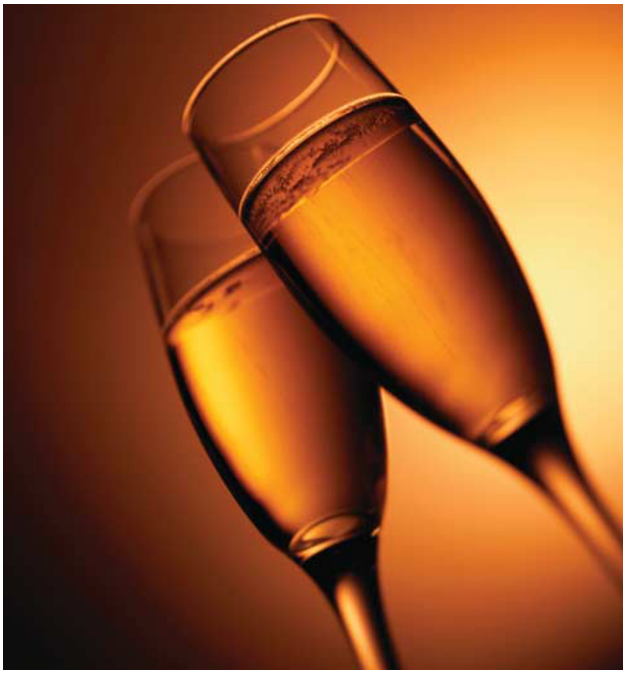
NATURAL KILLER CELLS

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/