INFLAMMATION

Neutrophil NETworks

A key function of neutrophils is to attack and kill bacteria at sites of infection. Bacteria are phagocytosed and fusion of phagocytic vacuoles with neutrophil granules exposes the bacteria to a collection of antimicrobial reagents, including lytic enzymes, cationic peptides that disrupt bacterial membranes and reactive oxygen species. Neutrophils can also kill bacteria extracellularly, and a new study from Volker Brinkmann and colleagues reveals that this is achieved by concentrating antimicrobial reagents in a fibrous network released by the neutrophil.

Neutrophils were stimulated with interleukin-8, phorbol myristate acetate or lipopolysaccharide. When the activated neutrophils were gently washed and fixed, and examined by high-resolution scanning electron microscopy, Brinkmann and colleagues observed fibrous extracellular structures outside the cells. These were termed neutrophil extracellular traps or NETs. The NETs were composed of DNA, histones and granule enzymes, such as neutrophil elastase, and were released from the cells as early as 10 minutes after activation. NETs were able to trap both Gram-positive and Gram-negative bacteria. Blocking the function of neutrophil proteases showed that they were required for the deactivation of bacterial virulence factors. NETs were also observed *in vivo* in samples taken from shigellosis infection of rabbits and appendicitis in humans.

Extracellular killing of bacteria carries the risk of damaging the host cells by exposing them to the neutrophil's antimicrobial toolkit. The authors suggest that NETs amplify the activity of antimicrobial components by concentrating them in the fibrous network, which should also reduce exposure of host tissue to these components. It remains to be determined whether NETs are formed by an active process or are an early stage in neutrophil cell death.

Elaine Bell

References and links

ORIGINAL RESEARCH PAPER Brinkmann, V. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004)

VACCINES

SPOT-on malaria target

Development of a malaria vaccine has been hindered by the difficulty of defining an immunological correlate of protection in humans. Now, a study in *Nature Medicine* reveals that protection against infection with *Plasmodium falciparum* (the causative agent of the most severe form of malaria) is associated with a peptide-specific $CD4^+$ T-cell response, which can be detected using an interferon-γ (IFN-γ) enzyme-linked immunosorbent SPOT (ELISPOT) assay.

Evidence from natural infection or vaccination with malarial liver-stage antigens indicates that antibodies and $CD4^+$ and $CD8^+$ T cells are involved in protective immunity; in particular, IFN-γ is thought to be a key component. However, despite the immunogenicity of current vaccine candidates, they have generally been selected on the basis of assays that do not distinguish between protected and unprotected individuals — such as T-cell proliferation or cytotoxicity.

To find an assay that provides a correlate of protection in the field, Hill and colleagues used several systems to measure the immune responses of ~200 non-immune or semi-immune Gambians, who were participating in a Phase IIb trial of a recombinant vaccine derived from the circumsporozoite protein. Although significant antibody and T-cell responses were elicited, most of these did not correlate with protection. By contrast, using a 'cultured' ELISPOT assay, in which cells were restimulated and clonally expanded *in vitro* prior to measuring IFN-γ production, $CD4^+$ T cells specific for a circumsporozoite-protein-derived peptide were found to be strongly associated with protection against naturally acquired infection and disease. Individuals in the unvaccinated group who showed similar responses to this peptide (resulting from previous natural exposure) were also found to be protected.

This study defines a cellular immune response that correlates with protection against malaria in both vaccinated and naturally exposed individuals. The peptide sequence is highly conserved across *P. falciparum* strains and has previously been shown to bind numerous HLA-DR alleles, making this epitope an enticing target for future vaccine studies. Clearly, assessment of an appropriate immune effector mechanism is important. The authors suggest that the cultured ELISPOT might measure the central-memory T-cell population, which was recently proposed to be more important in protective immunity to malaria than the short-lived effector-memory cell population measured in standard *ex vivo* ELISPOT assays.

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References and links

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FURTHER READING Tsuji, M. & Zavala, F. T cells as mediators of protective immunity against liver stages of *Plasmodium*. *Trends Parasitol.* **19**, 88–93 (2003)

WEB SITE

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http://www.jr2.ox.ac.uk/ndm/hill_%20group.htm