# Neutrophils and immunity: challenges and opportunities

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Abstract | Scientists who study neutrophils often have backgrounds in cell biology, biochemistry, haematology, rheumatology or infectious disease. Paradoxically, immunologists seem to have a harder time incorporating these host-defence cells into the framework of their discipline. The recent literature discussed here indicates that it is appropriate for immunologists to take as much interest in neutrophils as in their lympho-haematopoietic cousins with smooth nuclei. Neutrophils inform and shape immune responses, contribute to the repair of tissue as well as its breakdown, use killing mechanisms that enrich our concepts of specificity, and offer exciting opportunities for the treatment of neoplastic, autoinflammatory and autoimmune disorders.

**Danger theory**
A theory that the trigger for mounting an immune response consists of an injury to host cells, resulting in the release of alarm signals that activate antigen-presenting cells.

**Pattern-recognition theory**
A theory that the trigger for mounting an immune response consists of the recognition of 'microbial non-self' molecules by receptors expressed by innate immune cells.

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The function of the immune system is to prevent the takeover of the body by genomes other than that encoded in the germline[1]. Central to this function is the ability to kill. Given the fundamental conservation of biochemistry, there are few chemical mechanisms for killing a given target cell that lack the potential to kill some other life form[2]. Therefore, it is no surprise that the neutrophil, one of the body's main cellular components for the destruction of microorganisms, also damages cells and tissues of the host[3,4]. In fact, neutrophil-mediated tissue damage at infected sites is so common that the host takes stock of it when judging whether to mobilize an immune response. Indeed, tissue injury is one of the main sources of information that launches inflammation, which in turn launches immunity.

To view immunology as the host's participation in the competition between genomes helps explain what makes the neutrophil as fascinating as it is indispensable[5]. Surprisingly, some immunologists seem not to share this view. Say neutrophil, and they move on, thinking: inflammation, not immunity. They disrespect the cell's 'nonspecificity' and consider its best-studied behaviours — crawling, eating, and disgorging pre-packed enzymes and partially reduced molecules of oxygen — as rudimentary. Finally, scientists who are interested in anti-inflammatory therapeutics are discouraged from targeting neutrophils because it seems futile to try to suppress neutrophil-dependent tissue damage without the serious side effect of increasing the host's risk from infection[1].

The aim of this article is to dispel each of those views. The literature on neutrophils is so vast (PubMed finds over 76,000 papers on the subject) that few authors have attempted to cover it comprehensively[6,7]. This Review makes no such effort. On the contrary, large chunks of neutrophil biology are neglected here, including myelopoiesis, chemotaxis, transendothelial migration, synthesis of lipid mediators and much of signal transduction. Instead, selected studies are marshalled to support the following five tenets: that the neutrophil often has an important role in launching immune responses; that the neutrophil helps to heal tissues as well as destroying them; that the neutrophil gives instructions with as much specificity as a lymphocyte or neuron, albeit with specificity of a different kind; that the neutrophil integrates information, with a circuitry of awe-inspiring design, to tailor its responses to its spatial and temporal context; and that the neutrophil offers potential opportunities for selective pharmacological intervention, to both promote and restrain inflammation.

## Neutrophils as decision-shapers

Neutrophils help answer one of the central questions in immunology: what triggers an immune response? The danger theory holds that injured host cells release alarm signals that activate antigen-presenting cells (APCs)[8]. The pattern-recognition theory posits that 'microbial non-self' induces an innate immune response, which in turn triggers an adaptive immune response[9]. Both views are valid, but they leave much unexplained. For example, surgery injures large numbers of host cells but does not promote immune reactions to foreign compounds, such as antibiotics, that are present at the time. Non-pathogenic resident microorganisms belonging to several thousand
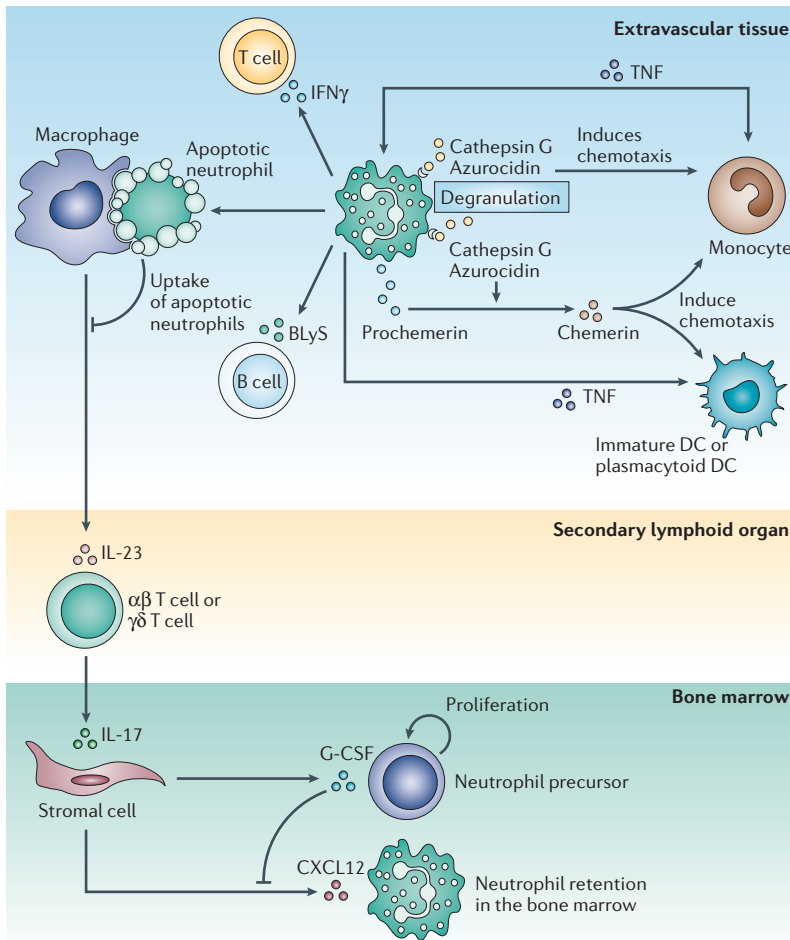
Figure 1 | **Neutrophils interact with monocytes, dendritic cells, T cells and B cells in a bidirectional, multi-compartmental manner.** Through cell–cell contact and secreted products, neutrophils recruit and activate monocytes, dendritic cells (DCs) and lymphocytes, and products of monocytes, macrophages and T cells activate neutrophils. Tissue macrophages ingesting apoptotic neutrophils produce less interleukin-23 (IL-23). IL-23 triggers T cells in secondary lymphoid tissues to produce IL-17. IL-17 triggers stromal cells in the bone marrow to produce granulocyte colony-stimulating factor (G-CSF). G-CSF promotes proliferation of neutrophil precursors and release of neutrophils into the circulation (see text for references). BLyS, tumour-necrosis factor-related ligand B-lymphocyte stimulator; CXCL12, CXC-chemokine ligand 12; IFNγ, interferon-γ; TNF, tumour-necrosis factor.

**Chemerin**
A dendritic-cell-attracting chemokine that is generated by neutrophil-dependent proteolytic activation of its precursor. Chemerin does not yet have a 'chemokine ligand' designation.

**Plasmacytoid DCs**
Immature dendritic cells (DCs) with a morphology resembling that of plasma cells. Plasmacytoid DCs produce type I interferons in response to viral infection.

species express pathogen-like microbial patterns, but they only elicit maturation of the neonatal immune system[10] and tolerance, not an immune response.

A third view is that a normal immune response results from the ongoing detection of signals that report injury and signals that report infection[1]. Although many different molecular signals might be involved, they can be considered 'binary' in the sense that most of them arise as a consequence of one of these two events, and it generally requires at least one signal from each class to launch a response. This binary control begins with inflammation. Except in autoinflammatory disorders, the triggering and continuation of an inflammatory response generally require the simultaneous receipt of molecular signals directly or indirectly reporting tissue damage and the presence of a genome different from

that of the host. Signals reporting injury and infection activate epithelial cells, mast cells, macrophages, endothelial cells, platelets and neutrophils. Binary signalling is propagated as these cells recruit, activate and programme APCs through further binary signals, such as cytokines and microbial products, cytokines and CD40 ligation, or microbial products and products of necrotic host cells. Finally, T cells are activated and programmed by antigen-receptor ligation together with signals from APCs, and B cells are activated by antigen-receptor ligation together with signals from T cells. Therefore, inflammation imprints the immune response with a pattern of information flow: the integration of signals of two or more distinct classes derived directly or indirectly from injury and infection.

As a key component of the inflammatory response, neutrophils make important contributions to the recruitment, activation and programming of APCs (FIG. 1). Neutrophils generate chemotactic signals that attract monocytes and dendritic cells (DCs), and influence whether macrophages differentiate to a predominantly pro- or anti-inflammatory state[11–13]. For example, neutrophils proteolytically activate prochemerin to generate chemerin, one of the few chemokines that attracts both immature DCs and plasmacytoid DCs[14]. Neutrophils also produce tumour-necrosis factor (TNF) and other cytokines that drive DC and macrophage differentiation and activation[12,13,15]. Neutrophil activation of DCs is fostered by cell–cell contact, in which the specific carbohydrates on CD11b engage DC-specific ICAM3-grabbing non-integrin (DC-SIGN)[15]. Moreover, neutrophils secrete TNF-related ligand B-lymphocyte stimulator (BLyS)[16], which helps to drive proliferation and maturation of B cells, and interferon-γ, which helps to drive differentiation of T cells and activation of macrophages[17]. On a per-cell basis, neutrophils make fewer molecules of a given cytokine than do macrophages or lymphocytes, but neutrophils often outnumber mononuclear leukocytes at inflammatory sites by one to two orders of magnitude, and they can therefore be important sources of cytokines such as TNF at the crucial juncture at which the decision is made to mount an immune response. However, neutrophils can also function as powerful suppressors of T-cell activation. For example, in patients with advanced cancer, activated neutrophils can impair T-cell receptor (TCR) ζ-chain expression and cytokine production[18].

The ability of neutrophils to augment or inhibit lymphocyte expansion and activation at sites of inflammation, draining lymph nodes and in the spleen is reciprocated by the adaptive immune system's control of the rate of neutrophil production in the bone marrow. This can be appreciated by looking upstream of the cytokine granulocyte colony-stimulating factor (G-CSF), which is an essential regulator of neutrophil production through several mechanisms. Stromal-cell-derived G-CSF triggers bone-marrow neutrophils to release matrix metalloproteinase 9 (MMP9), which solubilizes KIT ligand, helping to mobilize progenitor cells[19]. G-CSF also acts directly on the progenitors to increase their proliferation, while suppressing stromal-cell expression of CXC-chemokine ligand 12 (CXCL12, also known as

SDF1), which helps to retain neutrophils in the bone marrow[20]. In turn, G-CSF production is regulated by interleukin-17 (IL-17), which is produced by subsets of γδ and αβ T cells (FIG. 1). T-cell production of IL-17 is governed by IL-23, which is released from extravascular macrophages. The release of IL-23 is suppressed when macrophages ingest apoptotic neutrophils[21].

The homeostatic feedback loop among macrophages, T cells and neutrophils described earlier illustrates the fallacy of visualizing vertebrate immunity as two separate systems — innate and adaptive — that take turns over the course of an encounter with antigen. Instead, elements of more and less ancient evolutionary origin work together from before the initiation of an immune response[21], into its first hours[22], and through to its resolution. Resolution of an immune response is marked not only by the generation of immunological memory but also, in many cases, by the innate immune system's help in healing a wound.

## Demolition and reconstruction
Wounding is a frequent initiator of immune responses because it introduces both types of precipitants — injury and infection — except in the unnatural instance of surgery with aseptic technique. Although neutrophils might delay the closure of artificial, microbiologically sterile wounds[23,24], they have three important roles in healing natural wounds[25]: sterilization of microbes, generation of signals that slow the rate of accumulation of more neutrophils, and instigation of a macrophage-based programme that switches the state of damaged epithelium from pro-inflammatory and non-replicative, to anti-inflammatory and replicative.

The principal contribution of neutrophils to wound healing is microbial sterilization. Wounds tend to heal poorly and lead to lethal outcomes in individuals with insufficient neutrophils[5]; in individuals with neutrophils that cannot adhere to the endothelium or extracellular matrix and therefore fail to accumulate in infected sites[26,27]; in individuals with a vasculature that is insufficient to deliver neutrophils, for example persons with full-thickness burns; or in individuals with neutrophils that have deficiencies in microbicidal machinery.

It is not realistic to expect neutrophils to find and eat each bacterium in a wound before any bacteria have had time to escape into the lymph or blood. Neutrophils that sense tissue damage plus infection but fail to encounter a bacterium within a short time, fire off their arsenal into the extracellular space[28]. *In vitro*, this period of restraint ranges from ~15 to ~45 minutes, which is thought to be the approximate time it takes for a neutrophil to emigrate from the blood into the extravascular tissues. When restraint is abandoned, what ensues is the liquefaction of tissue — that is, the formation of pus — through neutrophils' release of proteases, their activation of proteases that are expressed in a latent form by cells resident in the tissues, and their oxidative inactivation of anti-proteases (proteins that specifically bind and inactivate proteases)[3,4]. Tissue breakdown is usually viewed as detrimental, and therefore neutrophils are often cast in a pejorative light. However, early, small-scale neutrophil-mediated tissue destruction is usually a life-saving process. It serves to

disassemble the collagen fibrils that impede neutrophil–bacterial contact and puts pressure on surrounding tissue. This might help to collapse lymphatics and capillaries, thereby cutting off bacterial escape routes and trapping the microbes in a toxic soup.

At the same time, neutrophils also generate signals with four key actions: to retard their own accumulation; to suppress their own activation; to promote their own death; and to attract and programme macrophages to stop the damage and orchestrate repair. Among the main endogenously derived anti-inflammatory compounds are metabolites of fatty acids. These include neutrophil-derived lipoxins, as well as macrophage-derived resolvins and protectins that are produced in response to the ingestion of apoptotic neutrophils[29], all of which block neutrophil recruitment. Proteinaceous anti-inflammatory factors such as secretory leukocyte protease inhibitor (SLPI)[30] are also important (FIG. 2), and neutrophils, as well as macrophages[30] and epithelial cells, produce SLPI. Despite producing SLPI, activated neutrophils can oxidatively inactivate this anti-inflammatory factor[31]. However, SLPI protects itself from oxidative inactivation by suppressing the neutrophil respiratory burst[32]. Moreover, SLPI also puts a brake on tissue proteolysis by inhibiting neutrophil elastase. Macrophages and epithelial cells also make an epithelial-cell-growth-promoting cytokine, proepithelin (PEPI; also known as progranulin). PEPI synergizes with SLPI in inhibiting neutrophil activation[31]. Although neutrophil elastase degrades PEPI to generate pro-inflammatory epithelins, SLPI protects PEPI in two ways: by binding and inhibiting neutrophil elastase, and by binding and shielding PEPI from degradation by neutrophil elastase. This helps to prevent the products of degradation of PEPI from promoting epithelial-cell production of CXCL8 (also known as IL-8)[31], which is one of the most important neutrophil attractants. Moreover, intact PEPI signals to epithelial cells to proliferate and close the wound[31,33].

## Specificity in command
Neutrophil-derived chemokines, cytokines and eicosanoids signal with a type of specificity that is familiar to immunologists: that of ligand–receptor, protein–protein or lipid–protein interactions. But neutrophil specificity does not stop there. One of the main functions of neutrophils is to undergo the respiratory burst: an abrupt, non-mitochondrial reduction of oxygen to forms that are less reduced than water and far more reactive, known as reactive oxygen intermediates (ROIs). Respiratory burst products and their derivatives include superoxide, singlet oxygen, ozone, hydrogen peroxide ($H_2O_2$), hypohalous acids, chloramines and hydroxyl radicals[34] (FIG. 3). These species can interact with an unlimited number of macromolecules. This seems to make a travesty of specificity, as this term is most often used in immunology, when it characterizes the crowning achievement of the adaptive immune system, that is, the ability of TCRs, B-cell receptors and antibodies to make distinctions so precise that they only permit the recognition of a single macromolecule among tens of thousands.

**Pus**
A collection of liquefied tissue containing many living or dead neutrophils and bacteria.

**Latent form**
A form in which activity is not yet expressed, pending activation by an event such as redistribution from a particular compartment or proteolytic processing.

**Eicosanoids**
Biologically active compounds that are primarily derived from arachidonic acid, in part through cyclooxygenases and lipoxygenases, including prostaglandins, prostacyclins, thromboxanes, leukotrienes and lipoxins.

**Reduction of oxygen**
Donation of electrons to molecular oxygen. Donation of up to three electrons gives rise to reactive oxygen intermediates (ROIs), whereas donation of four electrons gives rise to water. The term 'intermediate' in ROI refers to oxygen whose reduction state is intermediate between that of oxygen ($O_2$) and water ($H_2O$).
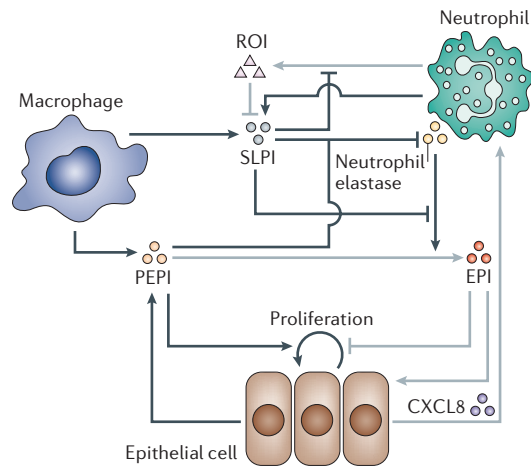
Figure 2 | **Neutrophils have a key role in wound healing both by controlling microbial contamination and by attracting monocytes and/or macrophages.** The illustration shows one pathway out of many that are operative in wounds. Neutrophil- and macrophage-derived secretory leukocyte protease inhibitor (SLPI) blocks neutrophil elastase. SLPI alone, and in synergistic combination with macrophage- and epithelial-cell-derived proepithelin (PEPI), blocks cytokine-induced release of proteolytic enzymes and reactive oxygen intermediates (ROIs) by neutrophils. These actions diminish the neutrophil-dependent proteolytic conversion of PEPI to epithelins (EPIs), decreasing the ability of EPI to promote epithelial-cell production of CXC-chemokine ligand 8 (CXCL8; also known as IL-8), an important neutrophil chemoattractant. Intact PEPI promotes epithelial-cell proliferation, speeding closure of the wound. Black arrows indicate processes involved in tissue repair and regeneration. Grey arrows indicate processes involved in host defence.

Of necessity, however, there are different kinds of specificity. The infectious challenges faced by the immune system are so diverse and dire that they can only be met by a response in which collateral damage occurs as a matter of course[35,36]. No person who lives outside a bio-containment suite reaches maturity without their neutrophils having formed pus on multiple occasions, which is to say, destroying some of their tissue, and often saving their life in the process. In the familiar sense of the term, this is an ultimate example of nonspecificity. In fact, the specificity of ROIs is just as exquisite as that of antibody, but it is manifest at the atomic level, rather than the molecular level[2,37]. For example, ROIs lead to oxidation of sulphurs to sulphoxides and of sulphydryls to disulphides or to sulphenic, sulphinic or sulphonic acids. These are specific outcomes of the interaction of ROIs with specific atoms. That the atomic targets of ROIs are crucial to the function of a wide array of molecules probably accounts for the evolutionary selection of this means of host defence.

Indeed, there are two important innovations of innate immunity and they both involve distinctive forms of specificity. First, molecular specificity in the recognition of relatively invariant and widely distributed microbial macromolecules is imparted by Toll-like receptors, lectins

and lectin receptors, scavenger receptors, C-reactive protein, and complement and its receptors. Second, specific, covalent chemical reactions by ROIs and reactive nitrogen intermediates with a subset of atoms that are shared by, and crucial for, the function of diverse microbial molecules, characterizes one of innate immunity's important effector functions. The first innovation makes it difficult for a pathogen to evolve to a state that escapes detection, and the second makes it difficult for a pathogen to evolve to a state that escapes destruction[38]. The price of these twin innovations in specificity is tissue damage. But tissue damage is not wasted: it is used as information and incorporated into the central decision algorithm of the immune response. The immune system cannot function and the host cannot live without these two distinctive types of specificity.

Equally fundamental to an appreciation of neutrophil biology is the following principle: the same products that kill also signal. Homeostatic signalling depends on appropriately timed, brief exposures to low concentrations of mediators. When present at the wrong time, for too long and in high concentrations, the same mediators can kill. In fact, across biology, killing is frequently the outcome of excessive and inappropriate signalling, whether the sender–receiver pairs are host-versus-microorganism, microorganism-versus-host, host-cell-versus-host-cell, or microbial-cell-versus-microbial-cell[2]. ROIs, for example, are essential mediators of signalling by many cytokine and hormone receptors, such as those for insulin, platelet-derived growth factor, fibroblast growth factor, nerve growth factor, TNF and angiotensin[2]. The best-studied molecular mechanism for ROI-mediated signalling is the temporary inactivation of tyrosine phosphatases by reversible oxidation of their active-site cysteine sulphydryls[39]. Moreover, neutrophil proteases also regulate physiological processes[19]. In short, a neutrophil-rich inflammatory site is buzzing with information for every cell present; only at the epicentre is the command for all the cells to die.

## Fate decisions

Cytotoxic T cells and natural killer (NK) cells can potentially kill any nucleated host cell. Neutrophils match that capacity and top it with their additional abilities to destroy non-nucleated cells and connective tissue. In mammals, only neutrophils are licensed to liquefy any part of the body. Therefore, some of the main messages sent by neutrophils in inflammatory sites can be paraphrased as follows. To microorganisms and host cells that stand in the way: die. To themselves: if the site still seems infected, summon reinforcements until the crucial concentration of neutrophils that is required to clear a given tissue volume of bacteria is attained[40]; and if infection has been brought under control, wrap yourself in a phosphatidylserine flag and wait for macrophages to remove your apoptotic corpse for the safe disposal of your unexploded weapons[41].

Neutrophils' decisions over their own fate are co-ordinated through transcription factors. Hypoxia-inducible factor 1 (HIF1) regulates both the antibacterial activity of neutrophils[42] and their apoptosis[43]. Forkhead box O3A (FOXO3A) postpones neutrophil apoptosis by

Phosphatidylserine
A lipid whose exposure on the outer leaflet of the plasma membrane generally correlates with apoptosis of the cell and promotes its uptake by other cells.
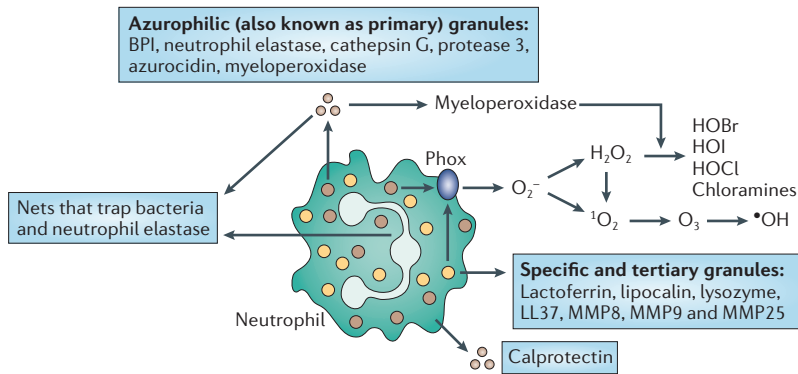
Figure 3 | **Neutrophils deliver multiple anti-microbial molecules.** Microbicidal products arise from most compartments of the neutrophil: azurophilic granules (also known as primary granules), specific granules (also known as secondary granules) and tertiary granules, plasma and phagosomal membranes, the nucleus and the cytosol. BPI, bactericidal permeability increasing protein; $H_2O_2$, hydrogen peroxide; HOBr, hypobromous acid; HOCl, hypochlorous acid; HOI, hypoiodous acid; MMP, matrix metalloproteinase; $^1O_2$, singlet oxygen; $O_2^-$, superoxide; $O_3$, ozone; $^\bullet$OH, hydroxyl radical; phox, phagocyte oxidase.

suppressing transcription of CD95 ligand (CD95L; also known as FASL)[44]. A protein called survivin also has an important role in delaying the death of neutrophils at inflammatory sites[45].

Neutrophils decide the fate of microorganisms in two general ways: by taking away and by giving. 'Taking away' means withholding essential factors that bacteria require, for example iron. The simplest approach is for neutrophils to secrete the iron-binding protein lactoferrin into the phagosome and the extracellular environment. However, lactoferrin faces tough competition from bacterial iron-binding molecules, a diverse array of compounds known as siderophores. The neutrophil in turn counters this 'counterpunch' by secreting lipocalin-2, a protein that embraces bacterial siderophores, preventing them from returning to the mother ship — the bacterium — with their cargo[46]. The dedication of neutrophils to delivering cytotoxic messages to bacteria (that is, to 'giving') is evident from two perspectives: their supply of redundant effector molecules and their use of multiple subcellular compartments as a source. Therefore, little of the neutrophil is wasted. As neutrophils migrate along a concentration gradient of a stimulus, they progressively discharge two distinct sets of granules, activate a killing cascade at the plasma membrane, extrude their nuclear proteins, and finally pour out their cytosol, all with anti-microbial effects (FIG. 3). Even the nuclei of neutrophils contribute to host defence by extruding their chromatin, which forms extracellular nets decorated with proteases from the azurophil granules[47]. Cytosol released by necrotic neutrophils delivers large amounts of calprotectin, a bacteriostatic heterodimer of MRP8 and MRP14 (REF. 48). Neutrophils also release potently bactericidal phospholipases[49].

*Payloads: prepacked or made on demand.* The first granules to be discharged are peroxidase-negative, and these include specific granules (also known as secondary granules) and tertiary granules (also known as

gelatinase granules), which contain overlapping sets of proteins[50]. In these two types of granules are found the gang of 'L's' — lactoferrin, lipocalin, lysozyme and LL37. LL37 is a chemotactic and antimicrobial peptide released from its precursor, the cathelicidin CAP18 (cationic antimicrobial protein 18), by protease 3. Also found in the peroxidase-negative granules are three 'M's' — MMP8, MMP9 and MMP25. By degrading laminin, collagen, proteoglycans and fibronectin, these MMPs are thought to have an important role in facilitating neutrophil recruitment and tissue breakdown[50].

As the concentration of secretagogues increases, the next granules to be emptied are peroxidase-positive and azurophilic (also known as primary). Their bulk contents are four α-defensins and myeloperoxidase (MPO), the iron-containing enzyme that colours pus green. The defensins are small, cyclic polypeptides that trade off their relatively low molar potency against their high abundance and broad spectrum as antimicrobial agents[51]. MPO converts the relatively innocuous $H_2O_2$ into much more powerful antiseptics: hypochlorous acid (which is the active ingredient in bleach), hypobromous acid and hypoiodous acid. Hypochlorous acid reacts with amines to give rise to longer-lived, bactericidal chloramines[34]. The same granules deliver a potent protein antibiotic that is active against Gram-negative bacteria, the lipopolysaccharide-binding bactericidal permeability increasing protein (BPI)[52], and four broad-spectrum antibiotic proteins termed serprocidins[53]. The serprocidins include three serine proteases — cathepsin G, neutrophil elastase and protease 3 — and a homologue that lacks proteolytic activity but retains equipotent antibacterial activity, which was independently identified as azurocidin[53] and CAP37 (REF. 54). The antimicrobial molar potency of serprocidins is comparable to that of formulary antibiotics, but unlike pharmaceuticals, the enzymatically active serprocidins also degrade most of the components of extracellular matrix. This bifunctionality highlights that tissue breakdown is part of the antimicrobial strategy of neutrophils.

Meanwhile, radical biology takes place at the plasma membrane and its offshoot, the membrane of the nascent phagosome. Studies of neutrophils have had a crucial role in establishing the fundamental but initially heretical principle that biochemical reactions can generate compounds with unpaired electrons (also known as radicals) as functional products[55,56]. Phagocyte oxidase (phox), which is the main ROI-producing enzyme expressed by neutrophils, continues to lead the way in illuminating the biochemistry of the broadly distributed family of ROI-producing, signal-capacitating enzymes that are termed NADPH oxidases (NOXs)[57].

As a biochemical cellular component, phox is so complex that its study has dominated the field of neutrophil biology for decades. For a description of the constituents of phox, their assembly and the diverse experimental formats that investigators have used to study the activation of phox, see Supplementary information S1 (box).

Deficiencies of phox were discovered because they led to death from infection[58]. The phenotype of phox deficiency, together with the bactericidal action of

**Siderophores**
Low-molecular-weight bacterial compounds that chelate iron and deliver it to the bacterium through specific receptors.

**Azurophilic**
Staining with azure (blue) components of the Romanowski-type stains used in standard evaluations of haematological specimens. In neutrophils, the earliest-formed set of granules, which contain many antibiotic proteins and proteases, are azurophilic.
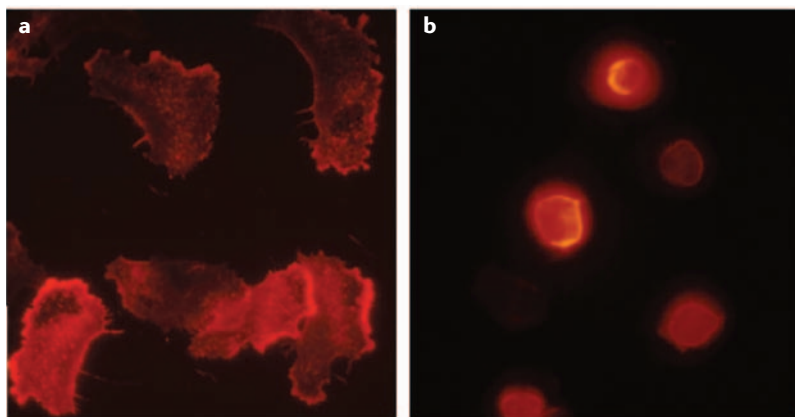
Figure 4 | **Neurophils spread and reorganize their cytoskeleton after activation by dual stimuli through integrins and cytokine receptors. a** | Polymerized actin is stained with rhodamine-phalloidin. Neutrophils spread when they are allowed to adhere to matrix-protein-coated glass and are also treated with tumour-necrosis factor (TNF) (100 ng ml$^{-1}$ for 30 min). **b** | Spreading is not seen when neutrophils are allowed to adhere to matrix protein-coated glass but are not given TNF, or when they are given TNF in suspension.

ROIs *in vitro*[34], convinced most investigators that the production of ROI by phox is an essential microbicidal mechanism. ROIs can also contribute to desorption of bactericidal neutrophil proteases from their proteoglycan matrix in the granules[59]. Indeed, an inability to reproduce the frequently reported bactericidal actions of $H_2O_2$ *in vitro* led one group to propose that the only role of phox is to mobilize neutrophil proteases[59]. However, neutrophils can release proteases under anaerobic conditions that preclude the action of phox, or when they are congenitally phox-deficient[60], and macrophages lack the proteases in question but can kill microbes in a phox-dependent manner[61]. In addition, MPO, which does not kill by itself, powerfully augments the killing action of ROIs, and deficiency of MPO, or addition of MPO inhibitors, reduces killing by neutrophils[34]. Furthermore, bacteria reacted to phagocytosing neutrophils by transcribing antioxidant defense genes[62], and *Staphylococcus aureus* deprived of an antioxidant protein became markedly more susceptible to phox-dependent killing by neutrophils[63]. In short, phox and the azurophil granule proteases are each important microbicidal mechanisms. They can function as mutually redundant, synergistic or stand-alone means of defence, depending on the experimental conditions.

**Move, stop and kill: a sense of time and place**

Given the beneficial and deleterious impacts of neutrophil-mediated destruction of microorganisms and host tissue, it is not surprising that there has been an enormous amount of research on control mechanisms that govern two of the most important engines of destruction by neutrophils: the respiratory burst and the degranulation response. The overview of signalling mechanisms offered below is not meant to be complete, but aims to illustrate the richness of the topic and to set the stage for the argument that the intricacy of neutrophil intracellular signalling affords opportunities for selective intervention.

**Cytochalasin**
A fungal metabolite that inhibits actin polymerization. Cytochalasin has been widely used *in vitro* to promote activation of neutrophils studied in suspension.

The cellular engineering problems solved by neutrophil signalling systems include the following. The cell must remain non-sticky as it hurtles through the arterial and arteriolar circulation; then it must squeeze through capillaries smaller in diameter than itself, without allowing collision, friction or distortion to activate it. A fraction of the population must adhere tightly enough to the normal endothelium of post-capillary venules to resist being washed away in the circulation, but loosely enough to roll while scouting for evidence of tissue damage and microbial infection. If such evidence is received, the cell must crawl to a boundary between endothelial cells, penetrate the junctions and the underlying basement membrane without damaging these structures, move up a chemotactic gradient and decide whether its original information remains valid. If the answer is negative, the cell must execute itself by apoptosis. If the answer is positive, the cell must attempt to engulf and destroy microbes. If it cannot locate microbes quickly, it must attempt to destroy them at a distance by releasing every weapon at its disposal.

One experimental system for studying neutrophil activation *in vitro* (others are noted in Supplementary information S1 (box)) consists of administration of soluble, physiological stimuli to neutrophils that are adherent to two-[28] or three-dimensional[40] surfaces coated with extracellular matrix proteins. The most striking feature of such systems is the magnitude of responses to soluble, physiological stimuli, such as TNF, used as single agents (that is, without cytochalasins and without being combined with other stimuli). In contrast to results with cells in suspension, the responses of adherent neutrophils last over an hour and lead to the accumulation of nearly as much antimicrobial product as can be elicited by bacteria or phorbol esters. For example, neutrophils that are adherent to biological surfaces respond to soluble, physiological stimuli by releasing about two orders of magnitude more $H_2O_2$ than neutrophils in suspension responding to the same stimuli[28].

Under such experimental conditions, two kinds of signals exert binary control over the respiratory burst and degranulation: signals resulting from integrin engagement with extracellular-matrix proteins on cellular or acellular surfaces, and signals transmitted through cell-surface receptors for inflammatory factors, such as formylated peptides, and cytokines and chemokines, including TNF, G-CSF, granulocyte/macrophage colony-stimulating factor (GM-CSF), CC-chemokine ligand 3 (CCL3; also known as MIP1α), CXCL8 and the complement component C5a[28,64,65]. The evidence that adhesion in general[28], and ligation of integrins in particular[66], controls the responses of cells to cytokines (FIG. 4) was one of the first examples of a principle that is now recognized as widespread in cell biology: the joint control of cell behaviour by integrin and non-integrin receptors[67]. The control of physiological, non-phagocytic, full-scale neutrophil activation by binary signals is an example of the binary control that is characteristic of inflammation and immunity.

*Responses of adherent neutrophils to TNF.* Responses of adherent neutrophils to one of the most important soluble pro-inflammatory agonists, TNF, do not depend on transcription or translation[28]. As such, these responses might have little to do with the canonical view of TNF signalling based on studies of other cell types. Because TNF is crucial to the pathogenesis of certain inflammatory disorders, such as rheumatoid arthritis, and given that neutrophils are the most abundant cell in the joint space in rheumatoid arthritis, it is exciting to realize that pathways of TNF signal transduction remain to be characterized that are likely to be of substantial pathophysiological relevance.

After ligation of TNF by TNF receptor 1 (TNFRI) and/or TNFRII, $\beta_2$-integrins are rapidly activated in wild-type and phox-deficient neutrophils in a manner that can be blocked by the flavoprotein inhibitor diphenylene iodonium (DPI) and mimicked using exogenous $H_2O_2$ (REF. 68). This was interpreted as implicating the production of very small amounts of $H_2O_2$ by an unspecified flavin-dependent enzyme. The result is transient inhibition of tyrosine phosphatase(s), whose cysteine-dependent active sites are susceptible to reversible oxidation by $H_2O_2$ (REF. 39). Such inhibition leads to increased phosphorylation and activation of at least three sets of tyrosine kinases: SRC-family kinases, haematopoietic cell kinase (HCK) and FGR[69]; spleen tyrosine kinase

(SYK)[70,71]; and protein tyrosine kinase 2 (PYK2)[70,72]. Also crucial at this early stage is the activation of phosphatidylinositol 3-kinase (PI3K)[70], which binds to TNFRs[73]. Inhibitor studies indicate that some of the foregoing events are mutually upstream of each other[70]. This apparent paradox can be resolved by postulating iterative interactions that constitute a feed-forward mechanism. Onset of the respiratory burst is closely related in time to tyrosine phosphorylation of the guanine nucleotide exchange factor VAV[74,75], which leads to the release of RAC2 from GDP dissociation inhibitor 1 (GDI1; also known as RHOGDI) and allows RAC2 to anchor the assembly of phox at the membrane.

Meanwhile, the actin-based cytoskeleton, which in suspended neutrophils is a spherical meshwork beneath the plasma membrane, dissolves. Actin polymers reassemble as stress fibres near the adherent surface of the neutrophil (FIG. 4) and interconnect the podosomes. The podosomes are sites of clustered integrins whose intracellular domains are associated with the most abundant of the accumulating tyrosine phosphoproteins, including paxillin[76,77]. This reorganization of the actin cytoskeleton represents an extended form of what happens locally at the base of the phagocytic cup during ingestion of particles, and serves to remove a physical barrier that, in suspended neutrophils, might make it difficult for granules to reach the plasma membrane.

For neutrophils to spread fully on matrix proteins, they need to shed CD43 (also known as leukosialin), a heavily sialylated molecule that seems to have an important role in keeping suspended neutrophils in their non-sticky state. Neutrophil elastase cleaves the ectodomain of CD43 near the plasma membrane. Therefore, a small degree of degranulation to release some neutrophil elastase is probably a precondition to a large degree of degranulation, which depends on cell spreading. (This is similar to the observation that a small degree of ROI generation from a source other than phox seems to be necessary before TNF can trigger phox)[68]. Albumin binds to CD43 and can protect CD43 from neutrophil elastase. In the bloodstream, high concentrations of albumin might help to prevent spontaneous neutrophil spreading and immobilization. In inflammatory sites, secreted neutrophil elastase is more abundant than in blood plasma, and neutrophil elastase inhibitors and albumin are much less abundant. Therefore, CD43 is cleaved and neutrophils can spread[78]. Like neutrophil elastase, cathepsin G can also regulate neutrophil activation[79], and it will be interesting to test if the mechanism also involves shedding of CD43.

A third process initiated in adherent neutrophils exposed to TNF is a nearly instantaneous elevation in the concentration of intracellular $Ca^{2+}$ (REFS 80–82). The functional impact has been understood to pertain chiefly to actin reorganization[83]. However, neutrophils were recently discovered to contain an enzyme termed soluble adenylyl cyclase (sAC)[82], which is characterized by its lack of a transmembrane domain, independence of G proteins and synergistic activation by $Ca^{2+}$ and bicarbonate. Activation of sAC by TNF led to activation of RAP1A and phox[82] (FIG. 5). Activation of sAC might
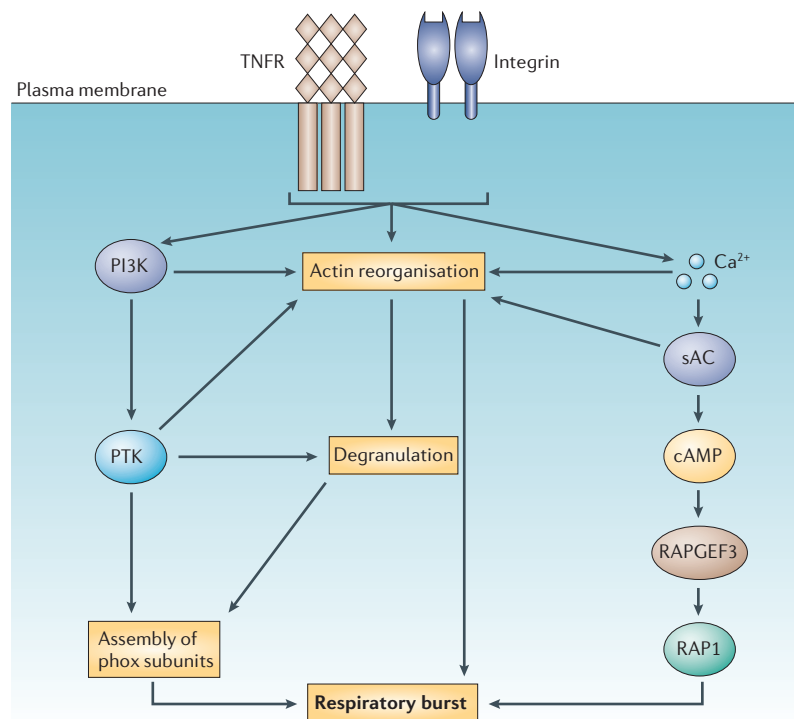


Figure 5 | **Signalling proceeds through parallel, as well as intersecting, pathways in adherent neutrophils responding to tumour-necrosis factor.** Tumour-necrosis factor (TNF)-triggers increased intracellular $Ca^{2+}$ concentrations and activation of phosphatidylinositol 3-kinase (PI3K), the protein tyrosine kinases (PTKs) — spleen tyrosine kinase, protein tyrosine kinase 2, haematopoietic-cell kinase and FGR — soluble adenylyl cyclase (sAC) and the small G protein RAP1. These effects are all unexpected, based on studies of TNF-induced signal transduction in other cells. The role of RAP guanine nucleotide exchange factor 3 (RAPGEF3; also known as EPAC1) is not yet determined. Other pathways are also activated but are not shown here. TNFR, TNF receptor.
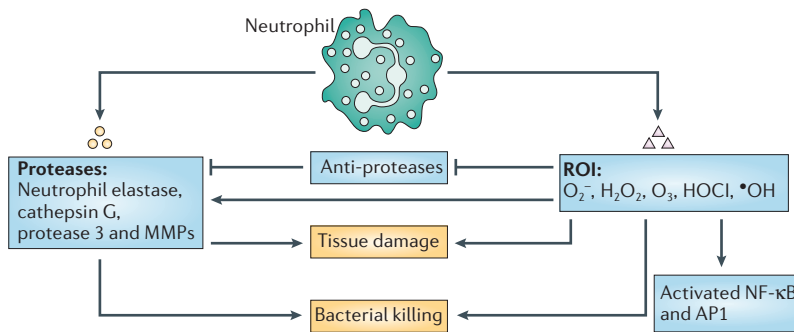
Figure 6 | **Interdependence of the two main classes of neutrophil tissue-damaging products creates opportunities for anti-inflammatory strategies.** Interruption of neutrophil accumulation or shortening of neutrophil survival in the tissues risks suppressing all neutrophil-dependent antimicrobial functions in extravascular sites. Inhibition of an individual protease might be relatively ineffective, whereas inhibition of the action of phagocyte oxidase (phox) risks overwhelming infection. However, inhibition of the activation of phox by inflammatory mediators, if at the same time preserving phox activation by bacteria, might lead to reduced oxidative damage to proteins that would normally increase their susceptibility to proteolysis. This could also lead to reduced oxidative activation of proteases in neutrophils and in other cells; reduced oxidative inactivation of anti-proteases, with resulting partial inhibition of many proteases at once; and reduced oxidative induction of pro-inflammatory transcription factors. AP1, activator protein 1; $H_2O_2$, hydrogen peroxide; HOCl, hypochlorous acid; MMP, matrix metalloproteinase; $O_2^-$, superoxide; $O_3$, ozone; •OH, hydroxyl radical; NF-κB; nuclear factor-κB.

**Tumour-lysis syndrome**
The immediate medical consequences of rapid destruction of large numbers of tumour cells, including the release of their intracellular potassium ions.

**Leukocyte-adhesion deficiency**
(LAD). A rare hereditary disease that is characterized by recurrent infection and delayed wound healing as a consequence of defective leukocyte adhesion. LAD type I is caused by mutations of $β_2$-integrin; LAD type II is caused by a defect in fucose metabolism that results in a failure to express sialyl-Lewis X, the ligand for endothelial-cell (E)-selectin and platelet (P)-selectin.

**Chronic granulomatous disease**
A genetic deficiency of phagocyte oxidase (phox), associated with recurrent, life-threatening bacterial and fungal infections.

therefore be another important consequence of the TNF-induced transient increase in the level of $Ca^{2+}$.

Until recently, it was thought that whatever activated a neutrophil strongly enough to make it undergo a respiratory burst also forced it to degranulate. However, two interventions were recently found to block the TNF-induced respiratory burst without blocking degranulation: introduction into the cell of a dominant-negative fragment of PYK2 (REF. 84) and inhibition of the TNF-induced $Ca^{2+}$ increase by a compound termed neucalcin[82]. These findings open up a new way thinking about anti-inflammatory therapy.

**Implications for therapy**
The tissue-damaging power of neutrophils might be appalling to the rheumatologist, but it should be appealing to the oncologist. Whereas inflammation can contribute to tumour formation and progression[85], neutrophils and/or eosinophils can have an important role in tumour destruction in mice[86–88] and, under certain circumstances, might do so in humans[89]. Once a malignant tumour has become metastatic, it is fair game to try to induce within it a neutrophil-rich inflammatory response that is robust enough to destroy the tumour directly[89] or to damage its vasculature[86]. In a clinical study, a complement-fixing, neutrophil-recruiting tumour-specific monoclonal antibody was administered to eight patients with end-stage metastatic melanoma[90]. This was followed by a low dose of systemic TNF, in an effort to activate intra-tumoural neutrophils. In one patient, haemorrhagic necrosis ensued in all the tumour deposits throughout the body, leading to the first instance of tumour-lysis syndrome reported in a patient

with a solid tumour. Although only one of the patients responded in this way, and he later died of a fungal infection[90], neutrophil recruitment to and triggering within tumours deserves further investigation. Neutrophils might be especially valuable as anti-tumour effector cells when one considers their enormous numerical preponderance over tumour-specific cytotoxic T cells.

The usual goal of neutrophil-targeted pharmacology is not to increase inflammation but is instead to suppress it, for example in rheumatoid arthritis, osteoarthritis or chronic obstructive pulmonary disease (FIG. 6). Unfortunately, conventional approaches to neutrophil-based anti-inflammatory therapy have their drawbacks. Antibodies or chemokine antagonists that are designed to block neutrophil emigration from the vasculature into the tissues will, at their most effective, mimic leukocyte-adhesion deficiency, a frequently fatal set of syndromes that impair host defence against infection[26,27]. Antibodies directed against adhesion molecules expressed by lymphocytes have shown benefit in some settings[91] but have been associated with devastating infections in others[92–94]. Based on the fact that life-threatening infections generally appear much sooner in neutropaenic patients than in lymphopaenic patients, one might anticipate a greater risk of infection from the administration of agents that interfere with the recruitment of neutrophils to infected sites than has already been seen after use of agents that block lymphocyte recruitment. Efforts to hasten neutrophil apoptosis raise the same concern.

An alternative approach has been to block individual neutrophil enzymes. This might afford benefit, but the approach is confounded by the redundancy of the inflammatory pathways involved. In particular, inhibition of neutrophil elastase[95] might be relatively ineffective when one has not also inhibited cathepsin G[96], protease 3 and MMPs. It has not been feasible to inhibit many proteases at once because it was assumed that this would require developing many pharmacologically acceptable compounds and testing them together, which is unrealistic. Nor would one want to inhibit phox, because this would be expected to mimic chronic granulomatous disease, a condition whose original name began with the term 'fatal'[58].

The recent discovery of the $Ca^{2+}$-triggered sAC pathway in neutrophils might provide a route around these obstacles. A screen of a chemical library was conducted to identify compounds that block the activation of phox by TNF or formylated peptides but not by phorbol esters[82]. Compounds fulfilling these criteria did not inhibit phox itself because triggering of phox by phorbol esters was preserved; the compounds only inhibited activation of phox, and even then, only its activation by soluble inflammatory factors. One such compound, termed neucalcin for its ability to block the TNF-induced elevation of intracellular $Ca^{2+}$, did not inhibit the activation of phox by phagocytosis of bacteria, did not inhibit degranulation, and as expected from the foregoing results, did not inhibit killing of bacteria. Moreover, in the presence of neucalcin, neutrophils migrated normally across TNF-activated endothelial monolayers[82].

Many questions remain unanswered, including what is the identity of the molecular target of neu-calcin and what actions such compounds might exert *in vivo*. However, even at this early stage, the foregoing observations raise the possibility that inhibition of the non-bacterial, inflammatory activation of phox might provide an indirect route to partial inhibition of pro-teases at sites of inflammation. Inflammatory proteoly-sis has multiple components that involve neutrophils: first, neutrophils release their own proteases, in part through the action of phox[59]; second, neutrophils oxidatively activate some of the proteases provided by other cells; and last, proteases are opposed by anti-proteases. Proteolysis ensues when there is a disruption in the protease–antiprotease balance. At least three of the main tissue anti-proteases (SLPI, $\alpha$1-antitrypsin and $\alpha$2-macroglobulin) are subject to oxidative inactivation by neutrophils. Therefore, inhibition of the inflammatory

activation of phox might tilt the protease–anti-protease balance against proteolysis, without impairing neutrophil recruitment and antimicrobial activity.

## Conclusions

It is time to set aside the view that neutrophils are destruc-tive cells that arrive too early, lash out too blindly and live too briefly to be of interest to immunologists. Neutrophils are life-saving decision-makers that coach DCs, mono-cytes and lymphocytes and help the organism decide whether to initiate and maintain an immune response. They are unique in their capacity both to destroy and help heal any tissue in the body. Understanding the circuits that confer and control such behaviour is as challeng-ing a problem as any other in cell biology. Meeting that challenge holds therapeutic promise in diverse settings of intense interest to immunologists, from metastatic tumours to ravaged joints.

1. Nathan, C. Inflammation: points of control. *Nature* **420**, 846–852 (2002).
2. Nathan, C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signalling. *J. Clin. Invest.* **111**, 769–778 (2003).
3. Henson, P. M. & Johnston, R. B. Jr. Tissue injury in inflammation. Oxidants, proteinases, and cationic proteins. *J. Clin. Invest.* **79**, 669–674 (1987).
4. Weiss, S. J. Tissue destruction by neutrophils. *N. Engl. J. Med.* **320**, 365–376 (1989).
5. Lekstrom-Himes, J. A. & Gallin, J. I. Immunodeficiency diseases caused by defects in phagocytes. *N. Engl. J. Med.* **343**, 1703–1714 (2000).
6. Klebanoff, S. J. & Clark, R. A. *The Neutrophil: Function and Clinical Disorders* (Elsevier/North-Holland, Amsterdam, 1978).
7. Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P. & Halbwachs-Mecarelli, L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab. Invest.* **80**, 617–653 (2000).
8. Matzinger, P. The danger model: a renewed sense of self. *Science* **296**, 301–305 (2002).
9. Medzhitov, R. & Janeway, C. A. Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* **296**, 298–300 (2002).
10. Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. & Gordon, J. I. Host-bacterial mutualism in the human intestine. *Science* **307**, 1915–1920 (2005).
11. Chertov, O. *et al*. Identification of human neutrophil-derived cathepsin G and azurocidin/CAP37 as chemoattractants for mononuclear cells and neutrophils. *J. Exp. Med.* **186**, 739–747 (1997).
12. Bennouna, S., Bliss, S. K., Curiel, T. J. & Denkers, E. Y. Cross-talk in the innate immune system: neutrophils instruct recruitment and activation of dendritic cells during microbial infection. *J. Immunol.* **171**, 6052–6058 (2003).
13. Tsuda, Y. *et al*. Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*. *Immunity* **21**, 215–226 (2004).
14. Wittamer, V. *et al*. Neutrophil-mediated maturation of chemerin: a link between innate and adaptive immunity. *J. Immunol.* **175**, 487–493 (2005).
15. van Gisbergen, K. P., Sanchez-Hernandez, M., Geijtenbeek, T. B. & van Kooyk, Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J. Exp. Med.* **201**, 1281–1292 (2005).
16. Scapini, P. *et al*. Proinflammatory mediators elicit secretion of the intracellular B-lymphocyte stimulator pool (BLyS) that is stored in activated neutrophils: implications for inflammatory diseases. *Blood* **105**, 830–837 (2005).
17. Ethuin, F. *et al*. Human neutrophils produce interferon-$\gamma$ upon stimulation by interleukin-12. *Lab. Invest.* **84**, 1363–1371 (2004).
18. Schmielau, J. & Finn, O. J. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Cancer Res.* **61**, 4756–4760 (2001).
19. Heissig, B. *et al*. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* **109**, 625–637 (2002).
20. Semerad, C. L., Liu, F., Gregory, A. D., Stumpf, K. & Link, D. C. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. *Immunity* **17**, 413–423 (2002).
21. Stark, M. A. *et al*. Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* **22**, 285–294 (2005).
22. Diefenbach, A. *et al*. Type 1 interferon (IFN$\alpha$/$\beta$) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. *Immunity* **8**, 77–87 (1998).
23. Dovi, J. V., Szpaderska, A. M. & DiPietro, L. A. Neutrophil function in the healing wound: adding insult to injury? *Thromb. Haemost.* **92**, 275–280 (2004).
24. Martin, P. *et al*. Wound healing in the PU. 1 null mouse — tissue repair is not dependent on inflammatory cells. *Curr. Biol.* **13**, 1122–1128 (2003).
25. Singer, A. J. & Clark, R. A. Cutaneous wound healing. *N. Engl. J. Med.* **341**, 738–746 (1999).
26. Roos, D. & Law, S. K. Hematologically important mutations: leukocyte adhesion deficiency. *Blood Cells Mol. Dis.* **27**, 1000–1004 (2001).
27. Alon, R. & Etzioni, A. LAD-III, a novel group of leukocyte integrin activation deficiencies. *Trends Immunol.* **24**, 561–566 (2003).
28. Nathan, C. F. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J. Clin. Invest.* **80**, 1550–1560 (1987).
29. Serhan, C. N. Novel $\omega$-3-derived local mediators in anti-inflammation and resolution. *Pharmacol. Ther.* **105**, 7–21 (2005).
30. Jin, F. Y., Nathan, C., Radzioch, D. & Ding, A. Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. *Cell* **88**, 417–426 (1997).
31. Zhu, J. *et al*. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell* **111**, 867–878 (2002).
32. Grobmyer, S. R. *et al*. Secretory leukocyte protease inhibitor, an inhibitor of neutrophil activation, is elevated in serum in human sepsis and experimental endotoxemia. *Crit. Care Med.* **28**, 1276–1282 (2000).
33. He, Z., Ong, C. H., Halper, J. & Bateman, A. Progranulin is a mediator of the wound response. *Nature Med.* **9**, 225–229 (2003).
34. Klebanoff, S. J. Myeloperoxidase: friend and foe. *J. Leukoc. Biol.* **77**, 598–625 (2005).
35. Hedrick, S. M. The acquired immune system: a vantage from beneath. *Immunity* **21**, 607–615 (2004).
36. Casanova, J. L. & Abel, L. Inborn errors of immunity to infection: the rule rather than the exception. *J. Exp. Med.* **202**, 197–201 (2005).
37. Nathan, C. The moving frontier in nitric oxide-dependent signalling. *Sci. STKE* 2 Nov 2004 (doi:10.1126/stke. 2572004pe2572052).
38. Nathan, C. Nitric oxide as a secretory product of mammalian cells. *FASEB. J.* **6**, 3051–3064 (1992).
39. Tonks, N. K. Redox redux: revisiting PTPs and the control of cell signalling. *Cell* **121**, 667–670 (2005).
40. Li, Y., Karlin, A., Loike, J. D. & Silverstein, S. C. A critical concentration of neutrophils is required for effective bacterial killing in suspension. *Proc. Natl Acad. Sci. USA* **99**, 8289–8294 (2002).
41. Savill, J. & Haslett, C. Granulocyte clearance by apoptosis in the resolution of inflammation. *Semin. Cell Biol.* **6**, 385–393 (1995).
42. Peyssonnaux, C. *et al*. HIF-1$\alpha$ expression regulates the bactericidal capacity of phagocytes. *J. Clin. Invest.* **115**, 1806–1815 (2005).
43. Walmsley, S. R. *et al*. Hypoxia-induced neutrophil survival is mediated by HIF-1$\alpha$-dependent NF-$\kappa$B activity. *J. Exp. Med.* **201**, 105–115 (2005).
44. Jonsson, H., Allen, P. & Peng, S. L. Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. *Nature Med.* **11**, 666–671 (2005).
45. Altznauer, F. *et al*. Inflammation-associated cell cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils. *J. Exp. Med.* **199**, 1343–1354 (2004).
46. Flo, T. H. *et al*. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* **432**, 917–921 (2004).
47. Brinkmann, V. *et al*. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
48. Striz, I. & Trebichavsky, I. Calprotectin- a pleiotropic molecule in acute and chronic inflammation. *Physiol. Res.* **53**, 245–253 (2004).
49. Wright, G. W., Ooi, C. E., Weiss, J. & Elsbach, P. Purification of a cellular (granulocyte) and an extracellular (serum) phospholipase A2 that participate in the destruction of *Escherichia coli* in a rabbit inflammatory exudate. *J. Biol. Chem.* **265**, 6675–6681 (1990).
50. Faurschou, M. & Borregaard, N. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect.* **5**, 1317–1327 (2003).
51. Selsted, M. E. & Ouellette, A. J. Mammalian defensins in the antimicrobial immune response. *Nature Immunol.* **6**, 551–557 (2005).
52. Weiss, J., Elsbach, P., Olsson, I. & Odeberg, H. Purification and characterization of a potent bactericidal and membrane active protein from the granules of human polymorphonuclear leukocytes. *J. Biol. Chem.* **253**, 2664–2672 (1978).

53. Campanelli, D., Detmers, P. A., Nathan, C. F. & Gabay, J. E. Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties. *J. Clin. Invest.* **85**, 904–915 (1990).

54. Pereira, H. A., Shafer, W. M., Pohl, J., Martin, L. E. & Spitznagel, J. K. CAP37, a human neutrophil-derived chemotactic factor with monocyte specific activity. *J. Clin. Invest.* **85**, 1468–1476 (1990).

55. McCord, J. M. & Fridovich, I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**, 6049–6055 (1969).

56. Babior, B. M., Kipnes, R. S. & Curnutte, J. T. Biological defense mechanisms. The production of superoxide, a potential bactericidal agent. *J. Clin. Invest.* **52**, 741–744 (1973).

57. Lambeth, J. D. NOX enzymes and the biology of reactive oxygen. *Nature Rev. Immunol.* **4**, 181–189 (2004).

58. Good, R. A. *et al.* Fatal (chronic) granulomatous disease of childhood: a hereditary defect of leukocyte function. *Semin. Hematol.* **5**, 215–254 (1968).

59. Reeves, E. P. *et al.* Killing activity of neutrophils is mediated through activation of proteases by $K^+$ flux. *Nature* **416**, 291–297 (2002).

60. Roos, D., van Bruggen, R. & Meischl, C. Oxidative killing of microbes by neutrophils. *Microbes Infect.* **5**, 1307–1315 (2003).

61. Shiloh, M. U. *et al.* Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. *Immunity* **10**, 29–38 (1999).

62. Staudinger, B. J., Oberdoerster, M. A., Lewis, P. J. & Rosen, H. mRNA expression profiles for *Escherichia coli* ingested by normal and phagocyte oxidase-deficient human neutrophils. *J. Clin. Invest.* **110**, 1151–1163 (2002).

63. Liu, G. Y. *et al. Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J. Exp. Med.* **202**, 209–215 (2005).

64. Nathan, C. F. Respiratory burst in adherent human neutrophils: triggering by colony-stimulating factors CSF–GM and CSF–G. *Blood* **73**, 301–306 (1989).

65. Wolpe, S. D. *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J. Exp. Med.* **167**, 570–581 (1988).

66. Nathan, C. *et al.* Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. *J. Cell. Biol.* **109**, 1341–1349 (1989).

67. Nathan, C. & Sporn, M. Cytokines in context. *J. Cell Biol.* **113**, 981–986 (1991).

68. Blouin, E., Halbwachs-Mecarelli, L. & Rieu, P. Redox regulation of $\beta_2$-integrin CD11b/CD18 activation. *Eur. J. Immunol.* **29**, 3419–3431 (1999).

69. Mocsai, A., Ligeti, E., Lowell, C. A. & Berton, G. Adhesion-dependent degranulation of neutrophils requires the Src family kinases Fgr and Hck. *J. Immunol.* **162**, 1120–1126 (1999).

70. Fuortes, M., Melchior, M., Han, H., Lyon, G. J. & Nathan, C. Role of the tyrosine kinase pyk2 in the integrin-dependent activation of human neutrophils by TNF. *J. Clin. Invest.* **104**, 327–335 (1999).

71. Mocsai, A., Zhou, M., Meng, F., Tybulewicz, V. L. & Lowell, C. A. Syk is required for integrin signalling in neutrophils. *Immunity* **16**, 547–558 (2002).

72. Yan, S. R., Huang, M. & Berton, G. Signalling by adhesion in human neutrophils: activation of the p72syk tyrosine kinase and formation of protein complexes containing p72syk and Src family kinases in neutrophils spreading over fibrinogen. *J. Immunol.* **158**, 1902–1910 (1997).

73. Korchak, H. M. & Kilpatrick, L. E. TNFα elicits association of PI3-kinase with the p60TNFR and activation of PI3-kinase in adherent neutrophils. *Biochem. Biophys. Res. Commun.* **281**, 651–656 (2001).

74. Zhao, T., Benard, V., Bohl, B. P. & Bokoch, G. M. The molecular basis for adhesion-mediated suppression of reactive oxygen species generation by human neutrophils. *J. Clin. Invest.* **112**, 1732–1740 (2003).

75. Gakidis, M. A. *et al.* Vav GEFs are required for $\beta_2$ integrin-dependent functions of neutrophils. *J. Cell Biol.* **166**, 273–282 (2004).

76. Fuortes, M., Jin, W. W. & Nathan, C. Adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumour necrosis factor. *J. Cell Biol.* **120**, 777–784 (1993).

77. Fuortes, M., Jin, W. W. & Nathan, C. $\beta_2$ integrin-dependent tyrosine phosphorylation of paxillin in human neutrophils treated with tumour necrosis factor. *J. Cell Biol.* **127**, 1477–1483 (1994).

78. Nathan, C., Xie, Q. W., Halbwachs-Mecarelli, L. & Jin, W. W. Albumin inhibits neutrophil spreading and hydrogen peroxide release by blocking the shedding of CD43 (sialophorin, leukosialin). *J. Cell Biol.* **122**, 243–256 (1993).

79. Raptis, S. Z., Shapiro, S. D., Simmons, P. M., Cheng, A. M. & Pham, C. T. Serine protease cathepsin G regulates adhesion-dependent neutrophil effector functions by modulating integrin clustering. *Immunity* **22**, 679–691 (2005).

80. Richter, J., Ng-Sikorski, J., Olsson, I. & Andersson, T. Tumour necrosis factor-induced degranulation in adherent human neutrophils is dependent on CD11b/CD18-integrin-triggered oscillations of cytosolic free $Ca^{2+}$. *Proc. Natl Acad. Sci. USA* **87**, 9472–9476 (1990).

81. Schumann, M. A., Gardner, P. & Raffin, T. A. Recombinant human tumour necrosis factor α induces calcium oscillation and calcium-activated chloride current in human neutrophils. The role of calcium/calmodulin-dependent protein kinase. *J. Biol. Chem.* **268**, 2134–2140 (1993).

82. Han, H. *et al.* Calcium-sensing soluble adenylyl cyclase mediates TNF signal transduction in human neutrophils. *J. Exp. Med.* **202**, 353–361 (2005).

83. Bengtsson, T. *et al.* Actin dynamics in human neutrophils during adhesion and phagocytosis is controlled by changes in intracellular free calcium. *Eur. J. Cell. Biol.* **62**, 49–58 (1993).

84. Han, H., Fuortes, M. & Nathan, C. Critical role of the carboxyl terminus of proline-rich tyrosine kinase (Pyk2) in the activation of human neutrophils by tumour necrosis factor: separation of signals for the respiratory burst and degranulation. *J. Exp. Med.* **197**, 63–75 (2003).

85. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).

86. Rothstein, J. L. & Schreiber, H. Synergy between tumour necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. *Proc. Natl Acad. Sci. USA* **85**, 607–611 (1988).

87. Tepper, R. I., Pattengale, P. K. & Leder, P. Murine interleukin-4 displays potent anti-tumour activity *in vivo*. *Cell* **57**, 503–512 (1989).

88. Stoppacciaro, A. *et al.* Regression of an established tumour genetically modified to release granulocyte colony-stimulating factor requires granulocyte–T-cell cooperation and T cell-produced interferon γ. *J. Exp. Med.* **178**, 151–161 (1993).

89. Soiffer, R. *et al.* Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumour immunity in patients with metastatic melanoma. *Proc. Natl Acad. Sci. USA* **95**, 13141–13146 (1998).

90. Minasian, L. M. *et al.* Hemorrhagic tumour necrosis during a pilot trial of tumour necrosis factor-α and anti-GD3 ganglioside monoclonal antibody in patients with metastatic melanoma. *Blood* **83**, 56–64 (1994).

91. Feagan, B. G. *et al.* Treatment of ulcerative colitis with a humanized antibody to the $\alpha_4\beta_7$ integrin. *N. Engl. J. Med.* **352**, 2499–2507 (2005).

92. Berger, J. R. & Koralnik, I. J. Progressive multifocal leukoencephalopathy and natalizumab — unforeseen consequences. *N. Engl. J. Med.* **353**, 414–416 (2005).

93. Langer-Gould, A., Atlas, S. W., Green, A. J., Bollen, A. W. & Pelletier, D. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. *N. Engl. J. Med.* **353**, 375–381 (2005).

94. Kleinschmidt-DeMasters, B. K. & Tyler, K. L. Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon β-1a for multiple sclerosis. *N. Engl. J. Med.* **353**, 369–374 (2005).

95. Luisetti, M. *et al.* MR889, a neutrophil elastase inhibitor, in patients with chronic obstructive pulmonary disease: a double-blind, randomized, placebo-controlled clinical trial. *Eur. Respir. J.* **9**, 1482–1486 (1996).

96. de Garavilla, L. *et al.* A novel, potent dual inhibitor of the leukocyte proteases cathepsin G and chymase: molecular mechanisms and anti-inflammatory activity *in vivo*. *J. Biol. Chem.* **280**, 18001–18007 (2005).

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