–24-hour period after T-cell transfer. In this phase,

NATURAL KILLER CELLS

New role for killer gene identified

Natural killer (NK) cells are one of the main cell types involved in the rejection of allogeneic bone-marrow transplants. In this paper, Taniguchi and colleagues describe an activating NK-cell receptor — NKG2I — that has a crucial role in eliciting allograft rejection.

The NKG2 receptors are a family of lectin-like molecules expressed by NK cells that include NKG2D, which is functional as a homodimer, and NKG2A, NKG2C and NKG2E, which form heterodimers with CD94. Koike *et al.* set out to study which genes were preferentially expressed by NKT cells compared with conventional T cells. Not unsurprisingly, a subset of the genes were NK-cell receptors, including NKG2A, NKG2D and NK1.1. In addition, a new clone with a putative protein sequence indicative of CD94 and the NKG2 family was described, and named NKG2I. However, NKG2I is in fact identical to KLRE1 — a lectin-like NK-cell receptor identified in a study by Westgaard *et al.* and previously published in *The Journal of Experimental Medicine*.

Koike *et al.* found that NKG2I was highly expressed by NK cells and sequence analysis showed that the C-type lectin domain of NKG2I had 40% homology with CD94 and NKG2D, indicating that its structure might be similar to these molecules. Surprisingly, NKG2I lacked any signalling motifs or positively charged residues in its putative transmembrane region, which are characteristics of the NKG2 family, and showed little similarity to other family members in its putative ligand-binding domain, indicating that it is likely to interact with unique ligands.

Flow cytometric analysis using an NKG2I-specific monoclonal antibody showed that most NK cells in the spleen, liver and bone marrow express NKG2I, and immunoprecipitation studies indicated that this receptor probably forms a dimeric complex. Further studies are required to determine whether NKG2I is a homodimer or a heterodimer.

Previous experiments have indicated that CD94, presumably complexed with an NKG2 family member, is involved in NK-cell-mediated allograft rejection. Given the sequence similarity between NKG2I and CD94, the authors studied whether NKG2I was also involved in NK-cell allorecognition. Cross-linking of NKG2I on the cell surface of *in vitro* cultured NK cells induced interferon-γ production, whereas blocking of NKG2I inhibited alloreactive NK-cell cytotoxicity. The importance of NKG2I in the activation of alloreactive NK-cell responses was further confirmed *in vivo*, where NKG2I-specific antibodies compromised the rejection of an allogeneic bone-marrow transplant, without depleting the number of NK cells or modulating the expression of other NK-cell receptors.

This report identifies NKG2I as an activating NK-cell receptor involved in initiating pathways that lead to NK-cell-mediated allograft rejection.

By contrast, the earlier report by Westgaard *et al.* indicated that KLRE1 can dimerize with an unidentified partner containing an immunoreceptor tyrosine-based inhibitory motif and form an inhibitory receptor complex. These observations indicate that further studies are required to characterize fully the functions of this molecule; and defining all KLRE1/NKG2I binding partners is likely to be a first step in this process.

Karen Honey

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T cells formed stable contacts with DCs that lasted for more than an hour. The T cells expressed activation markers and secreted interleukin-2 and interferon-γ. In phase three, which started after 24 hours, the T cells dissociated from DCs, and underwent rapid migration and proliferation. When transgenic $CD4^+$ T cells were used, a similar three-phase pattern of interactions was observed.

It remains to be determined what mechanisms control the phase-to-phase transition and how the length of the interactions is controlled.

Elaine Bell

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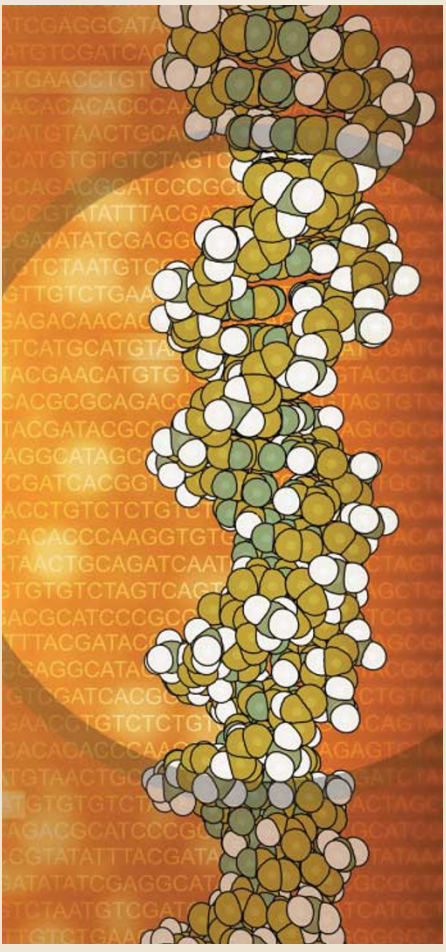

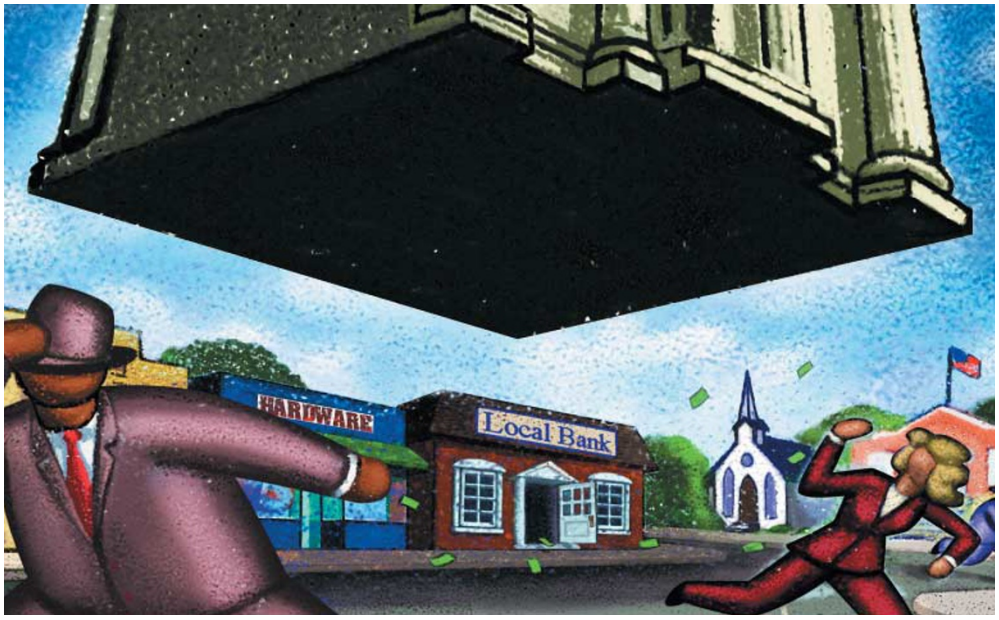

INNATE IMMUNITY

Microbes mind out!

To establish infection, microbial pathogens bind to components of the host extracellular matrix (ECM) and colonize the host. However, according to a recent paper in *Nature Immunology*, the secreted ECM protein mindin can also act as a pattern-recognition molecule and is crucial for the initiation of innate immune responses against invaders.

Although previous work has implied that ECM macromolecules might be actively involved in immune defence against microorganisms, this is the first time the role of individual components has been explored. He *et al.* first cloned mouse *Spon2*, which encodes mindin, by differential gene expression, and, having established that this gene is highly expressed in lymphoid tissues, generated mindin-deficient mice to investigate the role of this protein in immunity.

The immune system of mindin-deficient mice developed normally, but when the authors tested the response of mindin-deficient mice to lipopolysaccharide (LPS) — a pathogen-associated molecular pattern that is a component of the cell walls of Gram-negative bacteria — they found that these mice were resistant to LPS, unlike wild-type mice that all died from LPS-induced septic shock. In wild-type mice, macrophages rapidly produce inflammatory cytokines after exposure to LPS. However, this did not occur in the knockout mice, indicating that mindin is essential for this response. In addition, mindin-deficient macrophages failed to respond to a range of stimuli from Gram-positive and Gram-negative bacteria.

What effect does lack of mindin have on immune responses to bacteria? Mindin-deficient mice failed to clear *Haemophilus influenzae* but not *Pseudomonas aeruginosa* from the lungs, highlighting a role for mindin in the pulmonary clearance of some, but not all, bacterial pathogens. Mindin-deficient mice were also shown to be more sensitive to systemic infection with *Streptococcus pneumoniae*, but less sensitive to *Salmonella typhimurium* than wild-type mice.

These results indicate that mindin interacts with microbial pathogens and acts as a pattern-recognition molecule for immune cells. Finally, the authors confirmed this by showing that mindin can bind directly to both Gram-negative and Gram-positive bacteria, and can also act as an opsonin for macrophage phagocytosis of some pathogens.

So, mindin is essential for the initiation of innate immune responses to bacterial pathogens, and functions as a pattern-recognition molecule resident in the ECM, ready and waiting for bacterial invasion.

Jenny Buckland

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