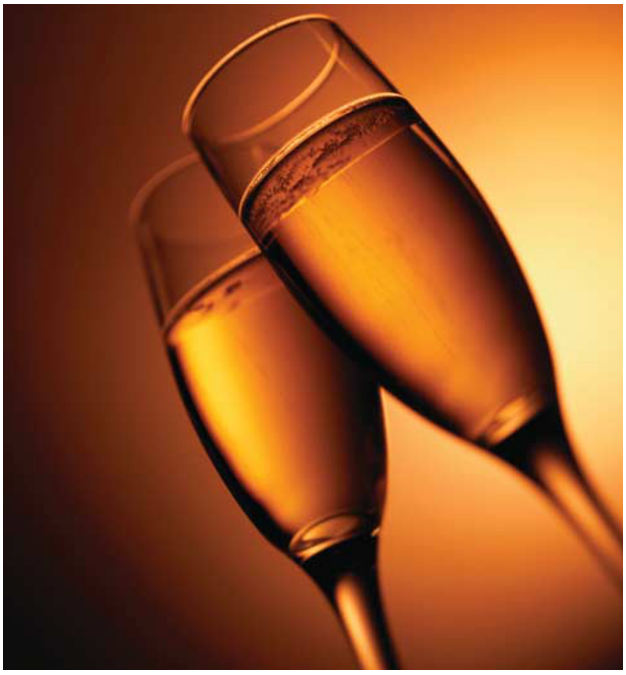
NATURAL KILLER CELLS

Matchmaking for natural killers



Finding the right partner has always been a tricky proposition, but a team led by Alessandro Moretta has found the perfect match for the natural killer (NK)-cell receptor DNAX accessory molecule 1 (DNAM1; also known as CD226). It seems that DNAM1 has two potential partners to choose from, depending on the situation.

NK cells are prevented from attacking normal tissue by the interaction between MHC class I molecules and inhibitory receptors. However, in the absence of MHC class I expression — for example, on tumour cells or virus-infected cells — ligation of activating receptors on NK cells triggers target-cell killing. In addition to the well-known triggering NK-cell receptors (such as NKp30, NKp44, NKp46 and NKG2D), cross-linking of cell-surface DNAM1 has also been shown to activate the cytotoxicity of NK cells. However, until now, the target-cell ligand to which DNAM1 binds has not been known.

Bottino *et al.* immunized mice with NK-cell-susceptible cell lines to generate monoclonal antibodies against their cell-surface molecules. These antibodies were then tested for their ability to inhibit the killing of these cell lines by NK cells *in vitro*. Experiments were carried out in the presence of an antibody to block the effects of NKG2D ligation. Four antibodies successfully blocked cytotoxic activity and were therefore likely to bind to molecules recognized by activating receptors other than NKG2D on NK cells.

To characterize these molecules, cell lysates were immunoprecipitated with each of the antibodies, then probed with the same or a different antibody. Three of the antibodies recognized the same molecule of ~70 kD, whereas the fourth antibody recognized two molecules of ~65 kD and ~60 kD. Mass spectrometry identified these proteins as poliovirus receptor (PVR) and the δ and α isoforms of nectin-2, respectively. This was confirmed by antibody staining of cells transfected with the gene encoding PVR or nectin-2. Fc fusion proteins containing the extracellular domain of PVR or nectin-2 were both shown to stain cells transfected with DNAM1, but not with other activating NK-cell receptors, which

INNATE IMMUNITY

Neutrophils can't take a complement

Previous reports have documented a correlation between excessive production of the complement component C5a during the onset of sepsis and poor clinical outcome associated with compromised innate immunity. And blockade of either C5a or its receptor has beneficial effects in experimental models of sepsis. Peter Ward and colleagues have now added further detail to this model by looking directly at the effects of C5a on the production of cytokines by neutrophils.

Neutrophils are important for innate responses to invading bacteria and they are one of the main sources of the pro-inflammatory cytokine tumour-necrosis factor (TNF) in the serum during inflammatory responses — for example, to bacterial lipopolysaccharide (LPS).

In an *in vitro* assay, C5a inhibited LPS-induced TNF production by blood neutrophils from normal rats. In an *in vivo* model of sepsis — caecal ligation/puncture (CLP) of rats — TNF production in response to LPS by blood neutrophils isolated during the first 12 hours of sepsis was markedly reduced compared with control neutrophils. The authors showed that this was due to C5a production by blocking endogenous C5a in the rats during sepsis with a C5a-specific antibody injected intravenously at the start of CLP. This led to increased levels of serum TNF and an increased TNF response of isolated neutrophils stimulated with LPS.

How does C5a mediate this effect on neutrophils? The promoter region of the *TNF* gene contains a binding site for nuclear factor-κB (NF-κB), and when neutrophils were treated *in vitro* with an inhibitor of NF-κB activation, their production of TNF in response to LPS was completely prevented. So, neutrophil production of TNF depends on NF-κB, the nuclear translocation and activity of which is negatively regulated by inhibitor of NF-κB (IκB). Neutrophils exposed to C5a *in vitro* had increased levels of IκBα, as did neutrophils isolated from CLP-induced septic rats. However, C5a did not increase transcription of the gene encoding IκBα, indicating that the increased protein levels might be due to reduced degradation. Regardless of the mechanism, an increased level of IκBα in response to C5a would result in decreased NF-κB activity and, therefore, decreased *TNF* transcription. This study provides one possible explanation for the poor outcome associated with high levels of C5a, by showing that this can inhibit some of the innate defences of neutrophils in response to bacteria. Therefore, C5a could be a new target for the treatment of sepsis.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Riedemann, N. C. *et al.* Regulation by C5a of neutrophil activation during sepsis. *Immunity* **19**, 193–202 (2003)

FURTHER READING Riedemann, N. C. *et al.* The enigma of sepsis. *J. Clin. Invest.* **112**, 460–467 (2003)

WEB SITE

Peter Ward's homepage:
http://www.pathology.med.umich.edu/faculty/Ward/

identifies these molecules as specific cell-surface ligands for DNAM1.

The killing of untransfected CHO-K cells by the NK-cell line NK92 depends on NKp30 (another orphan receptor). But, when these cells were transfected with the gene encoding PVR or nectin-2, NK-cell killing could only be partly inhibited by an NKp30-specific antibody, with the remainder of the cytotoxic activity being inhibited by an antibody specific for DNAM1. The functional interaction between DNAM1 and PVR and/or nectin-2 was also shown to lead to NK-cell activity for tumour target cells. These experiments showed that the killing of tumour cells by polyclonal NK cells depends on many triggering receptors, with the relative contribution of DNAM1 binding to PVR or to nectin-2 depending on the tumour type and ligand/receptor availability.

Kirsty Minton

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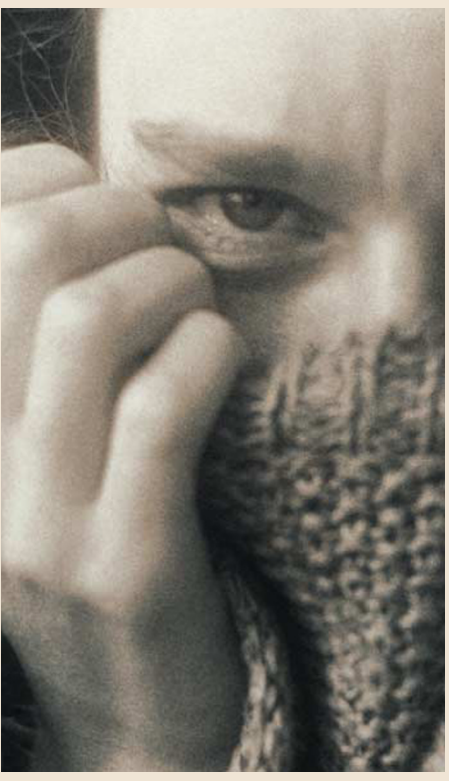

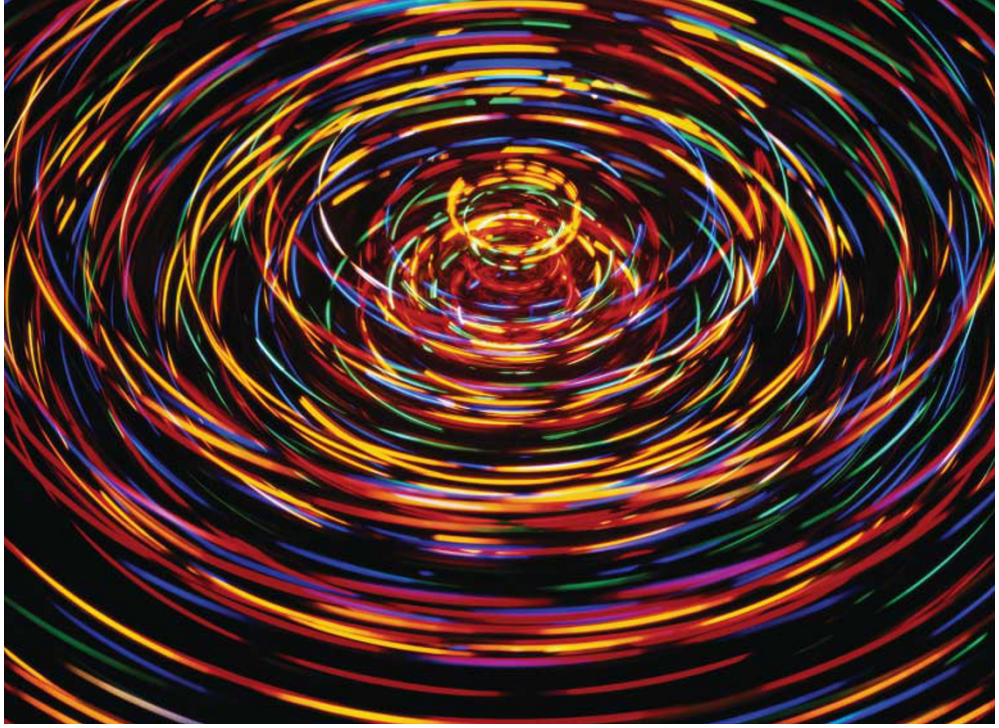

LYMPHOCYTE MIGRATION

Homing in on leukotrienes

To mediate an effective immune response, T cells must find their way to sites of infection or inflammation. Changes in T-cell homing patterns following activation are thought to result from changes in the expression of homing molecules, including adhesion molecules and chemokine receptors, however, data now show that this might not be the whole picture. Three articles in the October issue of *Nature Immunology* report that leukotriene B_4 (LTB_4) — an arachidonic-acid-derived pro-inflammatory lipid — is also involved in T-cell recruitment.

Andrew Luster and colleagues, who generated mice deficient for the main LTB_4 receptor BLT1, showed that LTB_4 could direct the recruitment of $CD4^+$ effector T cells. They showed that BLT1 was highly expressed by $CD4^+$ effector T-cell subsets differentiated *in vitro* under T helper 1 (T_H1)- or T_H2-polarizing conditions and after activation *in vivo*. Expression of BLT1 enabled both subsets of $CD4^+$ effector T cells to move by chemotaxis towards a LTB_4 gradient, whereas naive cells were unaffected by the presence of LTB_4. Both subsets of $CD4^+$ effector T cells also adhered to endothelial cells under flow when exposed to LTB_4. They used their BLT1-deficient mice in an asthma model to show that the recruitment of early $CD4^+$ and $CD8^+$ T cells to the airways after aerosol challenge of previously immunized mice was dependent on the expression of BLT1.

An important source of LTB_4 was shown by Vanessa Ott *et al.* to be activated mast cells — the sentinel cells of the tissues' early warning system. They showed that activated mast cells could induce the migration of $CD8^+$ effector T cells through the production of LTB_4. Therefore, LTB_4 seems to act as a link between the activation of innate immune cells and early recruitment of adaptive immune cells.

Ulrich von Andrian's group also investigated the response of $CD8^+$ T-cell subsets to LTB_4. $CD8^+$ naive, effector and central memory T cells have distinct migratory properties *in vivo*; naive and central memory T cells home to secondary lymphoid tissues, whereas effector T cells migrate efficiently to inflamed tissues. The authors showed that $CD8^+$ effector T cells express high levels of BLT1, whereas $CD8^+$ memory T cells express low levels. BLT1-expressing $CD8^+$ effector T cells moved by chemotaxis towards LTB_4, whereas naive and memory cells did not. By contrast, LTB_4 induced the rapid accumulation of both effector and memory T cells, but not naive cells, in postcapillary venules by promoting the transition from rolling to firm arrest. This effect depended on signals mediated through BLT1, as cells from the BLT1-deficient mice did not arrest in response to LTB_4. Using a model of peritonitis, they showed that wild-type effector cells were three times more efficient at migrating to the inflamed peritoneal cavity than BLT1-deficient effector cells, further indicating an important role for LTB_4–BLT1 interactions in the trafficking of $CD8^+$ effector T cells *in vivo*.

Lucy Bird

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