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INNATE IMMUNITY

Multitasking NET makers

Neutrophil extracellular traps (NETs) are fibrous networks of DNA and antimicrobial factors that are released by neutrophils to trap and kill pathogens. NETosis was thought to be associated with cell death, and thus it was assumed that NETosing neutrophils are non-functional. Reporting in *Nature Medicine*, Yipp *et al.* show that neutrophils rapidly undergo NETosis but remain functional, albeit with unusual cellular phenotypes, in response to infection *in vivo*.

Most studies of NETs so far have been carried out *in vitro*. NETosis was suggested to be slow and to occur as a feature of explosive cell death. In this study, the authors developed a model to visualize *in situ* NETosis, together with neutrophil behaviour and fate, during *Staphylococcus aureus* skin infection using spinning-disk confocal intravital microscopy.

They observed the rapid formation of sheets of extracellular DNA within the extravascular dermal parenchyma after acute *S. aureus* infection (and in response to killed bacteria), but not after sterile inflammation (induced by intradermal injection of the chemokine CXCL2). These sheets had all the characteristics of NETs. Furthermore, recruited neutrophils were shown to crawl through the infected tissue, thereby widening the distribution of the NETs, and no neutrophil cell death was observed during this early phase of the response. Disruption of NETs using exogenous DNase treatment resulted in increased bacteraemia and reduced numbers of bacteria at the site of injection, indicating that NETs are important for containing acute skin infection *in vivo*.

> “NETosis is tightly controlled by TLR2 and complement-mediated opsonization”

Both Toll-like receptor 2 (TLR2) and complement are known to be involved in immunity to *S. aureus*. Despite normal neutrophil recruitment to the skin in mice lacking either TLR2 or complement component C3, these cells did not release NETs. These and other data indicate that NETosis is tightly controlled by TLR2 and complement-mediated opsonization in this model.

But what is the phenotype of neutrophils undergoing NETosis? The authors identified three nuclear morphologies at the site of infection: normal nuclei, diffuse nuclei and absent nuclei. Three-dimensional imaging showed that NETosing neutrophils had a diffuse nucleus; anuclear neutrophils did not contain any stainable nucleic acids, suggesting that these cells had completed NETosis.

Although NETosing neutrophils crawled within the infected tissue, they had an unusual cell morphology with multiple pseudopods and a high crawling velocity. Importantly, neutrophils with diffuse nuclei could phagocytose bacteria, indicating that NETosing neutrophils remained functional.

Finally, human neutrophils were shown to release NETs and become anuclear in abscesses induced by Gram-positive bacteria. Of note, neutrophils with diffuse or absent nuclei still contained granules, suggesting that these cells retained the ability to kill bacteria through conventional mechanisms. Neutrophils that were isolated from healthy volunteers and then injected into the skin of mice had normal nuclei in the absence of *S. aureus* infection, but a large percentage of the cells had decondensed nuclei or became anuclear in the presence of infection.

Together, these data show that NETosis occurs rapidly *in vivo* in response to an acute infection with Gram-positive bacteria and is required to prevent the dissemination of the pathogen. Although neutrophils undergoing NETosis adopt an unusual cell morphology, they remain functional and do not immediately undergo cell death.

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ORIGINAL RESEARCH PAPER Yipp, B. G *et al.* Infection-induced NETosis is a dynamic process involving neutrophil multitasking *in vivo*. *Nature Med.* **18**, 1386–1393 (2012)