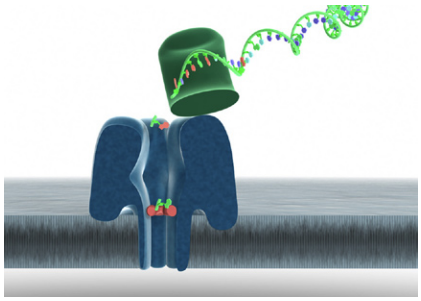

Pore progress: Oxford Nanopore's modified nanopore can distinguish between the four standard DNA nucleotides and methylated cytosine. The aim is to use an endonuclease (green) to feed the nucleotides to the pore one by one. (Photo: iemedia solutions.)

Coming soon to a doctor's surgery near you?

Hopes placed on the sequencing of 'the' human genome and the subsequent identification of many single-nucleotide polymorphisms (SNPs) have turned out to be misguided, as the causes of common diseases like cancer and heart disease have proven too complex to be addressed with simple SNP searches. Also, large-scale genomic differences like copy number variations (CNV) and insertions/deletions (indels) are now known to be equally important as SNPs, rendering the quest for genomic answers to medical problems ever more challenging.

There is, however, the hope that large-scale analysis of individual genomes may yield some of the answers that SNPs failed to provide. And the rapid progress in sequencing technology, with the costs dropping more rapidly than those of computer technology (still following Moore's Law), means that comparing thousands of individual genomes, thoughtfully chosen by epidemiological criteria, is now a perfectly viable approach to medical genomics.

At the more individual level, affordable genome analysis is also bound to aid diagnosis and therapy, especially in cancer. Soon it will be easy to compare the genome of a tumour with that of the patient's healthy cells, enabling doctors to find out both what went wrong and what treatment is most likely to succeed. And this, when it happens, will be the real genome revolution.

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Quick guide

Neutrophil extracellular traps

Borko Amulic and Garret Hayes

What are neutrophil extracellular traps, or NETs? Neutrophils, the most abundant immune cells in humans, are the first line of defense against invading pathogens. They employ a wide array of anti-microbial strategies, most notably phagocytosis, to attack and eliminate pathogens. In 2004 a new and unexpected anti-microbial activity of neutrophils was uncovered: upon encountering bacteria, neutrophils release a mesh-like structure capable of ensnaring and eliminating microbes (Figure 1). These web-like traps contain a backbone consisting of DNA/histones and are peppered with anti-microbial peptides that normally reside within the neutrophil granules. These structures were dubbed NETs and the process of NET formation is known as NETosis.

What is the role of NETs in immunity? NETosis is an anti-microbial strategy that results in neutrophil death and contributes to pathogen control and elimination. *In vitro*, the microbicidal properties of NETs have been clearly demonstrated: they trap pathogens and prevent them from dispersing, while simultaneously destroying them through exposure to a high concentration of anti-microbial effectors. These anti-microbial effectors include, as expected, the anti-microbial proteins of the neutrophil granules. Perhaps unexpectedly, the histones in NETs are also key components of the anti-microbial repertoire. It has long been known that histones are some of the most powerful anti-microbial agents that exist. Nevertheless, exactly how this anti-microbial activity of histones could be effectively harnessed was a long-standing and intractable mystery. The discovery of NETs presents a satisfying explanation for this apparent dilemma. Through NET formation, neutrophils provide histones with an opportunity to execute their alternative, non-structural function: microbial killing.

But isn't NETosis just apoptosis by another name? Actually, no! These two processes are mediated by different pathways, with NETosis involving a distinct set of events. The primary difference between these two cell-death routines is the dramatic change that occurs in the nucleus of neutrophils committed to NET formation. The hallmark of the process is nuclear envelope breakdown, followed by expansion and decondensation of the chromatin. This is accompanied by release of anti-microbial proteins from neutrophil granules; these proteins subsequently mix with and adhere to the decondensed chromatin. The process culminates in rupture of the plasma membrane and, finally, release of a NET. In contrast, apoptosis involves DNA fragmentation and nuclear shrinkage, without breakdown of membranes. Similarly, in another cell-death process, necrosis, DNA remains enclosed in a nuclear envelope, although the typical lobulation of the neutrophil nucleus is lost. Despite similarities with these other pathways, NETosis is clearly a distinct process from other cell-death mechanisms.

How are NETs formed? The molecular details of NET formation have only recently begun to be unravelled. The phenomenon is tightly linked to the production of reactive oxygen species (ROS) by neutrophil NADPH oxidase. Accordingly, patients with mutations in this enzyme fail to make NETs and suffer from debilitating infections. In addition, individuals lacking myeloperoxidase, another key enzyme in the ROS cascade, are also unable to make NETs, thus providing further evidence for a role for ROS in NET formation. Although important, ROS are not the only crucial players in NET formation. During NETosis, decondensation of chromatin is also critical for proper NET formation. Recently, a role for neutrophil elastase in the decondensation process was demonstrated; this serine protease was shown to partially degrade histones. Histone cleavage presumably leads to relaxation and decondensation of chromatin and is thus a pivotal event in the process of NET formation.

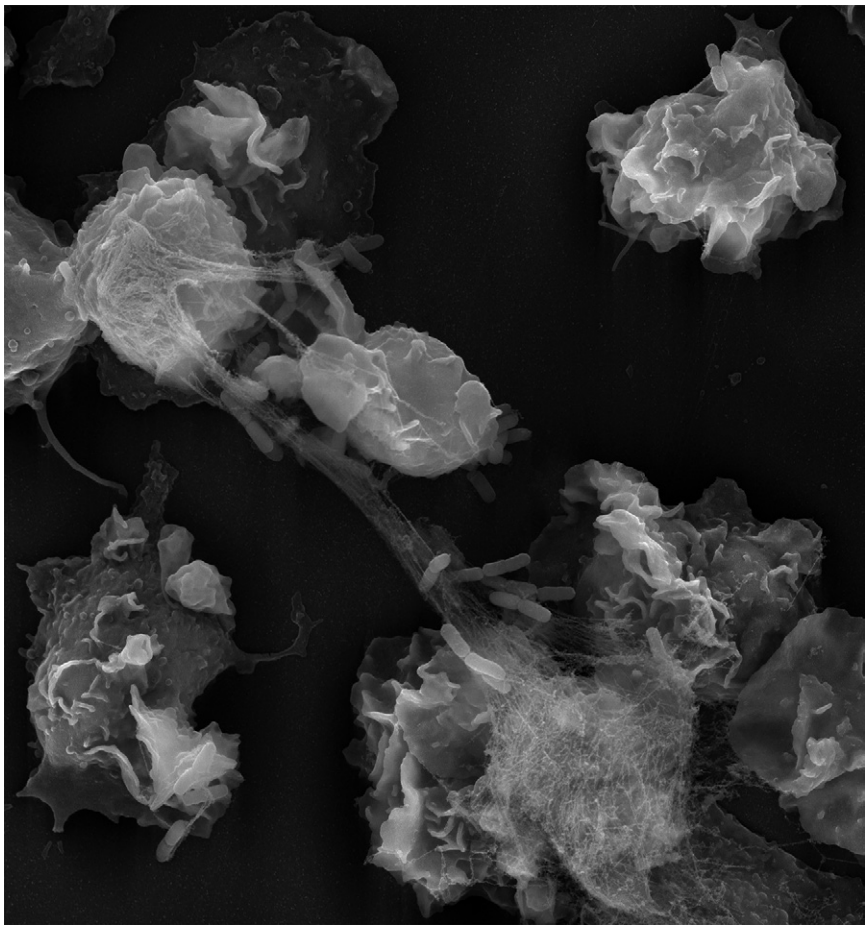

Figure 1. Scanning electron micrograph of *Shigella flexneri* trapped in a NET. The mesh-like structure of the NET is clearly visible between two still-intact neutrophils. Image courtesy of Volker Brinkmann.

So are microbes totally helpless against NETs? Evolutionary pressure has produced creative and varied microbial strategies for avoiding immune system defenses, and NETs are no exception in this regard. Certain bacterial species, such as *Streptococcus pneumoniae*, contain capsule assembly genes that decrease binding to NETs. Some Streptococci secrete DNase, which has been shown to degrade NETs *in vitro*. Bacterial mutants that cannot secrete DNase are accordingly much less virulent in mouse models of infection. Speculation abounds that other bacterial species might inhibit NET formation altogether by preventing ROS formation. The existence of these microbial evolutionary adaptations against NETs further underscores a salient role for NETs during the innate immune response to infection.

NETs seem to be quite helpful — but do they have a dark side? As with all inflammatory responses, NETosis must be carefully regulated in order to prevent unwanted damage; failure to do so can have dire repercussions. Previous studies have shown that serum DNase I degrades NETs in the bloodstream. Disruption of this process leads to the generation of anti-self antibodies and can therefore contribute to the development of autoimmune diseases, such as systemic lupus erythematosus (SLE). The release of neutrophil contents into the extracellular space during NETosis raises several pertinent questions. How does the body go about cleaning up NETs? What happens when an individual produces too many NETs? Is the production of NETs in some cases more harmful than helpful? Such questions relating to the regulation and resolution of NET production are some of the most pressing issues in current NET research.

What can NETs teach us about the biology of the cell? NET formation is a unique (and fascinating!) immunological phenomenon, analysis of which might provide insight into biological principles such as cell death, regulation of chromatin structure, nuclear envelope disassembly and membrane dynamics. However, neutrophils are short-lived, terminally differentiated cells that are notoriously difficult to manipulate genetically; novel techniques will have to be developed to fully explore the process of NETosis. An understanding of NET formation is also contingent on a better understanding of how reactive oxygen serves as a signalling molecule. NADPH oxidase homologs are expressed by a variety of different tissues and the concept of ROS as signalling molecules is emerging as an important paradigm in cell signalling. Future studies on NET formation will, without a doubt, assist in deciphering the role of ROS in cell biology and continue to provide critical insights into the function of these unique signalling molecules.

Where can I find out more?

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