Neutrophils in the activation and regulation of innate and adaptive immunity

Alberto Mantovani[*‡||], *Marco A. Cassatella*[§||], *Claudio Costantini*[§||] and *Sébastien Jaillon*[*||]

Abstract | Neutrophils have long been viewed as the final effector cells of an acute inflammatory response, with a primary role in the clearance of extracellular pathogens. However, more recent evidence has extended the functions of these cells. The newly discovered repertoire of effector molecules in the neutrophil armamentarium includes a broad array of cytokines, extracellular traps and effector molecules of the humoral arm of the innate immune system. In addition, neutrophils are involved in the activation, regulation and effector functions of innate and adaptive immune cells. Accordingly, neutrophils have a crucial role in the pathogenesis of a broad range of diseases, including infections caused by intracellular pathogens, autoimmunity, chronic inflammation and cancer.

Neutrophils have long been viewed as short-lived effector cells of the innate immune system, possessing limited capacity for biosynthetic activity and with a primary role in resistance against extracellular pathogens and in acute inflammation. These cells are classically characterized by their ability to act as phagocytic cells, to release lytic enzymes from their granules and to produce reactive oxygen intermediates (ROI) with antimicrobial potential[1,2]. In the 1990s, however, this limited view was challenged by the demonstration that neutrophils survive much longer than first suggested[3] and can be induced to express genes encoding key inflammatory mediators, including complement components, Fc receptors, chemokines and cytokines[4]. In addition, recent evidence suggests that neutrophils can also produce anti-inflammatory molecules and factors that promote the resolution of inflammation. The use of microarray-based approaches has added a new dimension to our knowledge of neutrophil biosynthetic potential that more broadly affects innate immune processes[5]. Furthermore, the development of new tools for the isolation of highly purified (>99.7%) neutrophils[6] — as well as 'on-chip' processing of mRNA and protein isolation for genomics and proteomics[7] — have been instrumental in extending our understanding to discriminate temporal transcriptional events of neutrophils within clinical settings. More recent evidence highlights that *de novo* induction of microRNAs might be part of the crucial regulatory circuits that control neutrophil gene expression[8]. Recent data have also suggested that neutrophils can be polarized towards distinct phenotypes in response to environmental signals[9]. Neutrophils have thus emerged as key components of the effector and regulatory circuits of the innate and adaptive immune systems[2], and this has led to a renewed interest in their biology.

It has also become apparent that neutrophils are important mediators of the T helper 17 (T_H17)-controlled pathway of resistance to pathogens, as well as of immunopathology[10]. Accordingly, interleukin-17 (IL-17) and related cytokines secreted by T_H17 cells induce mediators that promote granulopoiesis and consequent neutrophil proliferation and accumulation[10]. Moreover, T_H17 cell-derived cytokines (such as IL-17, CXC-chemokine ligand 8 (CXCL8; also known as IL-8), interferon-γ (IFNγ), tumour necrosis factor (TNF) and granulocyte–macrophage colony-stimulating factor (GM-CSF)) favour recruitment, activation and prolonged survival of neutrophils at inflammatory sites[6]. Therefore, T_H17 cells orchestrate and amplify neutrophil function in resistance against extracellular bacteria[10].

Here, we focus on how neutrophils are integrated in the activation, regulation and effector mechanisms of the innate and adaptive immune systems, and on their function as major determinants of diverse pathologies, beyond their long-known role in acute inflammatory responses and resistance to extracellular pathogens.

*Istituto Clinico Humanitas IRCCS, via Manzoni 56, 20089 Rozzano, Italy.
‡Department of Translational Medicine, Università degli Studi di Milano, via Manzoni 56, 20089 Rozzano, Italy.
§Department of Pathology and Diagnostics, University of Verona, 37134 Verona, Italy.
||All authors contributed equally to this work.
Correspondence to A.M. and M.A.C.
e-mails: alberto.mantovani@humanitasresearch.it; marco.cassatella@univr.it
doi:10.1038/nri3024

Role in innate immunity

Neutrophils are essential for innate immunity and resistance to pathogens, as illustrated by the debilitating and life-threatening conditions associated with congenital or acquired abnormalities in neutrophil life cycle or function. A comprehensive overview of the functions of neutrophils in the innate immune system is beyond the scope of this section and the reader is referred to previous reviews for a background[1,2,11]. Therefore, we focus here on new vistas that highlight the versatility and sophistication of these cells.

Activation of neutrophils. It has long been known that *N*-formyl peptides induce neutrophil chemotaxis and functional activation via the seven-transmembrane G protein-coupled receptor FPR1. The production of formylated proteins is restricted to bacteria and mitochondria[12], and therefore FPR1 fulfils the criteria of a pattern recognition receptor (PRR) recognizing microbial moieties and tissue damage. Indeed, mitochondria-derived formylated peptides, when injected, induce neutrophil recruitment to, and inflammation in, different tissues[12] (such as the lungs) and, in conjunction with intravascular CXCL2, guide neutrophils to sites of sterile inflammation[13].

Neutrophils express a vast repertoire of PRRs (in addition to FPR1), including all members of the Toll-like receptor (TLR) family with the exception of TLR3 (REF. 14); the C-type lectin receptors dectin 1 (also known as CLEC7A)[15] and CLEC2 (also known as CLEC1B)[16]; and cytoplasmic sensors of ribonucleic acids (RIG-I and MDA5)[17]. Of note, CLEC2 is not expressed by mouse neutrophils, a finding that cautions against extrapolation across species[16]. In addition, neutrophils express nucleotide-binding oligomerization domain protein 1 (NOD1)[18], although the expression and function of the NOD-like receptors (NLRs) that are components of the inflammasome have not been carefully studied. The sensing of pathogens and tissue damage through these PRRs, together with lymphoid cell-derived signals (see below), activates the effector functions of neutrophils[1,2]. These include the production of ROI[1,2,11], lytic enzymes and antimicrobial peptides, as well as more recently described functions (see below).

The expanding repertoire of neutrophil-derived cytokines. BOX 1 summarizes the cytokine repertoire that neutrophils can express. Here we focus on recent findings and perspectives and refer the reader to previous reviews for a background[4,19].

Cytokine production by neutrophils is controlled by regulatory mechanisms that act at different levels, including mRNA transcription[4], stability or translation (for example, through microRNA-mediated targeting, as in the case of mouse IFNγ[20]), as well as protein secretion. With regard to protein secretion, significant fractions of B cell-activating factor (BAFF; also known as BLYS), TNF-related apoptosis-inducing ligand (TRAIL), CXCL8, CC-chemokine ligand 20 (CCL20) and IL-1 receptor antagonist (IL-1RA) are not directly released following synthesis but are stored in intracellular pools. These cytokines are rapidly secreted only when neutrophils are acutely stimulated by secretagogue-like molecules (reviewed in REF. 19).

Granules
Neutrophils store an assortment of molecules in three types of granule (primary, secondary and tertiary). Primary granules are characterized by the accumulation of antimicrobial proteins and proteases, whereas secondary granules and tertiary granules are characterized by a high content of lactoferrin and gelatinase, respectively. In addition, secretory vesicles contain a reservoir of membrane-associated proteins.

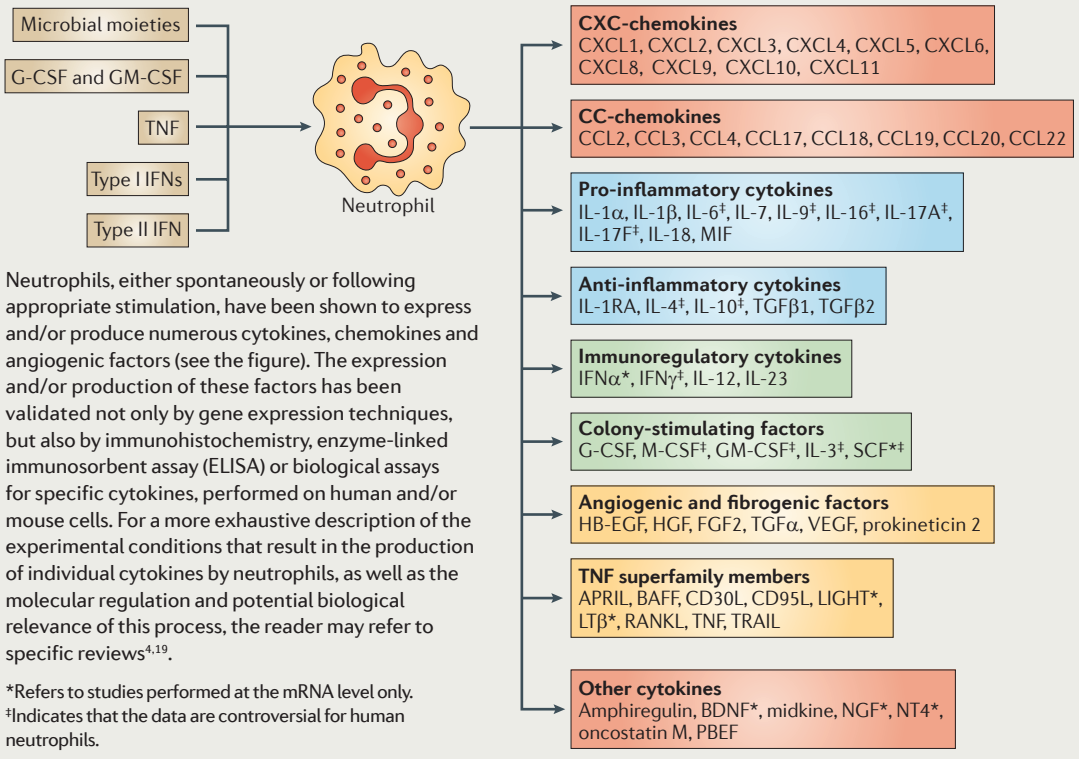
Reactive oxygen intermediates
(ROI). In the context of this Review, this term refers to various reactive oxygen species, including superoxide anions produced by phagocytes via the activation of the NADPH oxidase enzymatic system, and other compounds derived from superoxide anion metabolism, such as hydrogen peroxide and hydroxyl radicals. ROI are crucial for the antimicrobial activity of neutrophils.

MicroRNAs
Single-stranded RNA molecules of approximately 21–23 nucleotides in length that are thought to regulate the expression of other genes.

***N*-formyl peptides**
Bacteria initiate protein synthesis with *N*-formylmethionine, a modified form of the amino acid methionine. The only eukaryotic proteins that contain *N*-formylmethionine, and are therefore *N*-formylated, are those encoded by mitochondria.

Pattern recognition receptor
A germline-encoded receptor that recognizes unique and essential structures that are present in microorganisms, but absent from the host. In vertebrates, signalling through these receptors leads to the production of pro-inflammatory cytokines and chemokines and to the expression of co-stimulatory molecules by antigen-presenting cells.

Box 1 | **Neutrophil-derived cytokines**



Neutrophils, either spontaneously or following appropriate stimulation, have been shown to express and/or produce numerous cytokines, chemokines and angiogenic factors (see the figure). The expression and/or production of these factors has been validated not only by gene expression techniques, but also by immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) or biological assays for specific cytokines, performed on human and/or mouse cells. For a more exhaustive description of the experimental conditions that result in the production of individual cytokines by neutrophils, as well as the molecular regulation and potential biological relevance of this process, the reader may refer to specific reviews[4,19].

*Refers to studies performed at the mRNA level only.
‡Indicates that the data are controversial for human neutrophils.

Recent studies have shown that human neutrophils are a major source of cytokines that are crucial for the survival, maturation and differentiation of B cells. These molecules include BAFF[19] and a proliferation-inducing ligand (APRIL; the most closely related molecule to BAFF)[21]. Remarkably, neutrophils in the inflamed synovial fluid of patients with rheumatoid arthritis[22], in inflamed mucosa-associated lymphoid tissue (MALT)[21] or in various B cell malignancies and solid tumours[23] express and secrete high levels of APRIL. APRIL promotes the survival and proliferation of normal and malignant B cells. Therefore, neutrophil-derived APRIL could sustain autoantibody production, as in rheumatoid arthritis, or malignant growth and progression, as in B cell lymphoma[23].

Neutrophil-derived cytokines are also involved in bone resorption. Human and murine neutrophils have been shown to upregulate the expression of functionally active, membrane-bound RANKL (the ligand for receptor activator of NF-κB (RANK)) following activation *in vitro* and *in vivo*[24]. In addition, neutrophils from the synovial fluid of patients with exacerbated rheumatoid arthritis have been found to express high levels of RANKL[24] and, following interaction with osteoclasts, these neutrophils were shown to activate osteoclastogenesis in a RANKL-dependent manner[24]. Given the presence of large numbers of neutrophils at sites of inflammatory bone loss, as well as the expression by these neutrophils of other regulatory factors involved in bone remodelling, such as RANK and osteoprotegerin (also known as TNFRSF11B)[24,25], these cells might have the potential to orchestrate bone resorption in rheumatoid arthritis.

Differences in the capacity to express cytokines have been reported to occur between human and mouse neutrophils. In particular, whether human neutrophils can express IL-6, IL-17A, IL-17F and IFNγ, like their mouse counterparts, is the subject of conflicting reports[4,6,26,27]. The possible role of low numbers of contaminating monocytes in isolated neutrophil populations cautions against interpretation of some of these findings[4]. In addition, there is not a consensus in the literature as to whether human neutrophils produce IL-10. Although previous studies reported negative findings[28], lipopolysaccharide (LPS) and serum amyloid A have been reported to induce high levels of IL-10 by human neutrophils[29]. However, these findings could not be reproduced in other laboratories (REF. 30 and M.A.C. and A.M., unpublished observations), again highlighting the need for stringent purification procedures to control for monocyte contamination[4]. Interestingly, several studies have shown that mouse neutrophils produce IL-10 during pneumonia[31], methicillin-resistant *Staphylococcus aureus* infection[32] and disseminated *Candida albicans* infection[15]. The mechanisms underlying the differences between mouse and human neutrophils remain to be defined.

Thus, in response to different signals neutrophils express a vast and diverse repertoire of cytokines that are crucial to the role of neutrophils in innate and adaptive immune responses and to their role in defence and pathology.

Neutrophil extracellular traps. In addition to producing classical effector molecules, such as ROI[1,2,11] and cytokines (BOX 1), neutrophils can extrude extracellular fibrillary networks termed neutrophil extracellular traps (NETs)[33] (BOX 2). These networks are composed mainly of DNA, but also contain proteins from neutrophil granules. NETs act as a mesh that traps microorganisms and, in turn, facilitates their interaction with neutrophil-derived effector molecules. Importantly, NETs also contain some neutrophil-derived pattern recognition molecules (PRMs) with antibody-like properties.

Neutrophils as a source of pattern recognition molecules. The innate immune system includes a cellular and a humoral arm[34]. The humoral arm includes PRMs, such as collectins, ficolins and pentraxins[34]. These soluble PRMs act as antibody-like molecules that interact with conserved microbial structures (such as mannose-containing glycosidic moieties in the case of mannose-binding lectin) or with conserved proteins (such as enterobacterial outer membrane protein A, which is bound by pentraxin 3 (PTX3))[34]. Neutrophils are a ready-made reservoir of some PRMs, such as PTX3, peptidoglycan recognition protein short (PGRP-S; also known as PGRP1) and M-ficolin (also known as ficolin 1).

Mature neutrophils serve as a major reservoir of preformed PTX3, which can be rapidly released and partly localizes in NETs[35]. Neutrophil-associated PTX3 is essential for resistance against the fungal pathogen *Aspergillus fumigatus*[35]. PTX3 interacts with Fcγ receptors (FcγRs)[36] and has opsonic activity and activates the classical pathway of the complement cascade[35,37]. Leukocyte-derived PTX3 also has a regulatory function during neutrophil recruitment and inflammation by interacting with P-selectin, thereby inhibiting neutrophil extravasation[38].

PGRP-S and M-ficolin are stored in secondary and tertiary granules, are released by activated neutrophils and, at least in the case of PGRP-S, are localized in NETs[39–41]. PGRP-S binds to peptidoglycans, recognizes selected microorganisms and exerts bacteriostatic and bactericidal activities[39]. Ficolins, including M-ficolin, have the ability to interact with microbial glycosidic moieties, activate the lectin pathway of the complement cascade and exert opsonic activity[34]. However, in contrast to PTX3 and PGRP-S, a specific role for neutrophil-associated M-ficolin has not been defined.

Taken together, these observations indicate that, following activation, neutrophils contribute to the humoral arm of the innate immune response, by releasing soluble PRMs that enhance phagocytosis, activate complement (both the classical and lectin pathways) and regulate inflammation. In more general terms, the participation of these 'unsung heroes'[42] in mechanisms of innate resistance goes beyond the production of microorganism- and tissue-damaging molecules, to include a diverse, highly regulated, customized production of cytokines, together with the release of NETs and antibody-like PRMs.

Inflammasome
A molecular complex of several proteins — including members of the NOD-like receptor family — that upon assembly cleaves pro-interleukin-1β (pro-IL-1β) and pro-IL-18, thereby producing the active cytokines.

Osteoclastogenesis
A process whereby haematopoietic stem cells differentiate into multinucleated osteoclasts with bone-resorbing activity.

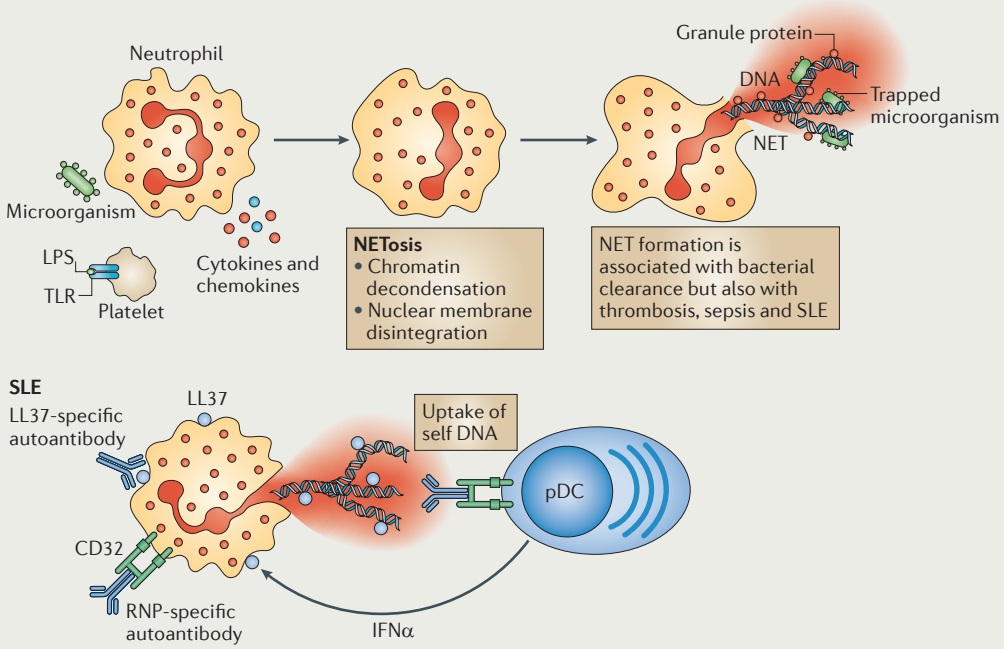
Complement cascade
There are three independent pathways that can lead to the activation of the complement cascade. The classical pathway is activated via C1q binding to immune complexes; the alternative pathway is triggered by direct C3 activation; and the lectin pathway is initiated by the interaction of mannose-binding lectin with the surface of microorganisms.

NETosis
A form of cell death that differs from classical apoptosis and necrosis, and that occurs during the formation of neutrophil extracellular traps.

Box 2 | **Neutrophil extracellular traps**

Neutrophil extracellular traps (NETs) are composed of nuclear components (such as DNA and histones)[33] and are decorated by proteins from primary granules (such as myeloperoxidase and neutrophil elastase[33]), secondary granules (such as lactoferrin[33] and pentraxin 3 (PTX3)[35]) and tertiary granules (such as matrix metalloproteinase 9 (MMP9)[33] and peptidoglycan recognition protein short (PGRP-S)[39,40]). Mitochondria can also serve as a source of DNA for NET formation[123]. NETs have been shown to trap microorganisms — such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella enterica* subspecies *enterica* serovar Typhimurium, *Candida albicans* and *Leishmania amazonensis* — and promote the interaction of these pathogens with granule-derived proteins and their subsequent disposal[33,124]. NET-localized molecules have a diverse repertoire of functions, including microbial recognition (for example, by PGRP-S and PTX3), antimicrobial activity (for example, by cathelicidin antimicrobial peptide (the uncleaved form of LL37) and bactericidal permeability-increasing protein) and tissue remodelling (for example, by elastase and MMP9). NET formation is a rapid, active process (occurring in minutes) that has been suggested to be mediated by a cell death-dependent process referred to as NETosis[125] (see the figure). Microorganisms have evolved strategies to escape NETs. For instance, M1 serotype strains of *Streptococcus pyogenes* and *Streptococcus pneumoniae* — which are known to cause the invasive infections necrotizing fasciitis and community-acquired pneumonia, respectively — express a DNase that impedes NET-mediated killing and promotes their virulence *in vivo*[126,127]. Thus, neutrophils produce 'poisonous' NETs to trap bacteria, whereas escape from NETs is an evolutionary strategy adopted by bacteria.

In systemic lupus erythematosus (SLE), the presence of autoantibodies specific for ribonucleoproteins (RNPs) and the antimicrobial peptide LL37 stimulates the release of NETs by neutrophils via CD32 (also known as FcγRIIB) and surface-expressed LL37, respectively. Autoantibodies specific for the antimicrobial peptides present in NETs promote the transport of DNA into plasmacytoid dendritic cells (pDCs) via CD32 and the production of interferon-α (IFNα) in a Toll-like receptor 9 (TLR9)-dependent manner. IFNα, in turn, enhances LL37 surface expression on neutrophils and amplifies the NET release that is induced by autoantibodies[97,98] (see the figure).



Cellular crosstalk

Once recruited into inflamed tissues (reviewed in REFS 1,43) neutrophils may engage in complex bidirectional interactions with macrophages, dendritic cells (DCs), natural killer (NK) cells, lymphocytes and mesenchymal stem cells (FIG. 1).

Control of neutrophil survival. Although neutrophils do not proliferate and have an estimated half-life of approximately 10–12 hours under *in vitro* culture conditions, signals such as adhesion, transmigration, hypoxia, microbial products and cytokines[3] can delay their programmed cell death and thus extend their survival *in vivo*. A prolonged lifespan, combined with an acquired ability to synthesize and release immunoregulatory cytokines, is probably essential for neutrophils to more efficiently eliminate damaging agents, as well as for them to interact with other cells. For example, macrophages can attract neutrophils to the site of injury and produce cytokines to control the lifespan and activity of the recruited cells (recently reviewed in REF. 43). In addition, human mesenchymal stem cells can affect the lifespan and activation of neutrophils[44,45]. TLR3- or TLR4-activated bone marrow-derived mesenchymal stem cells are more efficient than resting cells at mediating anti-apoptotic effects

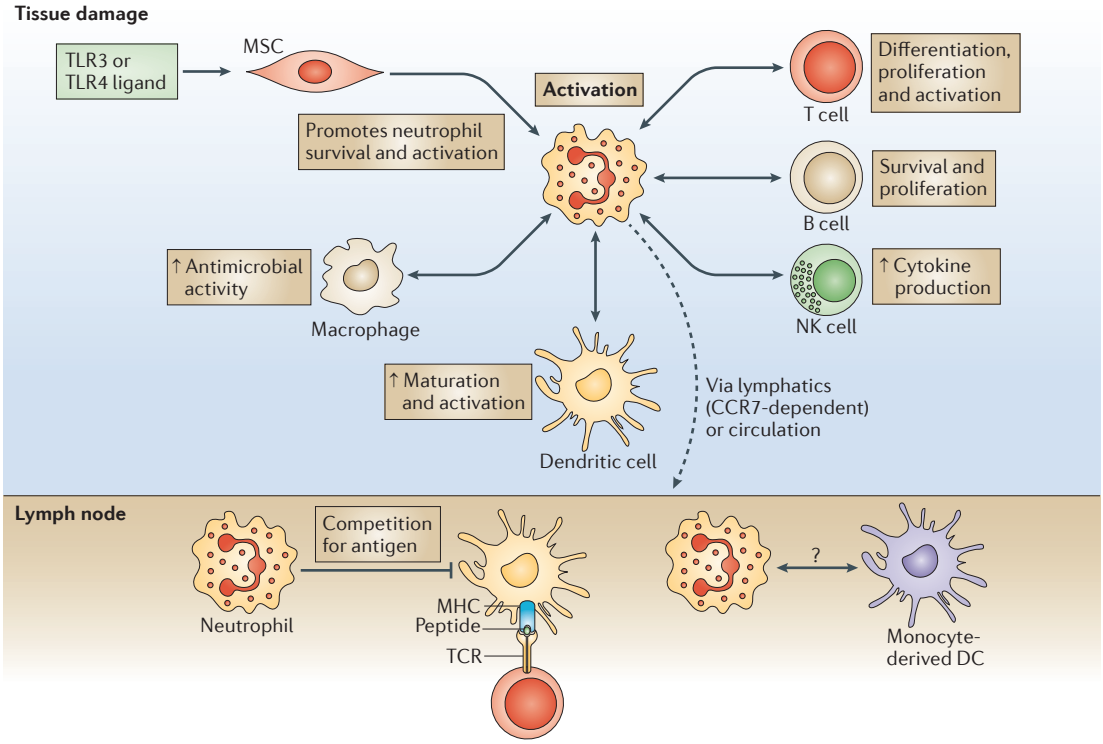


Figure 1 | **Neutrophils crosstalk with immune and non-immune cells in inflamed tissues and lymph nodes.** Circulating neutrophils are stimulated by systemic pathogens to crosstalk with platelets and endothelial cells, and this triggers the coagulation cascade (not shown). In the presence of tissue damage, neutrophils leave the circulation and crosstalk with both resident and recruited immune cells, including mesenchymal stem cells (MSCs), macrophages, dendritic cells (DCs), natural killer (NK) cells and B and T cells. The figure shows the main outcome(s) of the effects that MSCs exert on neutrophils and of neutrophil crosstalk with other cell types. Neutrophils can also migrate to the lymph nodes either via the lymphatics (in a CC-chemokine receptor 7 (CCR7)-dependent manner, similarly to tissue DCs) or via the circulation (similarly to monocytes). In the lymph nodes, neutrophils can interact with DCs to modulate antigen presentation. TCR, T cell receptor; TLR, Toll-like receptor.

on human neutrophils, thereby preserving a significant fraction of viable and functional neutrophils for up to 72 hours *in vitro*[44,45]. Such effects are mediated by IL-6, IFNβ and GM-CSF produced by TLR3-activated mesenchymal stem cells, and mostly through GM-CSF in the case of TLR4-activated mesenchymal stem cells[45]. Whether mesenchymal stem cells contribute to the modulation of neutrophil survival *in vivo* remains to be determined.

Immune cell crosstalk. The first evidence that neutrophils can cooperate with DCs came from studies showing that supernatant from cultures of mouse neutrophils stimulated with *Toxoplasma gondii* induces the maturation of bone marrow-derived DCs *in vitro*, as well as their production of IL-12 and TNF[46]. The *in vivo* relevance of such crosstalk was proved by observing that splenic DCs isolated from neutrophil-depleted mice infected with *T. gondii* showed reduced IL-12 and TNF production[46]. Human neutrophils have also been shown, at least *in vitro*, to induce the maturation of monocyte-derived DCs through contact-dependent interactions. These interactions involve CD18 and CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1)[47–49] on neutrophils, and DC-SIGN (DC-specific ICAM3-grabbing non-integrin) on monocyte-derived DCs[48,49]. As a result, monocyte-derived DCs that are matured by neutrophils acquire the potential to induce T cell proliferation and polarization towards a T_H1 cell phenotype[47,49]. In addition, neutrophils were found to frequently contact DC-SIGN$^+$ DCs in colonic mucosa from patients with Crohn's disease[49].

Interestingly, the crosstalk between human neutrophils and DCs does not always result in DC activation. For instance, neutrophil-derived elastase has been shown to decrease the allostimulatory ability of human monocyte-derived DCs[50]. Similarly, ectosomes released by human neutrophils — either following stimulation *in vitro* or at the site of inflammation *in vivo*[51] — inhibit the maturation of both monocyte-derived DCs[52] and monocyte-derived macrophages[53], possibly by increasing their production of the immunosuppressive cytokine transforming growth factor-β1 (TGFβ1)[52,53]. Indeed, ectosome-treated monocyte-derived DCs were shown to develop a tolerogenic phenotype, characterized by reduced phagocytic activity and expression of cell surface markers, as well as by an impaired capacity to produce cytokines and to induce T cell proliferation following LPS stimulation[52].

Ectosomes
Large membrane vesicles (> 100 nm diameter) that are secreted by budding or shedding from the plasma membrane.

SLAN
(6-sulpho LacNAc).
A carbohydrate modification of P-selectin glycoprotein ligand 1 (PSGL1). SLAN is expressed by a subset of dendritic cells found in human blood and is recognized by the monoclonal antibody MDC8.

The relevance of these *in vitro* studies on the crosstalk between neutrophils and DCs requires *in vivo* validation, for instance by direct imaging and stronger functional studies. It has recently been demonstrated that, in mice treated with LPS or Gram-negative bacteria, peripheral monocytes migrate to lymph nodes, where they differentiate into DCs to become the predominant antigen-presenting cells[54] (FIG. 1). Given that neutrophils can also migrate to and localize in lymph nodes (see below), one could envisage that monocyte-derived DCs and neutrophils interact in lymphoid organs as well as in the tissues.

Human neutrophils can also modulate the activation status of NK cells, either by themselves or in cooperation with other cell types. In the steady state, neutrophils are required for the maturation and function of NK cells, both in humans and mice (B. N. Jaeger, C. Cognet, S. Ugolini and E. Vivier, personal communications), which opens new perspectives on our understanding of the NK cell deficiency observed in patients with neutropenia-associated diseases. *In vitro*, neutrophils can modulate NK cell survival, proliferation, cytotoxic activity and IFNγ production via the generation of ROI and prostaglandins and/or the release of granule components (recently reviewed in REF. 55). By contrast, when interacting with DCs, human neutrophils specifically potentiate the release of IFNγ by NK cells, but do not regulate the cytotoxic activity of these cells[56].

Subset-specific differences in peripheral blood DCs affect the cooperation between neutrophils and NK cells[56] (FIG. 2). In humans, peripheral blood DCs can be divided in plasmacytoid DCs (pDCs) and myeloid DCs, which can be further divided into three subsets[57], namely CD1c$^+$ DCs, CD141$^+$ DCs and CD16$^+$ or 6-sulpho LacNAc (SLAN)$^+$ DCs[58]. It has been shown that, for some reasons yet to be determined, neutrophils potently enhance the release of IFNγ by NK cells cultured with SLAN$^+$ DCs, but not with CD1c$^+$ DCs or pDCs[56]. An *in vitro* tripartite network has been described, in which neutrophils promote the release of IL-12p70 by SLAN$^+$ DCs via a CD18–ICAM1 (intercellular adhesion molecule 1) interaction, and this IL-12p70 stimulates NK cells to produce IFNγ. The IFNγ, in turn, potentiates the interaction between neutrophils and SLAN$^+$ DCs and the release of SLAN$^+$ DC-derived IL-12p70, thus creating a positive feedback loop[56]. In addition, neutrophils can directly stimulate the production of IFNγ by NK cells; this is mediated through ICAM3 expressed by neutrophils and, probably, the CD18–CD11d complex expressed by NK cells[56,59] (FIG. 2). Importantly, the crosstalk between human neutrophils and NK cells is reciprocal, as culture of neutrophils with NK cells[60] or NK cell-derived soluble factors (such as GM-CSF and IFNγ[61]) promotes neutrophil survival, expression of activation markers, priming of ROI production and cytokine synthesis (as recently reviewed[55]). The potential pathophysiological relevance of a neutrophil–NK cell–SLAN$^+$ DC cellular network has been highlighted by immunohistochemistry studies, which have revealed the colocalization of neutrophils, NK cells and SLAN$^+$ DCs at the sites of several chronic inflammatory pathologies, including in the colonic mucosa of patients with Crohn's disease and in the skin lesions of patients with psoriasis[56]. Colocalization of neutrophils and NK cells has been also observed in the dermis of patients with acute febrile neutrophilic dermatosis (also known as Sweet's syndrome)[59].

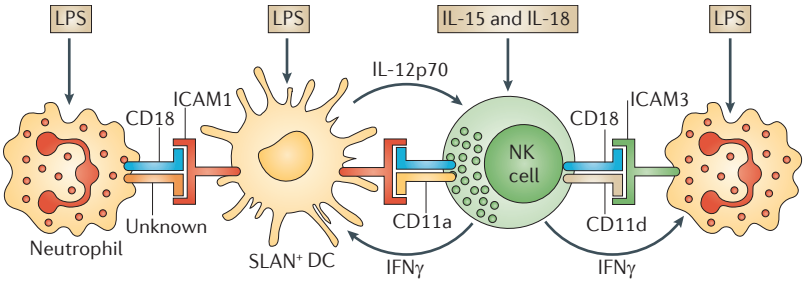


Figure 2 | **Crosstalk between neutrophils, NK cells and SLAN$^+$ DCs.** When neutrophils, 6-sulpho LacNAc (SLAN)$^+$ dendritic cells (DCs) and natural killer (NK) cells localize in tissues during inflammation, cell–cell interactions between these cells may occur. This results in crosstalk between activated neutrophils and SLAN$^+$ DCs (mediated by CD18 and intercellular adhesion molecule 1 (ICAM1)) and increased release of interleukin-12 p70 (IL-12p70) by SLAN$^+$ DCs. IL-12p70, in turn, enhances the production of interferon-γ (IFNγ) by activated NK cells. Concurrently, activated neutrophils directly stimulate the production of IFNγ by NK cells, probably through the engagement of CD11d–CD18 on NK cells by ICAM3. As a result, positive amplification loops for IL-12p70 and IFNγ production are created. LPS, lipopolysaccharide.

Human neutrophils can also crosstalk with B cells (as discussed above) and with T cells (FIG. 3). A first level of interaction between T cells and neutrophils is related to the ability of these cells to modulate each other's recruitment to inflamed tissues. It has been recently shown that activated neutrophils can attract T_H1 and T_H17 cells to sites of inflammation via the release of CCL2, CXCL9 and CXCL10 or CCL2 and CCL20, respectively[6]. In addition, activated T cells can recruit neutrophils, although the mechanism used by individual T cell subsets differs. For example, activated regulatory T (T_{Reg}) or T_H17 cells, but not T_H1 cells, can release CXCL8, which potently attracts neutrophils[6,62]. By contrast, γδ T cells promote the release of CXCL8 in culture with neutrophils, and this may amplify their own recruitment[63]. Also, human T_H1 cells, following stimulation *in vitro*, can potently recruit neutrophils[6], but the responsible mediator (or mediators) involved has not yet been identified.

A second level of interaction between neutrophils and T cells is related to the ability of these cells to modulate each other's functions (FIG. 3). Indeed, activated CD4$^+$ and CD8$^+$ T cells, including T_H17 cells, produce cytokines (such as IFNγ, GM-CSF and TNF) that modulate neutrophil survival and expression of activation markers in *in vitro* culture systems[6,27,63]. Similarly, γδ T cells strongly promote neutrophil survival and activation, as determined by upregulation of CD64 and HLA-DR expression (M. Davey and M. Eberl, personal communication). In addition, both IL-17A and IL-17F released by T_H17 cells stimulate epithelial cells to secrete granulopoietic factors (such as G-CSF and stem cell factor), as well as neutrophil chemoattractants (such as CXCL1, CXCL2, CXCL5 and CXCL8), which

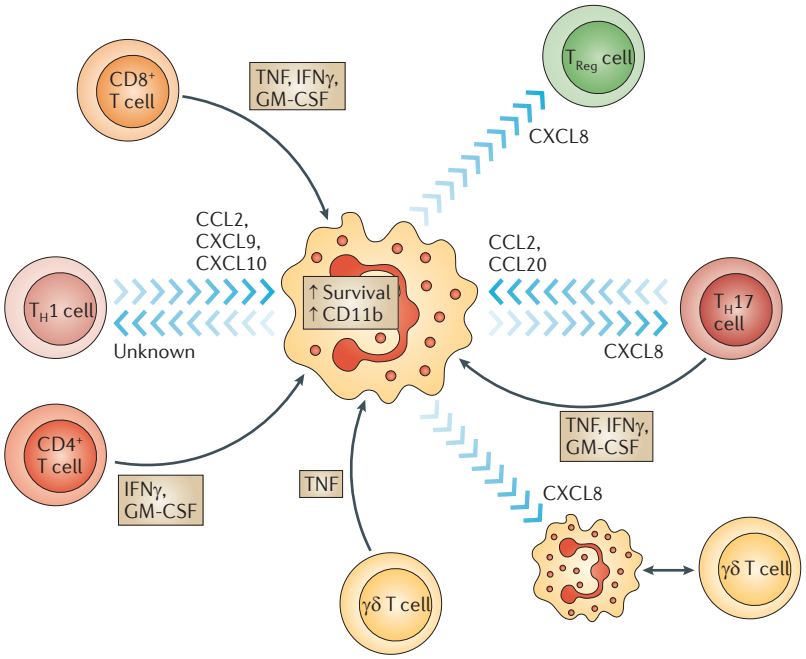


Figure 3 | **Interplay between neutrophils and T cells.** Human neutrophils (when appropriately activated) release chemokines that mediate the recruitment of T helper 1 (T_H1) and T_H17 cells. In the case of T_H1 cells, the chemokines involved are CC-chemokine ligand 2 (CCL2), CXC-chemokine ligand 9 (CXCL9) and CXCL10, whereas CCL2 and CCL20 mediate the recruitment of T_H17 cells. In turn, both T_H1 and T_H17 cells, as well as regulatory T (T_{Reg}) cells, can attract neutrophils via the release of CXCL8 or as yet unidentified chemokines. Co-culture of γδ T cells and neutrophils results in the production of CXCL8, which amplifies neutrophil recruitment. In addition, the activation of the various T cell populations results in the release of interferon-γ (IFNγ), granulocyte–macrophage colony-stimulating factor (GM-CSF) and, in some cases, tumour necrosis factor (TNF). These factors, in turn, promote the survival of neutrophils and their increased expression of CD11b.

thus amplify neutrophil recruitment and activation. Furthermore, it has been recently demonstrated that mouse neutrophils can be induced by T cells to express MHC class II molecules *in vitro* and consequently to promote the differentiation of antigen-specific T_H1 and T_H17 cells[64]. Moreover, human and mouse neutrophils were shown to cross-present exogenous antigens *in vitro*, and injection of mice with antigen-pulsed neutrophils promoted the differentiation of naive $CD8^+$ T cells into cytotoxic T cells[65].

Together, these data suggest that neutrophils are not just isolated players that quickly perform their actions before being substituted by more specialized cells. They also guide and support the innate and adaptive immune response throughout its development, through crosstalk with most, if not all, of the cellular mediators.

Regulation of adaptive immunity. Contrary to previously commonly held views that neutrophils become rapidly exhausted at peripheral sites, recent evidence suggests that neutrophils can migrate to lymph nodes following antigen capture at the periphery[66,67] in a CC-chemokine receptor 7 (CCR7)-dependent manner, similar to DCs[68] (FIG. 1). The functional relevance of neutrophils that have migrated to lymph nodes was assessed in mice using three different antigenic proteins. In such a model, neutrophils were found to suppress the B cell and $CD4^+$ T cell responses, but not the $CD8^+$ T cell response, to all three antigens[69]. However, neutrophil-derived ROI, nitric oxide and IL-10 were not involved in this suppression. In addition, neutrophils were found to interfere with the ability of DCs and macrophages to present antigen shortly after their migration into the lymph node, presumably by competing with the antigen-presenting cells for the available antigen[69] (FIG. 1). Moreover, subcutaneously injected antigen-pulsed neutrophils were shown to cross-prime naive $CD8^+$ T cells, suggesting an interaction between injected neutrophils and $CD8^+$ T cells in the draining lymph nodes[65].

Collectively, data from both *in vitro* culture systems and *in vivo* models highlight the complexity of the function of neutrophils in terms of the cells they interact with and their sites of action. On the one hand, neutrophils can influence the maturation of DCs and, in turn, the proliferation and polarization of T cells, and they can directly prime antigen-specific T_H1 and T_H17 cells *in vitro*[64]. On the other hand, neutrophils appear to exert an immunoregulatory role *in vivo* at both peripheral sites and lymph nodes.

Role in resolution of inflammation. Neutrophils are generally considered to be passive components of the resolution of inflammation, whose fate is death followed by rapid and silent elimination. However, recent evidence suggests that they are involved in the active induction of resolution through the production of pro-resolving lipid mediators[70]. During the late, final phases of acute inflammatory responses, neutrophils switch their eicosanoid biosynthesis from leukotriene B4 (LTB4) to lipoxin A4 (LXA4), which can inhibit neutrophil recruitment through its interaction with its G protein-coupled receptor LXA4R (also known as FPR2)[70]. Neutrophils can also contribute to the biosynthesis of resolvins (such as resolvin E1, resolvin E2, resolvin D1 and resolvin D2) and protectin D1, which are derived from omega-3 essential polyunsaturated fatty acids[70]. These pro-resolving lipid mediators and the recently described macrophage-derived compound maresin 1 inhibit neutrophil transendothelial migration and tissue infiltration[70–73] (FIG. 4). For instance, resolvin E1 interacts with the LTB4 receptor BLT1 (also known as LTB4R1) on neutrophils and blocks stimulation by LTB4 (REF. 74). Accordingly, in a mouse model of peritonitis, the anti-inflammatory effects of resolvin E1 were lost in BLT1-deficient mice[74].

The contribution of neutrophils to the resolution of inflammation also includes blocking and scavenging of chemokines and cytokines. Pro-resolving lipid mediators (such as LXA4, resolvin E1 and protectin D1) increase the expression of CCR5 by apoptotic neutrophils, which can then act as functional decoys and scavengers for CCL3 and CCL5 (REF. 75) (FIG. 4). Neutrophils have also been reported to express CC-chemokine receptor D6, a decoy receptor and scavenger for virtually all inflammatory CC-chemokines[76]. The type 2 IL-1 decoy receptor (IL-1R2) is expressed at high levels by

Resolvins
Lipid mediators that are induced in the resolution phase following acute inflammation. They are synthesized from the essential omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid.

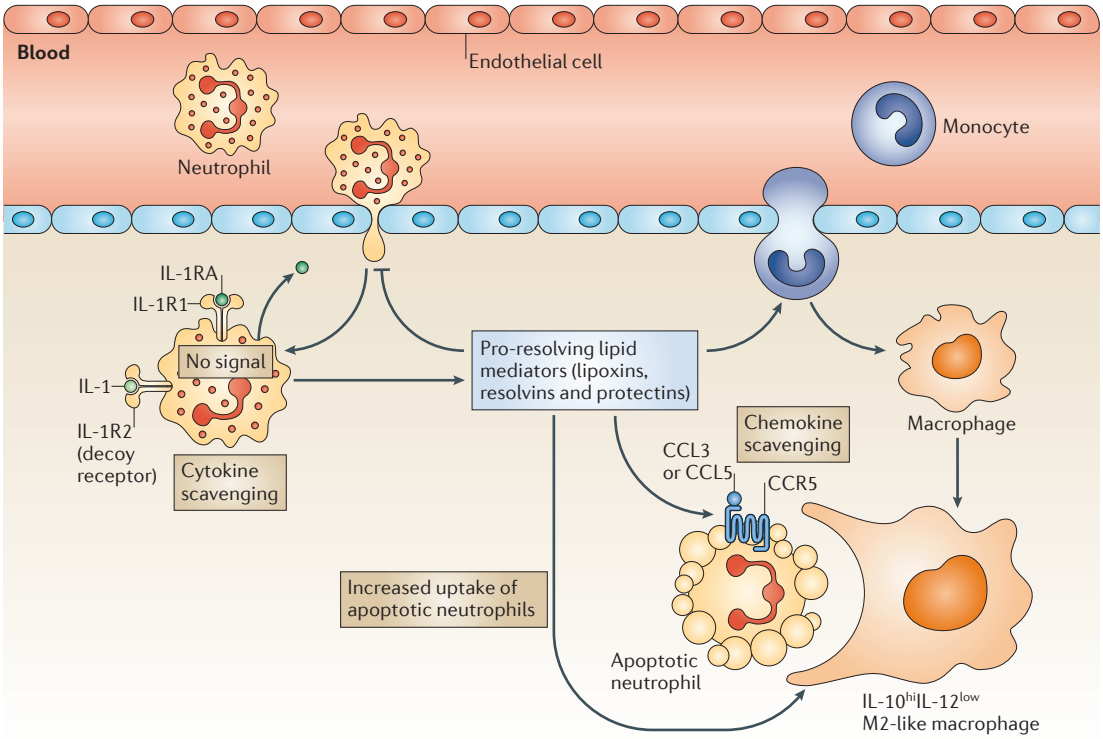


Figure 4 | **The role of neutrophils in the resolution of inflammation.** Neutrophils orchestrate the resolution phase of inflammation via different mechanisms, including chemokine and/or cytokine scavenging and the formation of pro-resolving lipid mediators (such as lipoxins, resolvins and protectins). The pro-resolving lipid mediators stop neutrophil infiltration and increase the uptake of apoptotic neutrophils by macrophages. They also amplify the expression of CC-chemokine receptor 5 (CCR5) by apoptotic neutrophils, and this, in turn, promotes the sequestration and clearance of CC-chemokine ligand 3 (CCL3) and CCL5. Neutrophil-derived interleukin-1 receptor antagonist (IL-1RA; which binds to and blocks IL-1R1) and the decoy receptor IL-1R2 (which traps IL-1) provide additional mechanisms to limit the pleiotropic pro-inflammatory effects of IL-1.

neutrophils and its expression is further augmented by anti-inflammatory signals, such as glucocorticoid hormones[77]. This decoy receptor (in both membrane-bound and released forms) binds IL-1 and prevents its interaction with the signal-transducing receptor IL-1R1 (REF. 77) (FIG. 4). Neutrophils — particularly those that have been stimulated with the anti-inflammatory cytokine IL-10 — are also a major source of IL-1RA, a soluble molecule that binds to IL-1R1 without inducing any intracellular signals[78] (FIG. 4). So, the production and expression of these decoy receptors and cytokines help to limit the pro-inflammatory effects of IL-1.

Finally, disposal of apoptotic neutrophils is an important step in the resolution of inflammation[79] that is finely regulated by the expression of eat-me signals, which trigger an anti-inflammatory programme in the engulfing phagocyte[80]. Indeed, the recognition and ingestion of apoptotic neutrophils shapes the functional phenotype of macrophages[79]. Phagocytosis of apoptotic neutrophils stimulates the engulfing phagocyte to develop an IL-10^{hi}IL-12^{low} M2-like phenotype[81] (FIG. 4), and this negatively regulates inflammation and promotes tissue repair[82,83].

Thus, neutrophils, acting at multiple levels, are part of the cellular network that orchestrates the resolution of inflammation.

Eat-me signals
Signals emitted by dying cells to facilitate their recognition and phagocytosis by neighbouring healthy cells.

The role of neutrophils in pathology

Given the broad functions of neutrophils that have recently been uncovered, it is not surprising that neutrophils have emerged as important players in the pathogenesis of numerous disorders, including infection caused by intracellular pathogens, autoimmunity, chronic inflammation and cancer.

Infection and chronic inflammation. Although it was traditionally believed that the main role for neutrophils was in the efficient elimination of extracellular pathogens, several results point to the participation of neutrophils also in the elimination of intracellular bacterial pathogens, such as *Mycobacterium tuberculosis*[84]. Such a characteristic may be due to several factors, including the enhanced microbicidal activity of neutrophils compared to macrophages[84] and the known difference in intraphagosomal pH between these phagocytes[85].

Consistent with the notion that neutrophils can also contribute to the host response towards intracellular pathogens, Berry *et al.*[86] identified a genetic signature involving 86 genes in blood neutrophils from patients infected with *M. tuberculosis*. This signature specifically consisted of transcripts that are induced by type I and type II IFNs, and it was associated with active tuberculosis disease[86]. Such an IFN-inducible signature is

not present in neutrophils from patients with group A *Streptococcus* or *Staphylococcus* spp. infection or Still's disease, indicating a specific involvement of neutrophils in the immune response to *M. tuberculosis*. Interestingly, in a mouse model of *M. tuberculosis* infection, neutrophils were required for the production of early, innate immune-derived IFNγ (probably by NK cells)[87]. Thus, mouse and human studies suggest a crucial role for neutrophils in immunity against the prototypical intracellular pathogen *M. tuberculosis*.

In addition to their functions during *M. tuberculosis* infection, neutrophils have emerged as important determinants of chronic inflammation. The tripeptide proline-glycine-proline (PGP) is a selective neutrophil chemoattractant that has been implicated in the persistence of chronic obstructive pulmonary disease (COPD)[88]. PGP is normally degraded in the lungs by the aminopeptidase activity of leukotriene A4 hydrolase (LTA4H), which is also responsible for the synthesis of the chemotactic molecule LTB4 (REF. 89). However, in the presence of cigarette smoke (the major risk factor for COPD), the aminopeptidase (but not the hydrolase) activity of LTA4H is inhibited, thereby resulting in PGP accumulation[88]. Under these conditions, the combined actions of LTB4 and PGP strongly promote neutrophil recruitment and chronic lung inflammation[89]. A similar mechanism may be at work in cystic fibrosis, which is characterized by a chronic neutrophilic inflammation[89].

New perspectives have also been obtained on the function and regulation of neutrophils in sepsis. IL-33, a member of the IL-1 family, has been shown to regulate neutrophil function during systemic inflammation. In a mouse model of polymicrobial sepsis, administration of IL-33 protected mice by reducing systemic inflammation[90]. IL-33 — via inhibition of G protein-coupled receptor kinase 2 (GRK2; also known as ADRBK1) — prevented the downregulation of CXC-chemokine receptor 2 (CXCR2) on circulating neutrophils and thus increased neutrophil migration to inflamed tissues and promoted bacterial clearance[90]. In addition, TLR4-activated platelets have been shown to bind to adherent neutrophils during sepsis and promote NET formation, which may lead to bacterial trapping but also to endothelial and tissue damage *in vitro* and *in vivo*[91,92] (BOX 2).

Moreover, following vessel injury and during systemic *Escherichia coli* infection, platelets induce NET formation, which promotes the coagulation cascade[92]. By promoting intravascular coagulation in liver sinusoids, neutrophil-promoted fibrin deposition was shown to prevent pathogen dissemination[92]. Neutrophils also have an important role in vascular pathology, including in atherosclerosis and thrombosis[93,94]. Thus, neutrophils can cooperate with platelets and endothelial cells to prevent pathogen dissemination via the vasculature but can also promote vascular inflammation and thrombosis.

Autoimmunity. In systemic lupus erythematosus (SLE) — a multiorgan autoimmune disease characterized by an IFN and granulopoiesis signature, as well as abnormal B and T cell function[95] — the degradation of NETs by DNase I, which is normally found in healthy human serum, is impaired in a subset (36.1%) of patients[96] (BOX 2). This defect is correlated with high levels of antinuclear antibodies (a hallmark of disease development), with the presence of NET-specific autoantibodies and with more frequent development of lupus nephritis[96]. Therefore, a defect in NET clearance could lead to a source of autoantigens and damage-associated molecular patterns (such as proteases) that are known to trigger and promote inflammation[80]. Accordingly, serum from patients with SLE was shown to contain immune complexes composed of autoantibodies specific for ribonucleoproteins, self DNA and antimicrobial peptides (such as cathelicidin antimicrobial peptide (the uncleaved form of LL37) and human neutrophil peptides (HNPs; also known as neutrophil defensins))[97,98], and all of these components are associated with NETs. These immune complexes block the degradation of self DNA and promote its uptake by pDCs through interactions between the autoantibodies and CD32 (also known as FcγRIIB)[97,98]. Following uptake, self DNA triggers TLR9 activation and the release of IFNα, which, in turn, induces further NET production by neutrophils[97,98] (BOX 2).

In small-vessel vasculitis (SVV), the presence of anti-neutrophil cytoplasmic antibodies (ANCAs) is a hallmark of the pathologies collectively known as ANCA-associated vasculitis, including Wegener's granulomatosis, microscopic polyangiitis and Churg–Strauss syndrome[99]. Interestingly, NETs are produced by ANCA-stimulated neutrophils and were found in glomeruli and in the interstitium of kidney biopsies from patients with SVV, where they may be involved in the damage to glomerular capillaries[100]. In addition, the production of NETs results in elevated levels of the autoantigens proteinase 3 (also known as myeloblastin) and myeloperoxidase, which are contained in the NETs, thereby providing additional autoantigens to further the autoimmune response[100].

In the K/BxN transgenic mouse model of inflammatory arthritis, neutrophil recruitment into the joints has been shown to be promoted by the chemotactic lipid LTB4 through its receptor BLT1, both of which are expressed by neutrophils[101]. Neutrophil activation by immune complexes in the joints promotes IL-1β production, which in turn stimulates synovial cells to produce chemokines, and this amplifies neutrophil recruitment into the joints[101]. Furthermore, CXCR2-dependent neutrophil activation and consequent induction of inflammation have been demonstrated in two mouse models of multiple sclerosis[102,103].

Cancer. Inflammatory cells are an essential component of the tumour microenvironment and play a role in tumour progression[104,105]. Neutrophil infiltration of tumours is generally not prominent, with tumour-associated macrophages (TAMs) being a major component of the infiltrate[105]. However, there is mounting evidence that the presence, functional characteristics and significance of tumour-associated neutrophils (TANs) may have been underestimated and therefore need careful reappraisal.

Chronic obstructive pulmonary disease
(COPD). A group of diseases characterized by the pathological limitation of airflow in the airway, including chronic obstructive bronchitis and emphysema. It is most often caused by tobacco smoking, but can also be caused by other airborne irritants (such as coal dust) and occasionally by genetic abnormalities, such as α1-antitrypsin deficiency.

Antinuclear antibodies
(ANAs). Heterogeneous autoantibodies specific for one or more antigens present in the nucleus, including chromatin, nucleosomes and ribonuclear proteins. ANAs are found in association with many different autoimmune diseases.

K/BxN transgenic mouse
A mouse strain formed by crossing NOD/Lt mice with C57BL/6 KRN T cell receptor-transgenic mice in which T cells recognize a peptide from the autoantigen glucose-6-phosphate isomerase (GPI). These mice develop a form of arthritis that is mediated, and can be transferred, by circulating antibody specific for GPI.

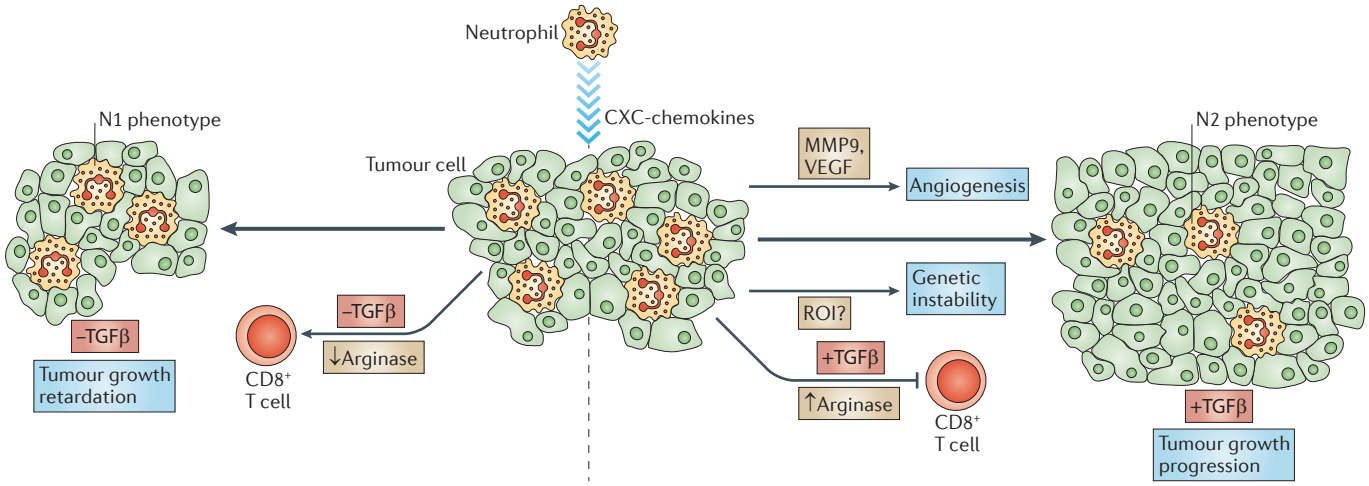


Figure 5 | **Tumour-associated neutrophils.** CXC-chemokines produced by tumour cells and tumour-associated macrophages promote neutrophil recruitment into tumours. Neutrophils may promote genetic instability (possibly through reactive oxygen intermediate (ROI) production) and stimulate angiogenesis (through the production of matrix metalloproteinase 9 (MMP9) and vascular endothelial growth factor (VEGF)). Neutrophils are driven by transforming growth factor-β (TGFβ) to acquire a polarized, pro-tumoural N2 phenotype (characterized by high levels of arginase expression). By contrast, inhibition of TGFβ promotes a reprogramming of neutrophils to an N1 phenotype. This is associated with higher cytotoxic activity, higher capacity to generate hydrogen peroxide, higher expression of tumour necrosis factor (TNF) and intercellular adhesion molecule 1 (ICAM1), and lower expression of arginase. In the presence of N1 neutrophils, $CD8^+$ T cell activation increases, and this results in effective antitumour activity[9].

Tumour cells often constitutively produce several inflammatory chemokines, including neutrophil-attracting CXC-chemokines[105] (FIG. 5). Indeed, activation of members of different classes of oncogenes results in the production of CXCL8 and related chemokines[105–107]. The relationship between TAN infiltration and prognosis in human cancer has not been systematically investigated[105], although there is suggestive evidence for a role for TANs in enhanced disease progression in specific human tumours[108,109]. For instance, TNF-driven tumour progression in ovarian cancer involves T_H17 cells, which promote neutrophil recruitment[110].

In a seminal study, Hans Schreiber and colleagues[111] found that neutrophil depletion resulted in inhibition of sarcoma growth. Since then, the involvement of neutrophils in the promotion and progression of cancer has been observed in various studies. The mechanisms described in these studies were diverse and included the release of granule-stored hepatocyte growth factor and the production of oncostatin M[112]. Surprisingly, elastase released from neutrophil primary granules is taken up into specific endosomal compartments of adjacent epithelial tumour cells, where it hydrolyses insulin receptor substrate 1 (IRS1). IRS1 binds to a subunit of phosphoinositide 3-kinase (PI3K) and blocks its interaction with the platelet-derived growth factor receptor (PDGFR)[113]. Therefore, in this setting, neutrophil-derived elastase unleashes the tumour-promoting activity of the PDGFR–PI3K pathway.

Type I IFNs have a crucial role in host antitumour immune responses, in particular in cancer immunoediting, and they are used for the treatment of several cancers. In tumour transplant models, enhancement of tumour growth, angiogenesis and metastasis were observed in IFNβ-deficient mice compared with control mice[114]. Interestingly, the number of TANs was increased in these mice, and the depletion of TANs reduced the tumour growth[114]. These results suggest that reducing the pro-tumour function of TANs is an important component of the anticancer activity of IFNβ.

TAMs and TANs are also potent drivers of tumour angiogenesis. Neutrophil-attracting CXC-chemokines are frequently present in tumours and promote angiogenesis[115]. Indeed, CXCL1 has been shown to have *in vivo* angiogenic activity that is mediated by neutrophil-derived vascular endothelial growth factor A (VEGF-A)[116]. In a genetically engineered mouse model of cancer (the RIP1-*Tag2* mouse model of pancreatic islet tumorigenesis), expression of matrix metalloproteinase 9 (MMP9) — which catalyses tumour angiogenesis by inducing VEGF expression within the neoplastic pancreatic tissue — was exclusively found in neutrophils, and neutrophil depletion inhibited the angiogenic switch[117]. A correlation was also found in human hepatocellular carcinoma between MMP9, neutrophils and angiogenesis[108]. Moreover, in a tumour xenograft model, G-CSF-induced neutrophil upregulation of BV8 (also known as prokineticin 2) was shown to promote tumour angiogenesis[118].

The process of myelopoiesis is profoundly modified during inflammation and cancer, and this leads to the appearance of altered mature myelocytes and of myeloid-derived suppressor cells (MDSCs)[119,120]. In general, although mature human neutrophils are not a major component of the MDSC activity, increased numbers of mature myelocytes have been shown to account for immune suppression in patients with renal cell carcinoma[119,120]. Moreover, in human melanoma, serum

Immunoediting
The process by which interaction of a heterogeneous population of tumour cells with the immune system generates tumour variants with reduced immunogenicity that might therefore escape from immune responses.

Angiogenesis
The development of new blood vessels from existing blood vessels. Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in granulation tissue formation. It is also a fundamental step for the growth of dormant tumours.

Myeloid-derived suppressor cells
A heterogenous collection of cells at different stages in the myeloid and monocytic differentiation pathway that have immunosuppressive functions. These cells include bona fide monocytes and neutrophils.

amyloid A1 protein (SAA1)-induced production of IL-10 by neutrophils was reported to suppress antigen-specific proliferation of $CD8^+$ T cells[29]. However, the finding of IL-10 production by activated human neutrophils could not be reproduced in other laboratories (REF. 30 and M.A.C. and A.M., unpublished observations) and thus requires further investigation.

Cancer has provided indications that neutrophils can exhibit considerable plasticity in response to environmental signals (FIG. 5). In a rat mammary adenocarcinoma model, co-injection of cancer cells with neutrophils from tumour-bearing rats, but not with neutrophils from normal rats, markedly increased metastasis[121]. This finding suggests that the tumour microenvironment profoundly shapes the functional status of neutrophils, a view confirmed by recent reports[30].

TGFβ acts as a promoter or suppressor of tumour initiation, progression and metastasis, depending on the context and stage of the tumour, and is also a regulator of neutrophil functions. In lung adenocarcinoma and mesothelioma models, inhibition of TGFβ enhanced the infiltration of TANs, as well as their tumour cytotoxicity and immunostimulatory profile (that is, higher expression of TNF, CCL3 and ICAM1 and lower expression of arginase 1)[9]. Interestingly, in tumour-bearing animals, depletion of neutrophils (including TANs) led to an increase in $CD8^+$ T cell activation, whereas, following TGFβ inhibition, depletion of neutrophils had the opposite effect[9]. Therefore, similarly to M1 and M2 macrophages[82], neutrophils have been proposed to polarize to N1 and N2 phenotypes. TGFβ promotes the polarization of TANs to a pro-tumoural N2 phenotype, whereas a shift towards an N1 phenotype with antitumoural properties occurs following TGFβ inhibition[9]. Thus, these results demonstrate that blocking TGFβ in tumours unleashes a $CD8^+$ T cell-dependent antitumoural response that involves the activation of neutrophils with antitumour properties. Accordingly, earlier studies indicated that neutrophils can exert antitumour activity *in vitro* and *in vivo*[122]. Thus, like macrophages, neutrophils can have opposing effects on tumour growth depending on environmental signals (such as TGFβ).

Conclusion and perspectives

Neutrophils have emerged as an important component of effector and regulatory circuits in the innate and adaptive immune systems. In contrast to the traditional view of these cells as short-lived effectors, evidence now indicates that they have diverse functions. By responding to tissue- and immune cell-derived signals and by undergoing polarization[9], neutrophils are reminiscent of macrophages[82]. Neutrophils engage in bidirectional interactions with different components of both the innate and adaptive immune systems and can differentially influence the response depending on the context.

Recent studies have also provided new insights on neutrophil effector functions. These gladiators of innate immunity can throw poisonous NETs and produce components of the humoral arm of the innate immune response. These new insights also raise new questions. Such unknowns include the degree of neutrophil diversity and plasticity, the molecular basis of this plasticity, and its relevance in the activation, expression and regulation of adaptive immune responses. Better tools (both genetic and antibody-based) are needed to dissect the function of neutrophils *in vivo*. The new perspectives opened by recent findings call for a reappraisal of the role of neutrophils in human pathology, especially in cancer. Finally, it is now time to reconsider neutrophils as a valuable therapeutic target in inflammatory pathology and cancer.

1. Borregaard, N. Neutrophils, from marrow to microbes. *Immunity* **33**, 657–670 (2010).
2. Nathan, C. Neutrophils and immunity: challenges and opportunities. *Nature Rev. Immunol.* **6**, 173–182 (2006).
3. Colotta, F., Re, F., Polentarutti, N., Sozzani, S. & Mantovani, A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* **80**, 2012–2020 (1992).
4. Cassatella, M. A. Neutrophil-derived proteins: selling cytokines by the pound. *Adv. Immunol.* **73**, 369–509 (1999).
5. Kobayashi, S. D. & DeLeo, F. R. Role of neutrophils in innate immunity: a systems biology-level approach. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **1**, 309–333 (2009).
6. Pelletier, M. *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* **115**, 335–343 (2010).
This paper provides the first evidence that human neutrophils and T_H17 cells, upon activation, can directly recruit each other, via specific chemokine release.
7. Kotz, K. T. *et al.* Clinical microfluidics for neutrophil genomics and proteomics. *Nature Med.* **16**, 1042–1047 (2010).
8. Bazzoni, F. *et al.* Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc. Natl Acad. Sci. USA* **106**, 5282–5287 (2009).
9. Fridlender, Z. G. *et al.* Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN. *Cancer Cell* **16**, 183–194 (2009).
This was the first study supporting the view that TANs can be polarized towards an 'N1' or an 'N2' phenotype, mirroring M1 and M2 macrophages.
10. Cua, D. J. & Tato, C. M. Innate IL-17-producing cells: the sentinels of the immune system. *Nature Rev. Immunol.* **10**, 479–489 (2010).
11. Segal, A. W. How neutrophils kill microbes. *Annu. Rev. Immunol.* **23**, 197–223 (2005).
12. Zhang, Q. *et al.* Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* **464**, 104–107 (2010).
13. McDonald, B. *et al.* Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science* **330**, 362–366 (2010).
Taking advantage of dynamic *in vivo* imaging to visualize the innate immune response, this report uncovers a multistep hierarchy of directional cues that guides neutrophil localization in a mouse model of sterile liver inflammation.
14. Hayashi, F., Means, T. K. & Luster, A. D. Toll-like receptors stimulate human neutrophil function. *Blood* **102**, 2660–2669 (2003).
15. Greenblatt, M. B., Aliprantis, A., Hu, B. & Glimcher, L. H. Calcineurin regulates innate antifungal immunity in neutrophils. *J. Exp. Med.* **207**, 923–931 (2010).
This paper elucidates that the increased susceptibility to fungal infections observed in patients treated with cyclosporine A, one of the most potent immunosuppressants available, is not the consequence of its broad inhibition of T cell responses but rather maps to the function of calcineurin B in neutrophils.
16. Kerrigan, A. M. *et al.* CLEC-2 is a phagocytic activation receptor expressed on murine peripheral blood neutrophils. *J. Immunol.* **182**, 4150–4157 (2009).
17. Tamassia, N. *et al.* Activation of an immunoregulatory and antiviral gene expression program in poly(I:C)-transfected human neutrophils. *J. Immunol.* **181**, 6563–6573 (2008).
18. Clarke, T. B. *et al.* Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Med.* **16**, 228–231 (2010).
19. Scapini, P., Bazzoni, F. & Cassatella, M. A. Regulation of B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS) expression in human neutrophils. *Immunol. Lett.* **116**, 1–6 (2008).
20. Yamada, M. *et al.* Interferon-γ production by neutrophils during bacterial pneumonia in mice. *Am. J. Respir. Crit. Care Med.* **183**, 1391–1401 (2011).
21. Huard, B. *et al.* APRIL secreted by neutrophils binds to heparan sulfate proteoglycans to create plasma cell niches in human mucosa. *J. Clin. Invest.* **118**, 2887–2895 (2008).
22. Gabay, C. *et al.* Synovial tissues concentrate secreted APRIL. *Arthritis Res. Ther.* **11**, R144 (2009).
23. Roosnek, E. *et al.* Tumors that look for their springtime in APRIL. *Crit. Rev. Oncol. Hematol.* **72**, 91–97 (2009).
24. Chakravarti, A., Raquil, M. A., Tessier, P. & Poubelle, P. E. Surface RANKL of Toll-like receptor 4-stimulated human neutrophils activates osteoclastic bone resorption. *Blood* **114**, 1633–1644 (2009).
This paper uncovers a new biological feature of human, as well as mouse, neutrophils. These cells are important in osteoclastogenesis, through their capacity to activate osteoclastic bone resorption.

25. Poubelle, P. E., Chakravarti, A., Fernandes, M. J., Doiron, K. & Marceau, A. A. Differential expression of RANK, RANK-L, and osteoprotegerin by synovial fluid neutrophils from patients with rheumatoid arthritis and by healthy human blood neutrophils. *Arthritis Res. Ther.* **9**, R25 (2007).
26. Ethuin, F. *et al.* Human neutrophils produce interferon γ upon stimulation by interleukin-12. *Lab. Invest.* **84**, 1363–1371 (2004).
27. Pelletier, M., Micheletti, A. & Cassatella, M. A. Modulation of human neutrophil survival and antigen expression by activated $CD4^+$ and $CD8^+$ T cells. *J. Leukoc. Biol.* **88**, 1163–1170 (2010).
28. Reglier, H., Arce-Vicioso, M., Fay, M., Gougerot-Pocidalo, M. A. & Chollet-Martin, S. Lack of IL-10 and IL-13 production by human polymorphonuclear neutrophils. *Cytokine* **10**, 192–198 (1998).
29. De Santo, C. *et al.* Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. *Nature Immunol.* **11**, 1039–1046 (2010).
30. Cassatella, M. A., Locati, M. & Mantovani, A. Never underestimate the power of a neutrophil. *Immunity* **31**, 698–700 (2009).
31. Zhang, X., Majlessi, L., Deriaud, E., Leclerc, C. & Lo-Man, R. Coactivation of Syk kinase and MyD88 adaptor protein pathways by bacteria promotes regulatory properties of neutrophils. *Immunity* **31**, 761–771 (2009).
A study demonstrating that, in mice, neutrophils represent a major source of IL-10.
32. 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).
33. Brinkmann, V. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
34. Bottazzi, B., Doni, A., Garlanda, C. & Mantovani, A. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu. Rev. Immunol.* **28**, 157–183 (2010).
35. Jaillon, S. *et al.* The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J. Exp. Med.* **204**, 793–804 (2007).
36. Lu, J. *et al.* Structural recognition and functional activation of FcγR by innate pentraxins. *Nature* **456**, 989–992 (2008).
37. Moalli, F. *et al.* Role of complement and Fcγ receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood* **116**, 5170–5180 (2010).
38. Deban, L. *et al.* Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nature Immunol.* **11**, 328–334 (2010).
39. Cho, J. H. *et al.* Human peptidoglycan recognition protein S is an effector of neutrophil-mediated innate immunity. *Blood* **106**, 2551–2558 (2005).
40. Dziarski, R., Platt, K. A., Gelius, E., Steiner, H. & Gupta, D. Defect in neutrophil killing and increased susceptibility to infection with nonpathogenic Gram-positive bacteria in peptidoglycan recognition protein-S (PGRP-S)-deficient mice. *Blood* **102**, 689–697 (2003).
41. Rorvig, S. *et al.* Ficolin-1 is present in a highly mobilizable subset of human neutrophil granules and associates with the cell surface after stimulation with fMLP. *J. Leukoc. Biol.* **86**, 1439–1449 (2009).
42. Parham, P. Innate immunity: the unsung heroes. *Nature* **423**, 20 (2003).
43. Soehnlein, O. & Lindbom, L. Phagocyte partnership during the onset and resolution of inflammation. *Nature Rev. Immunol.* **10**, 427–439 (2010).
44. Brandau, S. *et al.* Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. *J. Leukoc. Biol.* **88**, 1005–1015 (2010).
45. Cassatella, M. A. *et al.* Toll-like receptor-3-activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils. *Stem Cells* **29**, 1001–1011 (2011).
46. Bennouna, S. & Denkers, E. Y. Microbial antigen triggers rapid mobilization of TNF-α to the surface of mouse neutrophils transforming them into inducers of high-level dendritic cell TNF-α production. *J. Immunol.* **174**, 4845–4851 (2005).
47. Megiovanni, A. M. *et al.* Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. *J. Leukoc. Biol.* **79**, 977–988 (2006).
48. van Gisbergen, K. P., Ludwig, I. S., Geijtenbeek, T. B. & van Kooyk, Y. Interactions of DC-SIGN with Mac-1 and CEACAM1 regulate contact between dendritic cells and neutrophils. *FEBS Lett.* **579**, 6159–6168 (2005).
49. 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).
50. Maffia, P. C. *et al.* Neutrophil elastase converts human immature dendritic cells into transforming growth factor-β1-secreting cells and reduces allostimulatory ability. *Am. J. Pathol.* **171**, 928–937 (2007).
51. Hess, C., Sadallah, S., Hefti, A., Landmann, R. & Schifferli, J. A. Ectosomes released by human neutrophils are specialized functional units. *J. Immunol.* **163**, 4564–4573 (1999).
52. Eken, C. *et al.* Polymorphonuclear neutrophil-derived ectosomes interfere with the maturation of monocyte-derived dendritic cells. *J. Immunol.* **180**, 817–824 (2008).
53. Gasser, O. & Schifferli, J. A. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood* **104**, 2543–2548 (2004).
54. Cheong, C. *et al.* Microbial stimulation fully differentiates monocytes to DC-SIGN/$CD209^+$ dendritic cells for immune T cell areas. *Cell* **143**, 416–429 (2010).
55. Costantini, C. & Cassatella, M. A. The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity. *J. Leukoc. Biol.* **89**, 221–233 (2011).
56. Costantini, C. *et al.* Human neutrophils interact with both 6-sulfo $LacNAc^+$ DC and NK cells to amplify NK-derived IFNγ: role of CD18, ICAM-1, and ICAM-3. *Blood* **117**, 1677–1686 (2011).
This study provides a novel perspective on the cooperative strategies used by the innate immune system in humans by showing that neutrophils can amplify the crosstalk between NK cells and a specific subset of DCs.
57. MacDonald, K. P. *et al.* Characterization of human blood dendritic cell subsets. *Blood* **100**, 4512–4520 (2002).
58. Schakel, K. Dendritic cells — why can they help and hurt us. *Exp. Dermatol.* **18**, 264–273 (2009).
59. Costantini, C. *et al.* On the potential involvement of CD11d in co-stimulating the production of interferon-γ by natural killer cells upon interaction with neutrophils via intercellular adhesion molecule-3. *Haematologica* 28 Jun 2011 (doi:10.3324/haematol.2011.044578).
60. Costantini, C. *et al.* Neutrophil activation and survival are modulated by interaction with NK cells. *Int. Immunol.* **22**, 827–838 (2010).
61. Bhatnagar, N. *et al.* Cytokine-activated NK cells inhibit PMN apoptosis and preserve their functional capacity. *Blood* **116**, 1308–1316 (2010).
62. Himmel, M. E. *et al.* Human $CD4^+$ $FOXP3^+$ regulatory T cells produce CXCL8 and recruit neutrophils. *Eur. J. Immunol.* **41**, 306–312 (2011).
63. Davey, M. S. *et al.* Human neutrophil clearance of bacterial pathogens triggers anti-microbial γδ T cell responses in early infection. *PLoS Pathog.* **7**, e1002040 (2011).
64. Abi Abdallah, D. S., Egan, C. E., Butcher, B. A. & Denkers, E. Y. Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int. Immunol.* **23**, 317–326 (2011).
65. Beauvillain, C. *et al.* Neutrophils efficiently cross-prime naive T cells *in vivo*. *Blood* **110**, 2965–2973 (2007).
66. Abadie, V. *et al.* Neutrophils rapidly migrate via lymphatics after *Mycobacterium bovis* BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood* **106**, 1843–1850 (2005).
67. Chtanova, T. *et al.* Dynamics of neutrophil migration in lymph nodes during infection. *Immunity* **29**, 487–496 (2008).
This study uses two-photon microscopy to detail the behaviour of neutrophils in lymph nodes during infection.
68. Beauvillain, C. *et al.* CCR7 is involved in the migration of neutrophils to lymph nodes. *Blood* **117**, 1196–1204 (2011).
This study identifies a potential mechanism involved in the migration of neutrophils to the draining lymph nodes.
69. Yang, C. W., Strong, B. S., Miller, M. J. & Unanue, E. R. Neutrophils influence the level of antigen presentation during the immune response to protein antigens in adjuvants. *J. Immunol.* **185**, 2927–2934 (2010).
This study unravels a regulatory function of neutrophils in lymph nodes during an immune response.
70. Serhan, C. N., Chiang, N. & Van Dyke, T. E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Rev. Immunol.* **8**, 349–361 (2008).
71. Schwab, J. M., Chiang, N., Arita, M. & Serhan, C. N. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* **447**, 869–874 (2007).
72. Spite, M. *et al.* Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature* **461**, 1287–1291 (2009).
73. Serhan, C. N. *et al.* Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J. Exp. Med.* **206**, 15–23 (2009).
74. Arita, M. *et al.* Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. *J. Immunol.* **178**, 3912–3917 (2007).
75. Ariel, A. *et al.* Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. *Nature Immunol.* **7**, 1209–1216 (2006).
76. McKimmie, C. S. *et al.* Hemopoietic cell expression of the chemokine decoy receptor D6 is dynamic and regulated by GATA1. *J. Immunol.* **181**, 3353–3363 (2008).
77. Bourke, E. *et al.* IL-1 β scavenging by the type II IL-1 decoy receptor in human neutrophils. *J. Immunol.* **170**, 5999–6005 (2003).
78. Bazzoni, F., Tamassia, N., Rossato, M. & Cassatella, M. A. Understanding the molecular mechanisms of the multifaceted IL-10-mediated anti-inflammatory response: lessons from neutrophils. *Eur. J. Immunol.* **40**, 2360–2368 (2010).
79. Fox, S., Leitch, A. E., Duffin, R., Haslett, C. & Rossi, A. G. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. *J. Innate Immun.* **2**, 216–227 (2010).
80. Jeannin, P., Jaillon, S. & Delneste, Y. Pattern recognition receptors in the immune response against dying cells. *Curr. Opin. Immunol.* **20**, 530–537 (2008).
81. Filardy, A. A. *et al.* Proinflammatory clearance of apoptotic neutrophils induces an $IL\text{-}12^{low}IL\text{-}10^{high}$ regulatory phenotype in macrophages. *J. Immunol.* **185**, 2044–2050 (2010).
82. Biswas, S. K. & Mantovani, A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nature Immunol.* **11**, 889–896 (2010).
83. Bystrom, J. *et al.* Resolution-phase macrophages possess a unique inflammatory phenotype that is controlled by cAMP. *Blood* **112**, 4117–4127 (2008).
84. Silva, M. T. When two is better than one: macrophages and neutrophils work in concert in innate immunity as complementary and cooperative partners of a myeloid phagocyte system. *J. Leukoc. Biol.* **87**, 93–106 (2010).
85. Jankowski, A., Scott, C. C. & Grinstein, S. Determinants of the phagosomal pH in neutrophils. *J. Biol. Chem.* **277**, 6059–6066 (2002).
86. Berry, M. P. *et al.* An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* **466**, 973–977 (2010).
This study highlights the importance of genome analysis in the study of pathologies. Here, a genome-wide transcriptional signature shows that neutrophils and IFN signalling are linked to the pathogenesis of tuberculosis.
87. Pedrosa, J. *et al.* Neutrophils play a protective nonphagocytic role in systemic *Mycobacterium tuberculosis* infection of mice. *Infect. Immun.* **68**, 577–583 (2000).
88. Weathington, N. M. *et al.* A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. *Nature Med.* **12**, 317–323 (2006).
89. Snelgrove, R. J. *et al.* A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. *Science* **330**, 90–94 (2010).
Here, the authors uncover a novel mechanism whereby cigarette smoke perpetuates neutrophil chemotaxis, with implications for new therapeutic strategies to treat debilitating lung disorders.

90. Alves-Filho, J. C. *et al.* Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nature Med.* **16**, 708–712 (2010).
91. Clark, S. R. *et al.* Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Med.* **13**, 463–469 (2007).
92. Massberg, S. *et al.* Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nature Med.* **16**, 887–896 (2010).
This study demonstrates a novel effector function of neutrophils by providing unequivocal *in vivo* evidence that neutrophils can activate the coagulation cascade as an antimicrobial mechanism.
93. Baetta, R. & Corsini, A. Role of polymorphonuclear neutrophils in atherosclerosis: current state and future perspectives. *Atherosclerosis* **210**, 1–13 (2010).
94. Hidalgo, A. *et al.* Heterotypic interactions enabled by polarized neutrophil microdomains mediate thromboinflammatory injury. *Nature Med.* **15**, 384–391 (2009).
95. Bennett, L. *et al.* Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* **197**, 711–723 (2003).
96. Hakkim, A. *et al.* Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc. Natl Acad. Sci. USA* **107**, 9813–9818 (2010).
97. Garcia-Romo, G. S. *et al.* Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* **3**, 73ra20 (2011).
98. Lande, R. *et al.* Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA–peptide complexes in systemic lupus erythematosus. *Sci. Transl. Med.* **3**, 73ra19 (2011).
References 97 and 98 strengthen the concept that an interaction between neutrophils and other cell types might represent a crucial pathogenic event. Crosstalk between neutrophils and pDCs could represent the driving force for the development of SLE.
99. Chen, M. & Kallenberg, C. G. ANCA-associated vasculitides — advances in pathogenesis and treatment. *Nature Rev. Rheumatol.* **6**, 653–664 (2010).
100. Kessenbrock, K. *et al.* Netting neutrophils in autoimmune small-vessel vasculitis. *Nature Med.* **15**, 623–625 (2009).
101. Chou, R. C. *et al.* Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. *Immunity* **33**, 266–278 (2010).
This study provides a detailed picture of the diverse molecules that sequentially act on neutrophils to drive their migration in a mouse model of inflammation.
102. Carlson, T., Kroenke, M., Rao, P., Lane, T. E. & Segal, B. The Th17–ELR$^+$ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease. *J. Exp. Med.* **205**, 811–823 (2008).
103. Liu, L. *et al.* CXCR2-positive neutrophils are essential for cuprizone-induced demyelination: relevance to multiple sclerosis. *Nature Neurosci.* **13**, 319–326 (2010).
104. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
105. Mantovani, A., Allavena, P., Sica, A. & Balkwill, F. Cancer-related inflammation. *Nature* **454**, 436–444 (2008).
106. Borrello, M. G. *et al.* Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc. Natl Acad. Sci. USA* **102**, 14825–14830 (2005).
107. Sparmann, A. & Bar-Sagi, D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* **6**, 447–458 (2004).
108. Kuang, D. M. *et al.* Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J. Hepatol.* **54**, 948–955 (2011).
109. Wislez, M. *et al.* Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. *Cancer Res.* **63**, 1405–1412 (2003).
110. Charles, K. A. *et al.* The tumor-promoting actions of TNF-α involve TNFR1 and IL-17 in ovarian cancer in mice and humans. *J. Clin. Invest.* **119**, 3011–3023 (2009).
111. Pekarek, L. A., Starr, B. A., Toledano, A. Y. & Schreiber, H. Inhibition of tumor growth by elimination of granulocytes. *J. Exp. Med.* **181**, 435–440 (1995).
112. Queen, M. M., Ryan, R. E., Holzer, R. G., Keller-Peck, C. R. & Jorcyk, C. L. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. *Cancer Res.* **65**, 8896–8904 (2005).
113. Houghton, A. M. *et al.* Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. *Nature Med.* **16**, 219–223 (2010).
114. Jablonska, J., Leschner, S., Westphal, K., Lienenklaus, S. & Weiss, S. Neutrophils responsive to endogenous IFN-β regulate tumor angiogenesis and growth in a mouse tumor model. *J. Clin. Invest.* **120**, 1151–1164 (2010).
This study uncovers a novel function of IFNβ, and provides a better understanding of the therapeutic effect of IFNβ treatment during the early stages of cancer development. Specifically, the authors demonstrate that, in a transplantable tumour model, endogenous IFNβ inhibits tumour angiogenesis through repression of genes encoding pro-angiogenic and homing factors in tumour-infiltrating neutrophils.
115. Keeley, E. C., Mehrad, B. & Strieter, R. M. CXC chemokines in cancer angiogenesis and metastases. *Adv. Cancer Res.* **106**, 91–111 (2010).
116. Scapini, P. *et al.* CXCL1/macrophage inflammatory protein-2-induced angiogenesis *in vivo* is mediated by neutrophil-derived vascular endothelial growth factor-A. *J. Immunol.* **172**, 5034–5040 (2004).
117. Nozawa, H., Chiu, C. & Hanahan, D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc. Natl Acad. Sci. USA* **103**, 12493–12498 (2006).
118. Shojaei, F., Singh, M., Thompson, J. D. & Ferrara, N. Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proc. Natl Acad. Sci. USA* **105**, 2640–2645 (2008).
119. Rodriguez, P. C. *et al.* Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res.* **69**, 1553–1560 (2009).
120. Cuenca, A. G. *et al.* A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma. *Mol. Med.* **17**, 281–292 (2011).
121. Welch, D. R., Schissel, D. J., Howrey, R. P. & Aeed, P. A. Tumor-elicited polymorphonuclear cells, in contrast to "normal" circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. *Proc. Natl Acad. Sci. USA* **86**, 5859–5863 (1989).
122. Colombo, M. P. *et al.* Granulocyte colony-stimulating factor gene transfer suppresses tumorigenicity of a murine adenocarcinoma *in vivo*. *J. Exp. Med.* **173**, 889–897 (1991).
123. Yousefi, S., Mihalache, C., Kozlowski, E., Schmid, I. & Simon, H. U. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ.* **16**, 1438–1444 (2009).
124. Urban, C. F. *et al.* Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog.* **5**, e1000639 (2009).
125. Fuchs, T. A. *et al.* Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol.* **176**, 231–241 (2007).
126. Beiter, K. *et al.* An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps. *Curr. Biol.* **16**, 401–407 (2006).
127. Buchanan, J. T. *et al.* DNase expression allows the pathogen group A *Streptococcus* to escape killing in neutrophil extracellular traps. *Curr. Biol.* **16**, 396–400 (2006).

Acknowledgements
This work is supported by Associazione Italiana per la Ricerca sul Cancro (AIRC), special project 5 × 1000 and the European Research Council (to A.M.); S.J. is the recipient of a Mario e Valeria Rindi Fellowship from AIRC; M.A.C. is supported by Fondazione Cariverona and AIRC. We apologize to those colleagues whose work could not be cited here owing to space limitations.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Alberto Mantovani's homepage:
http://www.humanitasricerca.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF