Neutrophils in homeostasis, immunity and cancer

José Ángel Nicolás-Ávila[1], José M. Adrover[1] and Andrés Hidalgo[1,2 †]

[1]Area of Cell and Developmental Biology, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid 20829, Spain

[2]Institute for Cardiovascular Prevention, Ludwig Maximilians University, Munich 80539, Germany.

† Corresponding author: Andrés Hidalgo (ahidalgo@cnic.es)

Area of Cell and Developmental Biology, Fundación CNIC, Calle Melchor Fernández Almagro 3, 28029 Madrid, Spain. Phone: +34 91 4531200 (Ext. 1504). Fax: +34 91 4531245

Abstract

Neutrophils were among the first leukocytes described and visualized by early immunologists. Prominent effector functions during infection and sterile inflammation classically placed them low in the immune tree as rapid, mindless aggressors with poor regulatory functions. This view is currently under reassessment as we uncover new aspects of their life cycle, and identify transcriptional and phenotypic diversity that endows them with regulatory properties that extend beyond their lifetime in the circulation. These properties are revealing unanticipated roles for neutrophils in supporting homeostasis, as well as complex disease states such as cancer. We focus this review on these emerging functions in order to define the true roles of neutrophils in homeostasis, immunity and disease.

Introduction

Already in the nineteenth century early immunologists realized that a population of circulating cells, the *microphages* or *polynuclear* leukocytes, migrated to infected tissues and aggressively captured and digested microbes (Kay, 2016; Metchnikoff, 1893). These early studies on neutrophils have remained extremely influential to this day (Underhill et al., 2016), to the point that the study of these cells in the context of infection and acute inflammation has since dominated the field.

The intervening decades have seen much progress in our understanding of the biology of these leukocytes (reviewed in (Borregaard, 2010)). Neutrophils are now known to be produced in vast numbers through a series of progressively differentiated precursors in the bone marrow, where they accumulate before being released in a circadian fashion into the bloodstream. In the circulation, neutrophils patrol tissues and initiate aggressive responses upon encountering danger signals (sterile or triggered by microbes). This is accomplished by rapid migration into the damaged tissue (Nourshargh and Alon, 2014), followed by phagocytosis and release of reactive chemicals and proteases. These properties endow neutrophils with fabulous defensive capacities, but at the same time convert them into circulating “grenades” that can damage host vessels if they overreact at the wrong time and place (Phillipson and Kubes, 2011). Perhaps to minimize collateral damage, neutrophils display a short lifespan and clear into tissues for elimination every day (Adrover et al., 2016; Zhang et al., 2015). Refinements in the neutrophil’s life cycle have been introduced in recent years (summarized in Figure 1). These refinements have revealed an exquisite balance between mobilizing and retentive signals in the bone marrow and a marked influence of the commensal microbiota in normal neutrophil physiology, and have demonstrated that migration of neutrophils into healthy tissues can regulate normal tissue homeostasis.

We review here these emerging concepts and present them in the context of recent literature. We discuss the recently appreciated diversity of neutrophils, and argue that this diversity is important to support tissue homeostasis, but can be co-opted to perpetuate pathological states.

Neutrophil diversity

Heterogeneity is a fundamental property of cells that allows functional specialization and adaptation to changing environments. In the immune system this is best characterized for lymphocytes and innate lymphoid subsets, tissue-resident macrophages, and to a lesser extent monocytes. Differentiation of leukocytes into subsets requires, however, transcriptional plasticity and time to integrate environmental signals. Because of their relatively low transcriptional activity (Scapini et al., 1999) and short lifespan (Adrover et al., 2016), it has been generally believed that neutrophils are terminally differentiated, homogeneous cells once they leave the bone marrow. This view is rapidly changing and various subsets of neutrophils, mostly defined on the basis of surface markers, have been reported (Beyrau et al., 2012; Silvestre-Roig et al., 2016). Below we summarize major sources of diversity, as they reflect the remarkable capacity of neutrophils to adapt to and to anticipate environmental changes.

Chronic inflammation and immunosuppression. While neutrophils typically present high density and sediment with the red blood cell fraction in common Ficoll density gradients, under certain conditions they appear in the low density fraction together with mononuclear cells. These low density neutrophils (LDN; also referred to as low density granulocytes or LDG) were described for the first time in the blood of patients diagnosed with systemic lupus erythematosus (SLE), rheumatoid arthritis or rheumatic fever (Hacbarth and Kajdacsy-Balla, 1986). Subsequently, LDN were found in many other physiological or pathological settings in humans, including cancer (Brandau et al., 2011; Sagiv et al., 2015), psoriasis (Lin et al., 2011), asthma (Fu et al., 2014), sepsis (Morisaki et al., 1992), ANCA-associated vasculitis (Grayson et al., 2015), pregnancy (Ssemaganda et al., 2014), malaria (Rocha et al., 2015) or HIV infection (Cloke et al., 2012).

The properties of LDN depend on the context. For example, in patients with SLE they display heightened pro-inflammatory responses with increased TNF-α expression and production of type I Interferons that impair endothelial maturation (Denny et al., 2010); in contrast, LDN in patients with cancer, infected with HIV, or in pregnant women show immune-suppressor capacities. LDN in patients with tuberculosis show increased production of reactive oxygen species (ROS) (Deng et al., 2016), while in cancer patients they display reduced oxidative burst (Sagiv et al., 2015). Some of these features may be mechanistically associated, as local delivery of ROS by neutrophils suppresses the proliferation and activation of T cells (Pillay et al., 2012). Human LDN have also been reported to be prone to release extracellular traps (NETs) (Villanueva et al., 2011) and, interestingly, the presence of autoantigens in LDN-derived NETs has been proposed to sustain chronic autoimmune inflammation (Garcia-Romo et al., 2011; Khandpur et al., 2013).

The origin of LDN remains unclear. In SLE patients, LDN are a mixture of cells with segmented or banded nuclei, and of myelocyte-like cells, which has been interpreted as LDN being relatively-immature cells that leave the bone marrow during conditions of stress (Carmona-Rivera and Kaplan, 2013; Denny et al., 2010). Alternatively, because neutrophils can reduce their density upon degranulation, LDN may represent mature degranulated neutrophils in certain settings (Deng et al., 2016; Rocha et al., 2015). At least in the context of cancer or in a mouse

model of resolving inflammation, LDN can originate either by recruitment of immature cells from the bone marrow (a process similar to the one reported for granulocytic myeloid-derived suppressor cells, or G-MDSC; (Youn et al., 2008)) or from high-density mature neutrophils (Sagiv et al., 2015). Notably, this process appears to involve increase in volume rather than degranulation, and is instigated by TGF-β, an anti-inflammatory cytokine (Sagiv et al., 2015). This intriguing set of observations may explain the convergence of density loss and gain of immune-suppressive properties during resolving and non-resolving (i.e., cancer) inflammation. However, because lupus patients display chronic inflammation, LDN from these patients are unlikely to be immunosuppressive, suggesting that the acquisition of low-density and the immunosuppressive properties may result from independent processes. This possibility, however, needs to be formally demonstrated.

Migration modalities. While a general concept in neutrophil trafficking held that migration from the vascular lumen into extravascular tissues was unidirectional, a plethora of studies demonstrate that neutrophils can migrate back into the circulation, a process referred to as *reverse migration*. More specifically, neutrophils can display reverse *transendothelial* migration (rTEM) or reverse *interstitial* migration (rIM) depending on their initial location (Nourshargh et al., 2016). rTEM has been visualized in mice (Woodfin et al., 2011), while rIM has only been demonstrated in the transparent zebra fish model (Mathias et al., 2006).

Reverse transmigrating human neutrophils show high ICAM-1 and low CXCR1 expression *in vitro*, increased ROS production, and resistance to apoptosis compared with classically extravasated neutrophils. These neutrophils differ from ICAM-1^{LO} $CXCR1^{HI}$ circulatory neutrophils and ICAM-1^{LO} $CXCR1^{LOW}$ neutrophils in tissues and show higher vascular endothelial growth factor receptor (VEGFR) 1 expression, indicating a possible role for rTEM neutrophils in angiogenesis (Buckley et al., 2006). The abundance of this subset in healthy humans is low but increases up to 1-2% in patients with rheumatoid arthritis. Imaging studies in mice found that reverse transmigration relies on the type of stimulus used to elicit inflammation and is controlled by vascular junctional adhesion molecular C (JAM-C), whose blockade, cleavage or deletion favors rTEM (Colom et al., 2015). While the physiological relevance of reverse migration remains unclear, around 1% of circulating neutrophils acquire an ICAM-1^{HI} phenotype after ischemia-reperfusion and can be found in the lungs, where they produce ROS (Woodfin et al., 2011). These observations have led to the proposal that tissue-experienced neutrophils that return to the circulation can contribute to propagating inflammation (Woodfin et al., 2011; Yoo and Huttenlocher, 2011). Alternatively, rIM may enable neutrophils to transport captured antigens to lymph nodes to initiate adaptive immune responses (Maletto et al., 2006). In summary, the prolonged lifespan and altered function of intravasating neutrophils suggests that reverse migration is a functionally relevant source of heterogeneity.

NET-forming neutrophils. Neutrophils can release DNA-based structures under certain types of stress, a phenomenon called NETosis that was originally reported to allow capture and killing of bacteria (Brinkmann et al., 2004). Since its description, the capacity of neutrophils to undergo NETosis was found to vary widely across species or physiological states, as discussed below. This variability may be clinically relevant because the presence of NETs predisposes to

thrombosis and vascular inflammation, and NETs are a source of autoantigens (Stephenson et al., 2016).

Various types of inflammatory mediators and metabolites associated with states of chronic inflammation, such as interferons and glucose, have been shown to increase the susceptibility to release NETs, and consequently human neutrophils are more prone to form NETs during SLE or diabetes (Garcia-Romo et al., 2011; Wong et al., 2015). It is unclear which factors determine classical degranulation versus NET formation and whether these processes can coexist in a given cell. It is also unclear why some neutrophils are more susceptible to produce NETs than others: human neutrophils are faster and more efficient at releasing NETs than murine neutrophils (Ermert et al., 2009); mature (but not immature) neutrophils release NETs after stimulation with IFNs and C5a (Martinelli et al., 2004); human LDN are more prone to produce NETs during SLE (Villanueva et al., 2011), and aged neutrophils (i.e., those primed by microbiota-derived products in the steady-state) produce NETs more readily than neutrophils newly released from the bone marrow (Zhang et al., 2015). In contrast, NETosis of human neutrophils is impaired upon phagocytosis of apoptotic cells (Manfredi et al., 2015), or upon phagocytosis of microbes via Dectin-1, which prevents translocation to the nucleus of elastase (Branzk et al., 2014), a serine protease that initiates NETosis through histone degradation (Papayannopoulos et al., 2010). This may represent a strategy to prevent NET-induced damage when microbes are small enough to be phagocytosed. These series of observations suggest that heterogeneity in NET formation is established based on environmental demand, and responds to defensive and physiological needs.

Angiogenesis. Subsets of neutrophils have been shown to support angiogenesis during development, normal tissue repair and pathogenic states. A subset of murine neutrophils expressing VEGFR1, the receptor for VEGF-A, is efficiently recruited to non-vascularized hypoxic tissues and promotes angiogenesis by providing the metalloprotease MMP9 to facilitate vessel penetration (Christoffersson et al., 2012; Massena et al., 2015). This proangiogenic subset displays a $CD49d^{HI}$ $CXCR4^{HI}$ profile and is similar to neutrophils that support tumor vascularization (Jablonska et al., 2010). Interestingly, in the developing mouse embryo a distinct type of Ly6B.2+ neutrophils is instructed by the venular endothelium to secrete MMP9, thereby promoting arterial-venous alignment of the skin vasculature (Kidoya et al., 2015).

Activity towards tumors. Studies in murine models of cancer have been particularly revealing in terms of neutrophil diversity. Although neutrophils are generally pro-tumorigenic during the initiation and progression of tumors, blockade of TGF-β switches their function towards an anti-tumorigenic phenotype (Fridlender et al., 2009). These functionally antagonistic populations, referred to as N2 and N1 to mirror the nomenclature of macrophages with similar activity, differ in the expression of inflammatory (TNFα and ICAM-1) and angiogenic factors (VEGF and chemokines), as well as in their ability to inhibit or support, respectively, CD8+ T cells. Consequently, depletion of neutrophils has opposite effects on tumor growth depending on the type of neutrophil present at the time of depletion (Fridlender et al., 2009). The functional properties of neutrophils in cancer are described in more detail below.

The immunosuppressive properties of the pro-tumorigenic (N2) subset are also a reported feature of the so-called granulocytic myeloid-derived suppressor cells (G-MDSC), and these may actually be overlapping populations (Bronte et al., 2016)). Throughout this manuscript we avoid using the term G-MDSC to refer to these immunosuppressive, pro-tumoral neutrophils because it is unlikely that immune suppression is their only biological function, as noted recently (Coffelt et al., 2016).

Diurnal time. In the steady-state, circulating neutrophils undergo phenotypic changes from the time they are released from the bone marrow to their disappearance from the circulation. This natural phenotypic shift is referred to as neutrophil aging, and adjusts to daily light/dark, or diurnal, cycles (Casanova-Acebes et al., 2013). Neutrophils freshly released from the bone marrow are CD62L^{HI} CXCR4^{LO} and transition to a CD62L^{LO} CXCR4^{HI} phenotype during daytime in mice (Figure 1). Other cell markers, including CD49d, TLR4, ICAM-1, CD11c, CD24 and CD45, as well as transcriptional properties, nuclear morphology, granularity and cell size also change diurnally (Casanova-Acebes et al., 2013; Zhang et al., 2015). These alterations precede the daily clearance of neutrophils into tissues during the early night, and aged neutrophils are more prone to initiate an inflammatory response, produce ROS and undergo NETosis in murine models of sepsis or sickle cell anemia (Uhl et al., 2016; Zhang et al., 2015). Thus, neutrophil diversity induced by aging is thought to contribute to derailed activation and pathologic inflammation.

While the physiological role of diurnal aging remains unclear, it may reflect an adaptation of innate immunity to the varying likelihood of exposure to pathogens at different times of the day (Adrover et al., 2016). Interestingly, aging-induced diversity in neutrophils shares striking similarities with that of monocytes: inflammatory monocytes follow diurnal variations (Nguyen et al., 2013), and lose CD62L expression as they naturally convert into non-classical monocytes (Yona et al., 2013).

Microbial interactions. Given the prominent role of neutrophils to contain infections, the large repertoire of immune sensors with which they recognize microbial products (PAMPs), and the proposed co-evolution of microbes and immune cells (Martinez-Bakker and Helm, 2015), it is not surprising that microbes dictate multiple aspects of neutrophil biology. The commensal microbiota regulates steady-state granulopoiesis by regulating the production of G-CSF, and germ-free mice are therefore profoundly neutropenic (Bugl et al., 2013). Microbiota-derived diaminopimelic acid-bearing peptides which are agonists of the nucleotide-binding oligomerization domain containing 1 (NOD1) receptor regulate the lifespan of neutrophils (Hergott et al., 2016) and prime neutrophils for efficient immune response against infections (Clarke et al., 2010). In addition, endotoxins produced by the gut microbiota enter the bloodstream to drive diurnal phenotypic changes (i.e., neutrophil aging; (Zhang et al., 2015)) as well as differentiation of B-helper neutrophils in the spleen (Puga et al., 2011). These studies in mice suggest that neutrophil diversity induced by microbial products can impact both pathological and homeostatic states.

While studying the susceptibility of mice preconditioned by mild or severe systemic inflammatory response syndrome (SIRS) to *Staphylococcus aureus* infection, three types of neutrophils with

distinct morphological, cytokine-secreting and protective properties were identified in the circulation (Tsuda et al., 2004). Neutrophils from mice with mild SIRS conferred resistance to *S. aureus* and produced pro-inflammatory cytokines (IL-12), uniquely expressed TLR5 and 8, and were CD49d+. In mice rendered susceptible by severe SIRS, neutrophils produced the anti-inflammatory cytokine IL-10, uniquely expressed TLR7, and were $CD49d^{NEG}$ $CD11b^{HI}$, while neutrophils from unchallenged mice produced undetectable levels of cytokines and expressed low levels of CD49d and CD11b. Notably, each neutrophil subset induced different polarization profiles on macrophages *in vitro*, globally suggesting that neutrophil diversity is important to generate pro-inflammatory, microbicidal or immunosuppressed environments (Tsuda et al., 2004). This is additionally relevant as the susceptibility to infection may originate from the presence of neutrophils with suppressive capacities similar to those identified in tumors (Fletcher et al., 2015; Fridlender et al., 2009). Immunomodulatory properties induced by microbial products, however, may be also important to limit the severity of a response. Humans injected with low doses of endotoxin or after a trauma rapidly recruit in blood three phenotypically distinct subsets of neutrophils, which could be discriminated by varying expression of CD62L and CD16 (Pillay et al., 2012). One of these populations, which displayed a $CD62L^{LO}$ $CD16^{HI}$ ICAM-1^{HI} profile, produced higher levels of ROS and suppressed T cell proliferation and activation.

Overall, these studies suggest that neutrophil diversity might be beneficial. For example, immunosuppressive states may limit adaptive responses to autoantigens released during acute damage, while inflammatory subsets allow containment of invading microbes; compartmentalization of neutrophil phenotypes to different times of day, in contrast, may ensure vascular protection.

The neutrophil in homeostasis

Microscopic observation of inflamed tissues offers a stunning choreography of neutrophils rapidly migrating from vessels into the parenchymal spaces. This was readily appreciated by early immunologists, who established that granulocytes were primarily effectors of acute inflammatory responses (Kay, 2016). This dominant view, in our opinion, has historically precluded rigorous examination of additional functions. Homeostatic functions of neutrophils are likely because billions are released into the bloodstream, and naturally eliminated in tissues. Accumulating evidence suggests that these cleared neutrophils may be functional in healthy tissues before their elimination.

An illustrative example of a tissue populated by neutrophils in the steady-state is the lung. Contrasting with low numbers of neutrophils within most tissues in the steady-state, relatively large numbers are found in this organ in healthy mice and non-human primates (Devi et al., 2013). Retention in the lung is not passive (i.e., caused by physical constraints) because active signaling through CXCR4 is required, and the pulmonary endothelium constitutively expresses its ligand CXCL12. Neutrophils present in the pulmonary vascular and perivascular space are poorly migratory, but can be released into the bloodstream upon treatment with CXCR4 antagonists or epinephrine (Devi et al., 2013), or prompted to infiltrate the interstitium and

airspaces during inflammation (Kreisel et al., 2010). Thus, neutrophils appear to be strategically positioned in the lungs to either supply the circulation or to respond to injury. Whether similar strategies are adopted in other tissues is not known. A wealth of recent data, however, suggests that neutrophils that populate other tissues are also endowed with homeostatic functions as discussed below (and summarized in Figure 2).

Regulation of hematopoietic niches. Neutrophils are the most abundant cells in the bone marrow, as this is the main organ where granulopoiesis occurs and a large pool of mature neutrophils is stored before release into the bloodstream. Studies of mice in parabiosis or after cell transfer demonstrated, however, that the marrow is normally infiltrated by viable neutrophils (Casanova-Acebes et al., 2013; Martin et al., 2003). Infiltration of the marrow occurs with circadian frequency and exerts an inhibitory effect on the number and activity of stromal cells devoted to supporting haematopoiesis in this organ (Casanova-Acebes et al., 2013). These cells, collectively referred to as the hematopoietic niche, regulate essential aspects of hematopoietic precursors, including retention and mobilization (Morrison and Scadden, 2014). Interestingly, diurnal infiltration of neutrophils was causally coupled to the known circadian oscillations of these precursors in the bloodstream (Casanova-Acebes et al., 2013; Mendez-Ferrer et al., 2008). Although the mechanisms by which neutrophils regulate the bone marrow stromal niche remain partially obscure, phagocytosis by medullary macrophages and activation of Liver X receptors (LXRα and β), which are cholesterol-sensing transcription factors, were shown to be required for suppression of the stromal niche. Intriguingly, the observation that circadian regulation only affects the immature hematopoietic compartment suggests that infiltrating neutrophils target specific niches and populations within the marrow. This specificity is likely the consequence of a guided tropism of infiltrating neutrophils to defined regions of the marrow, for example towards tissue-resident macrophages rather than CXCL12-producing cells or the endosteum (Casanova-Acebes et al., 2013).

Similar neutrophil-driven mechanisms regulating murine haematopoiesis have been reported outside of the bone marrow. Phagocytosis of senescent neutrophils in extramedullary tissues by resident macrophages and dendritic cells inhibits transcription of IL23, a cytokine that in turn regulates IL17 production by subsets of T cells in tissues (Stark et al., 2005). Because IL17 controls production of G-CSF, this pathway sets a “neutrostat” system that limits granulopoiesis when neutrophils are normally eliminated in tissues (von Vietinghoff and Ley, 2008). Extramedullary regulation occurs in tissues that remain to be defined, and requires adhesion receptors (selectins and β2 integrins) and chemokines (CXCL2 and CXCL5) (Mei et al., 2012; Stark et al., 2005). Like in the bone marrow, activation of LXR as well as the phagocytic receptor Mertk is required to regulate IL23 expression, G-CSF levels and granulopoiesis (Hong et al., 2012).

Neutrophils can also influence bone marrow niches through direct secretion of bioactive lipids. Stimulation of the sympathetic tone in the bone marrow of G-CSF-treated mice causes niche inhibition and stem cell mobilization (Katayama et al., 2006), but also stimulates neutrophils to release prostaglandin E2. This lipid has positive effects on pre-osteoblasts, a component of the hematopoietic niche, and therefore limits the negative suppressive functions of G-CSF (Kawano

et al., 2016). Therefore, neutrophils are multi-layered regulators of hematopoietic niches capable of integrating circadian and neural inputs.

Modulation of core metabolism. Low levels of neutrophils are present in the liver and brown adipose tissue at steady-state, but rapidly increase during experimental obesity in mice (Talukdar et al., 2012). Although obesity relates to an inflammatory state, some discoveries made in this context support a possible role of neutrophils in modulating central metabolism. For instance, neutrophil-derived elastase, a protease stored in azurophilic granules, can degrade Insulin receptor substrate 1 (IRS1) in adipocytes and hepatocytes, which induces insulin resistance and promotes lipogenesis and cholesterol synthesis (Talukdar et al., 2012). This agrees with the increased neutrophil elastase activity and decreased serum levels of α1-antitrypsin, an elastase inhibitor, in obese mice and humans (Mansuy-Aubert et al., 2013). These observations raise the possibility that basal neutrophil infiltration in the liver and brown fat regulates multiple aspects of their metabolic functions, for example by regulating AMPK signaling, fatty acid oxidation and energy expenditure through the elastase/α1AT pathway (Mansuy-Aubert et al., 2013).

Menstrual cycle and pregnancy. The physiology of the female reproductive tract (FRT) during the menstrual cycle represents a paradigm of the supportive function of neutrophils to normal organ function. Like other mucosal surfaces, the FRT must couple tolerance and immune defense, with the added challenge of successive cycles of defense against potential pathogenic invasion of the lower tract and tolerance towards sperm and the fetus (Wira et al., 2015).

The balance between the main female sex hormones, estradiol and progesterone, in the different phases of the menstrual cycle dictate the composition of immune cells and protection along the cycle (Wira et al., 2015). Many neutrophils and monocytes are present in the human endometrium during the premenstrual and menstrual periods, especially in areas of tissue breakdown (Poropatich et al., 1987). Physiological angiogenesis, a prominent feature of the FRT during the menstrual cycle, is supported by VEGF-producing neutrophils during the proliferative stage, as the endometrium rapidly grows (Gargett et al., 2001; Heryanto et al., 2004). Early studies in humans established that progesterone allows infiltration of the endometrial stroma by neutrophils during the luteal phase and during pregnancy (King et al., 1989). Progesterone additionally orchestrates the physiological infiltration of murine neutrophils into the vaginal lumen during the luteal phase to confer immune protection, whereas estradiol prevents infiltration during the follicular phase to favor fertilization (Lasarte et al., 2016). These cycles of enhanced or impaired neutrophil influx in the lower tract are ensured by dynamic gradients of the chemokine CXCL1 along the stroma and vaginal epithelium (Lasarte et al., 2016).

During the luteal phase, a temporal endocrine structure derived from ovarian follicles forms and secretes sex hormones that are critical for establishing and supporting pregnancy. This structure, the corpus luteum, appears with each menstrual cycle and is infiltrated by large numbers of neutrophils (Brannstrom et al., 1994b). These are attracted by IL-8 produced locally to support angiogenesis (Jiemtaweeboon et al., 2011), and neutrophils continue infiltrating the

corpus luteum after successful implantation of the zygote (Brannstrom et al., 1994a). Infiltrating neutrophils may be additionally important in the luteolytic phase (Shirasuna et al., 2012).

Studies in humans have shown that CD66b^{HI} neutrophils migrate into the decidua basalis during mid-gestation. Decidual-derived IL-8 programs these neutrophils for expression of VEGF-A and Arginase-1, suggesting pro-angiogenic and tolerogenic properties similar to those reported for tumor-associated neutrophils (Amsalem et al., 2014). The immunosuppressive activity of arginase-producing neutrophils, characterized by low density (see previous discussion on LDN), is likely important in term placentae for implantation and growth of the fetus as neutrophil levels are elevated in the blood of pregnant women (Kropf et al., 2007). Arginase levels are even higher in LDN present in the human cord blood when compared with neutrophils in the placenta (Ssemaganda et al., 2014) and studies in rats showed that neutrophils are located directly at the fetal-maternal interface and in the mesometrial triangle, where they associate with regions of high IL-10 expression, a cytokine that dampens the pro-inflammatory action of NK cells (Tessier et al., 2015). Collectively, these studies suggest an important role of neutrophils in the angiogenic and immunomodulatory processes that take place along the menstrual cycle and pregnancy in a way that, intriguingly, resembles what occurs in tumors.

Neutrophils as immune regulators. Although neutrophils have been classically regarded as endpoint effector cells in immunity, increasing evidence has revealed important helper properties for both adaptive and innate immunity (Figure 3).

Infections by helminths elicit type 2 responses that rely on a defined set of cytokines thought to be primarily produced by Th2 lymphocytes and non-neutrophilic granulocytes (Anthony et al., 2007). Although neutrophils are generally perceived as type 1 early effector cells, a recent study demonstrated that they efficiently promote anti-parasitic immunity. In a model of *N. brasiliensis* challenge, neutrophils recruited to infected lungs acquire a type 2 transcriptional profile, with expression of the proto-typical type 2 cytokine IL-13. Neutrophil-borne IL13 in turn promoted conversion of pulmonary macrophages towards a helminthcidal phenotype (Chen et al., 2014). Macrophage polarization driven by neutrophils in this model lasted for many weeks and allowed helminth clearance upon macrophage transfer or secondary infection, thus providing direct evidence that neutrophils can provide immune training to **macrophages**. Although the origin and relationship of these “type 2” neutrophils with other subsets is unknown, the immature appearance of their nuclei intriguingly resembles that of immunosuppressive neutrophils in cancer (Fridlender et al., 2009), or neutrophils circulating at night (Casanova-Acebes et al., 2013). Whether the programming of neutrophils towards a type 2 phenotype occurs in the infected tissue or in the bone marrow, as well as the molecular underpinnings of the process are important issues that merit future work.

Neutrophils can shape adaptive immune responses by modulating **T cell** function in several ways; they can support or inhibit lymphocyte activation and proliferation, or guide their migration in the complex anatomy of tissues. An example of the latter comes from elegant studies on influenza infection of the respiratory tract (Lim et al., 2015). In this model, neutrophils precede and facilitate CD8+ T-cell recruitment and allow correct navigation of CXCR4+ T cells through the interstitial space of the infected trachea by shedding subcellular fragments enriched in

CXCL12. These long-lasting trails, which are important for efficient viral clearance (Lim et al., 2015), could conceivably guide other immune subsets in multiple inflammatory contexts.

Another mechanism by which neutrophils may support T cell function is through local delivery of antigen to T cells in lymph nodes. In the presence of an inflammatory stimulus, murine neutrophils can uptake antigen and gain access to draining lymph nodes primarily though lymphatic vessels (Abadie et al., 2005; Maletto et al., 2006). Activation of human or mouse neutrophils induces expression of CCR7, which is in turn required for homing to lymph nodes (Beauvillain et al., 2011). Once within the lymph nodes, neutrophils bearing antigen-loaded MHCI molecules can cross-prime T cells and induce effector functions on these cells (Beauvillain et al., 2007). The overall significance of neutrophils as antigen-presenting during antigen-driven immune responses, however, remains unclear.

Besides these activating functions, numerous lines of evidence indicate suppressive functions of neutrophils towards T cells (Leliefeld et al., 2015). These suppressive functions are mostly accomplished by the production and release of reactive oxygen species (ROS) and arginase. One way by which neutrophil-derived ROS suppress T cells is by inactivation of coffilin, an actin remodeling protein, resulting in impaired formation of the immune synapse and cell activation (Klemke et al., 2008). In addition, because regulatory T cells are less sensitive to ROS (Mougiakakos et al., 2009), production of ROS by neutrophils effectively creates an immunosuppressive environment. On the other hand, arginase, an enzyme present in neutrophil granules, depletes L-arginine thereby preventing proliferation and cytokine synthesis of human T cells (Munder et al., 2006). Neutrophil degranulation can also dampen T cell responses by releasing proteases that degrade both cytokines and their receptors on T cells (Bank and Ansorge, 2001).

Large numbers of neutrophils are found in the spleen of healthy mice and humans. Splenic neutrophils express a distinct $CD62L^{LO}$ $CD11b^{HI}$ $ICAM1^{HI}$ phenotype and localize proximal to **B cells** in the marginal zone, where they form NET-like structures (Puga et al., 2011). This population of neutrophils promotes survival, class switch, somatic hyper-mutation and antibody production of B cells through a mechanism that involves production of BAFF, APRIL, IL-21 and Pentraxin 3 (Chorny et al., 2016; Puga et al., 2011). In agreement with these findings in mice, marginal zone B cells are reduced in the spleen of neutropenic humans, and display impaired maturation and immunoglobulin production during T cell-independent responses (Puga et al., 2011). Interestingly, the B-helper neutrophils only appear postnatally, upon mucosal colonization by the commensal microbiota, and are instructed by IL10 produced by splenic sinusoids (Puga et al., 2011), or by GM-CSF produced by innate lymphoid cells (Magri et al., 2014). While some of the findings in humans remain controversial (Nagelkerke et al., 2014), these data are important as they reveal neutrophil specialization in the spleen. Similar modulation of B cell activation has been reported in murine lymph nodes, in which dendritic cells and prostaglandin E2 induce recruitment of BAFF-producing neutrophils during inflammation (Parsa et al., 2016). Contrasting with these trophic functions, activation of invariant natural killer (iNK) cells by neutrophils restricts autoantibody production by splenic B cells (Hagglof et al., 2016), therefore adding complexity to the regulatory functions of neutrophils on adaptive immunity.

Finally, neutrophils support differentiation and maturation of **natural killer cells** (NK), an innate effector cell type with potent cytotoxic functions during type 1 responses. Analysis of mice from a mutagenesis screening found that NK cells in neutropenic mice are immature and hypo-responsive, a finding that was corroborated in humans (Jaeger et al., 2012). Paradoxically, neutrophils have also been shown to impair maturation and cytokine production of mouse and human NK cells through a mechanism proposed to require cell-cell contact (Wingender et al., 2012) and cleavage of NKp46 by cathepsin G (Valayer et al., 2016).

As the list of regulatory functions of neutrophils in the immune system continues to expand, we note the remarkable ambivalent effects on every immune cell subset inspected thus far. Yet again, the different and even antagonistic functions exerted by neutrophils on specific immune cell subsets likely reflect their functional diversity. This in turn highlights the notion that neutrophils are highly adaptable to physiological needs, a feature that contributes to disease, as discussed below.

The neutrophil as instigator of chronic disease

The causal contribution of neutrophils to multiple chronic pathologies has only recently emerged. We discuss here several of these non-resolving chronic inflammatory processes in which neutrophils are prominent players, and place special emphasis on cancer, a disease that best exemplifies how disease may exploit neutrophil diversity to perpetuate itself. We make the point that early pathogenic stimuli co-opt the normally beneficial properties of neutrophils (presented in the previous section) to propagate disease.

Atherosclerosis is an inflammatory disease of large and medium-sized arteries that underlies a large number of acute cardiovascular manifestations, and is triggered by excessive cholesterol levels. Among immune cells, macrophages were pointed as almost exclusive culprits of plaque buildup in affected vessels (Libby et al., 2013). Although at much lower frequency, neutrophils can also be found in developing or established lesions in humans and mouse models, and their numbers correlate with features of rupture-prone plaques (Chevre et al., 2014; Ionita et al., 2010). Experimental increase or decrease in neutrophil numbers showed significant correlation with the atherosclerotic plaque size (Drechsler et al., 2010; Zernecke et al., 2008), a finding that agrees with the clinical correlation between neutrophil counts in blood and susceptibility to cardiovascular disease (Coller, 2005). The profile of pro-atherogenic neutrophils is clearly pro-inflammatory; they are recruited early in the arterial lesions and may incite inflammation by capturing activated platelets (Chevre et al., 2014), and by depositing cytokines and alarmins that attract monocytes (Wantha et al., 2013). Although the lack of satisfactory murine models of plaque rupture and thrombosis have precluded direct examination, the toxic and tissue-degrading activity of neutrophils over the activated endothelium (Saffarzadeh et al., 2012) together with correlative studies in human plaque samples (Ionita et al., 2010; Naruko et al., 2002) argue that neutrophils may be also active in the final stages of atherothrombosis.

Undefined factors present in the plasma of atherosclerotic mice induce NETosis (Knight et al., 2014), a process that requires histone deamination by the peptidylarginine deiminase 4 (PAD4). Granular proteins and histones present in NETs promote thrombosis (Fuchs et al., 2010) and activate human plasmacytoid dendritic cells (pDC) to produce IFNα (Garcia-Romo et al., 2011). Not surprisingly, blocking NETosis by continued inhibition of PAD4 delays atherogenesis and protects from arterial thrombosis in murine models (Knight et al., 2014). Additionally, NETosis induced by cholesterol crystals present in athero-prone mice or humans prime macrophages to produce IL1β, a cytokine that stimulates Th17 activation and perpetuates the influx of inflammatory cells (Warnatsch et al., 2015).

Neutrophils have been also shown to participate in systemic lupus erythematosus (SLE), an autoimmune disorder previously believed to exclusively engage adaptive immunity. Self-reactive antibodies in the plasma of SLE patients induce NETosis (Lande et al., 2011)). The released protein-DNA complexes cause activation of pDC through TLR signaling, which produce INFα, IL-6 and TNFα to further promote auto-antibody production, thereby engaging in a self-feeding loop that perpetuates disease (Garcia-Romo et al., 2011; Lande et al., 2011). Alterations in the normal mitochondrial disposal in neutrophils from SLE patients and release of oxidized mitochondrial nucleoids can also drive interferon production and generation of autoantigens (Caielli et al., 2016). This series of observations may explain the marked susceptibility of SLE patients to atherosclerosis (Denny et al., 2007), and illustrate the capacity of neutrophils to expel intracellular proteins (in NETs) and to amplify inflammation as driving forces during chronic inflammatory disorders.

Chronic low-grade inflammation underlies insulin resistance in type 2 diabetes (Gregor and Hotamisligil, 2011). As discussed above, neutrophils infiltrate the adipose tissue and livers of obese mice and secrete elastase that degrades IRS1 in adipocytes or hepatocytes, resulting in loss of insulin signaling and elevated transcription of inflammatory genes. Consequently, mice deficient in neutrophil elastase show protection from obesity-induced inflammation and improved glucose tolerance (Talukdar et al., 2012), while neutrophil infiltration is required for liver steatosis and glucose intolerance (Gonzalez-Teran et al., 2016). Notably, neutrophils can also delay wound healing, a clinical manifestations in diabetic patients. High glucose levels renders neutrophils susceptible to NETosis. NETs, in turn, are responsible for delayed healing, as this defect was corrected in PAD4-deficient mice, or by treatment with DNAse (Wong et al., 2015).

In the central nervous system, derailed inflammation is prevented by tight sealing of the blood-brain barrier, yet neurodegenerative disorders are now known to have a strong inflammatory component (Czirr and Wyss-Coray, 2012). Elegant recent studies have identified a causal role for neutrophils in some of the most prevalent neurodegenerative disorders. Brains from transgenic mice that reproduce the cardinal features of Alzheimer's disease are infiltrated by neutrophils (Baik et al., 2014), a process that requires specific activation of the integrin LFA-1 by amyloid β peptides. Infiltrating neutrophils cast NETs and produce IL17, and are ultimately responsible for cognitive loss in these models because their depletion, or inhibition of LFA-1, improved memory and long-term cognitive performance (Zenaro et al., 2015). Epilepsy, a prevalent form of chronic neurological disorder characterized by recurrent seizures, was also

shown to involve leukocyte recruitment as well as increased vascular permeability (Rossi et al., 2011). Notably, depletion of neutrophils (using a not fully specific antibody) reduced both acute and recurrent seizures in mice (Fabene et al., 2008). Thus, neutrophils together with brain-resident macrophages (microglia) contribute to long-term dysfunction of the central nervous system.

Neutrophils in cancer

Cancer is a chronic disease that critically relies on the interplay of tumoral cells with their environment. Cancer presents with inflammation and the inflammatory response is an important factor for the development of tumors (Mantovani et al., 2008). Compared to other immune cells, neutrophils have traditionally received little attention in the field, partly because their limited lifespan seemed at odds with the chronic nature of cancer. Experimental evidence generated in the past decade, however, unambiguously support a causal role for neutrophils in the initiation, growth and metastatic spread of tumors (Figure 4). A large body of clinical evidence also indicates that neutrophils are involved in tumor progression, and a negative association between number of tumor-associated neutrophils (TAN) and prognosis has been evidenced for many types of cancer including melanoma, renal carcinoma, colorectal cancer, gastric or pancreatic ductal carcinoma, hepatocellular carcinoma, intrahepatic cholangiocarcinoma or head and neck cancer (Shen et al., 2014). Meta-analyses of data from over 40,000 patients have further demonstrated significant correlation between circulating neutrophil counts (or rather, neutrophil-to-lymphocyte ratios) and overall survival of patients with solid and hematological tumors (Templeton et al., 2014).

Early tumorigenesis. Besides the initial genetic predisposition of parenchymal cells, the development of tumors requires environmental stress. Many experimental models of cancer rely on chemical irritants that induce inflammation. Pre-oncogenic mutations also initiate recruitment of inflammatory cells, which in turn secrete factors that favor proliferation and inhibit apoptosis of premalignant cells (Elinav et al., 2013). This is the case of myeloid cell-derived IL6 which stimulates Stat3 signaling in various tumor models in mice (Elinav et al., 2013; Grivennikov et al., 2009). Among inflammatory cells, neutrophils are particularly important for tumor initiation: ligands for CXRC2 (a receptor with highest expression on neutrophils) are abundant in early mouse and human tumors, and deficiency in CXCR2 or neutrophil depletion potently inhibits tumor formation in mice (Jamieson et al., 2012; Katoh et al., 2013). Neutrophils additionally support early tumorigenesis through secretion of oxygen and nitrogen radicals that cause DNA damage (Knaapen et al., 2006; Mangerich et al., 2012). Neutrophil-derived proteases, namely MMP9 and elastase, have also been implicated in tumor initiation, by either favoring early angiogenesis (Deryugina et al., 2014) or directly unleashing tumor proliferation (Houghton et al., 2010), respectively.

Tumor growth. The anti-tumoral properties of neutrophils were appreciated over 30 years ago when purified neutrophils were shown to kill tumoral cells (Gerrard et al., 1981). During the early stages of cancer, human and murine neutrophils perform primarily anti-tumoral functions by stimulating T cell responses and releasing pro-inflammatory factors such as IL-8, TNFα and IL-

6, which amplify the tumoricidal response (Eruslanov et al., 2014; Mishalian et al., 2014). The importance of anti-tumoral responses driven by neutrophils is highlighted by the observation that depletion of neutrophils in certain settings results in enhanced tumoral growth in mice (Fridlender et al., 2009; Kousis et al., 2007; Suttmann et al., 2006). In various cancer models, signals delivered in part by the MET proto-oncogen expressed in mouse and human neutrophils promote migration towards the tumor and production of nitric oxide, resulting in global anti-tumoral effects (Finisguerra et al., 2015). These series of studies, however, contrast with the growing appreciation that tumor-associated neutrophils (TANs) are pro-tumorigenic. The possibility that neutrophils switch both their phenotype and localization from peripheral to intratumoral as the tumor progresses may add to the current discrepancy in various experimental models (Mishalian et al., 2013).

In the context of experimental cancer, multiple lines of evidence indicate that signals delivered directly or indirectly by the tumor instruct pro-tumoral properties in neutrophils. TGFβ produced by the tumor polarizes neutrophils to a permissive phenotype (Fridlender et al., 2009); consequently, inhibition of TGFβ recruited a population of anti-tumoral (N1) neutrophils with distinct phenotypic, morphological and transcriptional properties, such that depletion of this neutrophil subset enhanced tumor growth. In other mouse models, tumors subvert the G-CSF neutrostat by inducing IL17 expression in intratumoral γδ T cells (Coffelt et al., 2015) . In this case, G-CSF acts in concert with IL-6 to both enhance neutrophil recruitment and to switch the properties of neutrophils from cytotoxic to pro-angiogenic in a process that requires STAT3 activation (Yan et al., 2013).

While the phenotype of pro-tumoral neutrophils is not precisely defined, certain mechanisms are now accepted to mediate this supportive function (Figure 4). In mice, TAN can secrete matrix metalloproteinase-9 (MMP9), which releases VEGF and FGF2 from the extracellular matrix, thereby promoting angiogenesis (Bergers et al., 2000; Nozawa et al., 2006). MMP9 expression in neutrophils is triggered by tumor-borne CXCL8, which potentiates angiogenesis and cleavage of CXCL8 to create a more active form, thereby creating a positive feed-back loop (Van den Steen et al., 2000). Neutrophils can also directly secrete VEGF or Bv8 to directly promote tumor vascularization (Jablonska et al., 2005; Shojaei et al., 2008). Alternatively, production of arginase 1 by neutrophils inhibits CD8+ T cell expansion and this prevents anti-tumoral immune responses in mice (Fletcher et al., 2015). Interestingly, in a model of autochthonous lung adenocarcinoma, neutrophils were shown to deliver elastase into mouse and human tumor cells, which targeted IRS1 for degradation and promoted cell proliferation (Houghton et al., 2010). Together, these observations suggest that an elaborate cellular program endows neutrophils with the ability to support tumor growth. Future studies need to define how this program varies among different types of tumors, and to what extent neutrophil programming is directly driven by the tumor.

A reverse reprogramming effect has also been reported and shown to be exerted by interferons (IFN); IFNγ and GM-CSF induce an anti-tumoral phenotype in human neutrophils capable of cross-presenting antigens and triggering and augmenting T cell responses (Singhal et al., 2016). Mice deficient in IFNβ show enhanced tumor-infiltration by N2-like neutrophils, suggesting that this cytokine counteracts the pro-tumoral functions of neutrophils by repressing

expression of Stat3 and c-Myc (Jablonska et al., 2010). IFNβ has also been shown to regulate CXCR2-mediated recruitment of neutrophils into tumors (Jablonska et al., 2014) and to limit the lifespan of mouse and human TAN (Andzinski et al., 2016).

Metastasis. As in the case of tumor growth, there is evidence for antagonistic and supportive functions of neutrophils during tumor spread. Neutrophils can mediate anti-metastatic activity by eliminating tumor cells at the pre-metastatic site (Granot et al., 2011; Lopez-Lago et al., 2013). Most evidence, however, suggests that neutrophils favor metastasis (Coffelt et al., 2016; Wu et al., 2015). Studies in mice have shown that these pro-metastatic functions are accomplished through suppression of CD8+ cytotoxic T cell responses in breast cancer (Coffelt et al., 2015) or inhibition of NK cell activity in the pulmonary microcirculation (Spiegel et al., 2016), by trapping circulating carcinoma cells through NETs thus favoring the invasion and formation of micrometastases in the liver or lung (Cools-Lartigue et al., 2013; Park et al., 2016), or by direct bridging interactions between cancer cells and the liver parenchyma through Mac-1 (Spicer et al., 2012). Other pro-metastatic mechanisms involve the production of survival factors by infiltrating neutrophils that favor invasion or survival of the metastasis-initiating cells. For example, secretion of leukotrienes favors metastatic growth in the lung through selective expansion of cells with high tumorigenic potential (Wculek and Malanchi, 2015), the alarmins S100A8/9 improve survival and chemoresistance (Acharyya et al., 2012), and production of IL1β activates the tumoral vasculature to enhance the migration of cancerous cells in mice (Spiegel et al., 2016).

An outstanding issue in this rapidly growing field is how neutrophils are recruited by the tumor in the first place. Tumors induce granulopoiesis through mechanisms that are not fully understood, although the signals appear to emanate from the primary tumor itself, as surgical removal of the tumor normalizes granulopoiesis in mice (Coffelt et al., 2015). In some models, tumors can enhance granulopoiesis by IL1β which elicits IL17 expression locally by γδT cells and production of G-CSF (Coffelt et al., 2015). Tumor-associated mesenchymal cells also contribute to recruiting neutrophils to the tumor by producing strong CXCR1/2 agonists (Sparmann and Bar-Sagi, 2004; Yu et al., 2016). Finally, while deployment of neutrophils from the bone marrow in most models is likely essential, another important source of tumoral neutrophils (and other myeloid cells) is the spleen, as evidenced by potent impairments in tumor growth after splenectomy (Cortez-Retamozo et al., 2012).

The various mechanisms by which neutrophils support tumor progression, including angiogenesis, suppression of immune responses, tissue remodeling and release of proliferative factors are remarkably similar to those occurring during homeostasis in the FRT. In contrast, the chronic inflammatory state that characterizes metabolic, neurodegenerative or autoimmune disease appears to exploit the armamentarium used by neutrophils to combat microbial pathogens. Defining how early signals co-opt one or the other properties of neutrophils represents an avenue of tremendous therapeutic potential.

Concluding remarks

We have reviewed here a substantial body of work generated in the last decade that, after a century of fundamental discoveries, provides a more nuanced understanding of the biology of neutrophils. These findings are important because they are re-shaping our perception of the role of these leukocytes in immunity, and because they identify new mechanisms by which neutrophils contribute to chronic disease.

Although outstanding questions and areas of future research are outlined in the text, we emphasize three themes that, in our opinion, deserve immediate attention. First, while the concept of diversity provides a useful framework to define how the varying properties of neutrophils contribute to different pathological and physiological states, direct evidence linking phenotype with function is still lacking. Second, the source of this diversity in homeostatic and diseased states needs to be assessed. Defining whether mature extramedullary neutrophils are plastic enough to undergo reprogramming, or whether programs are imprinted in progenitors that develop in the bone marrow, might enable the generation of neutrophils with immunotherapeutic potential. Finally, the dynamics and functions of neutrophils in healthy tissues remain understudied. The observation that some tissues are normally infiltrated and regulated by neutrophils raises the possibility that there are many more physiological processes under their control than anticipated.

Immunologists of the nineteenth century were fascinated by the dynamism and protective power of *microphages*. Many decades later, as our knowledge of neutrophils changes, our fascination with these leukocytes endures.

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Figure legends

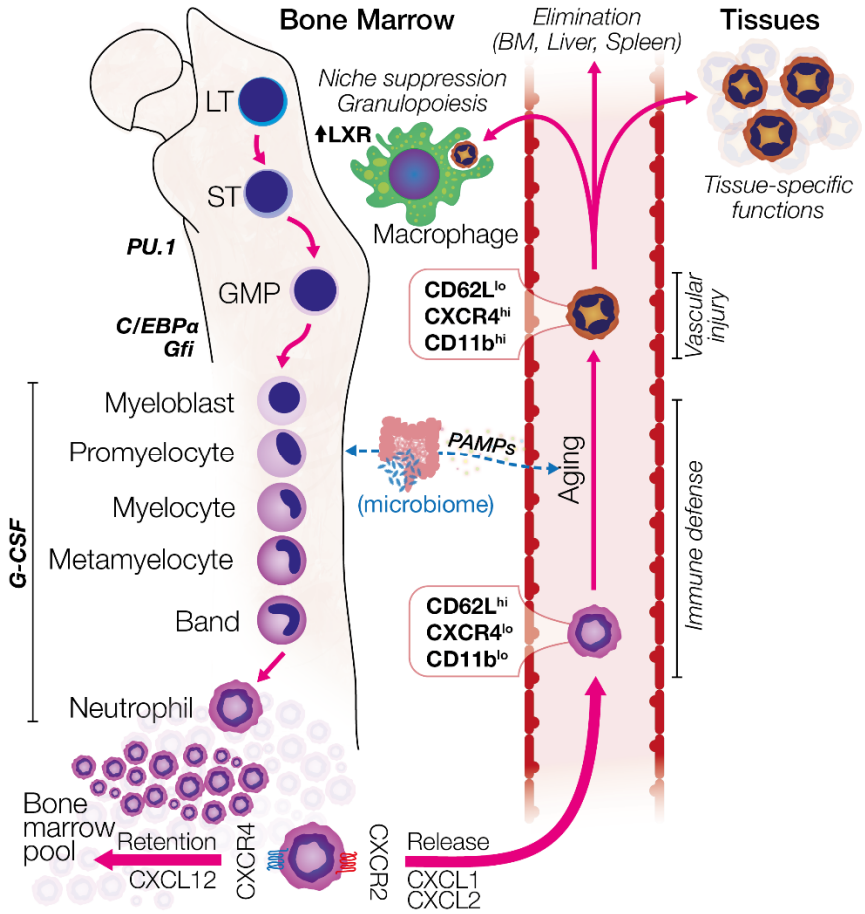

Figure 1. A revised life cycle model of neutrophils.
Neutrophils are produced in the bone marrow through a series of progenitors, from the most immature long- or short-term (LT/ST) stem cells and granulocyte-macrophage progenitors (GMP), to committed progenitors which finally generate mature neutrophils. This process of proliferation and differentiation is driven at different stages by several transcription factors (PU.1, C/EBPα, Gfi and C/EBPε), the cytokine G-CSF, and is regulated by the microbiome. Newly generated neutrophils are stored in the marrow for several days before being released into the circulation. Release is orchestrated by opposing retentive and mobilizing signals controlled by CXCR4 and CXCR2, respectively. Once in the circulation, neutrophils quickly change phenotype and function through a process of *aging* that is driven by products released by the commensal microbiota (PAMPs) and promotes vascular injury. After about 12 hours, neutrophils are ready to leave the bloodstream and are targeted to the bone marrow, liver and spleen for elimination. In the bone marrow and in some tissues, neutrophils are phagocytosed by resident macrophages to regulate granulopoiesis or inhibit the hematopoietic niche, both of which require activation of LXR nuclear receptors. Alternatively, neutrophils may infiltrate other tissues to execute tissue-specific functions. G-CSF, granulocyte colony-stimulating factor; PAMP, pathogen-associated molecular patterns.

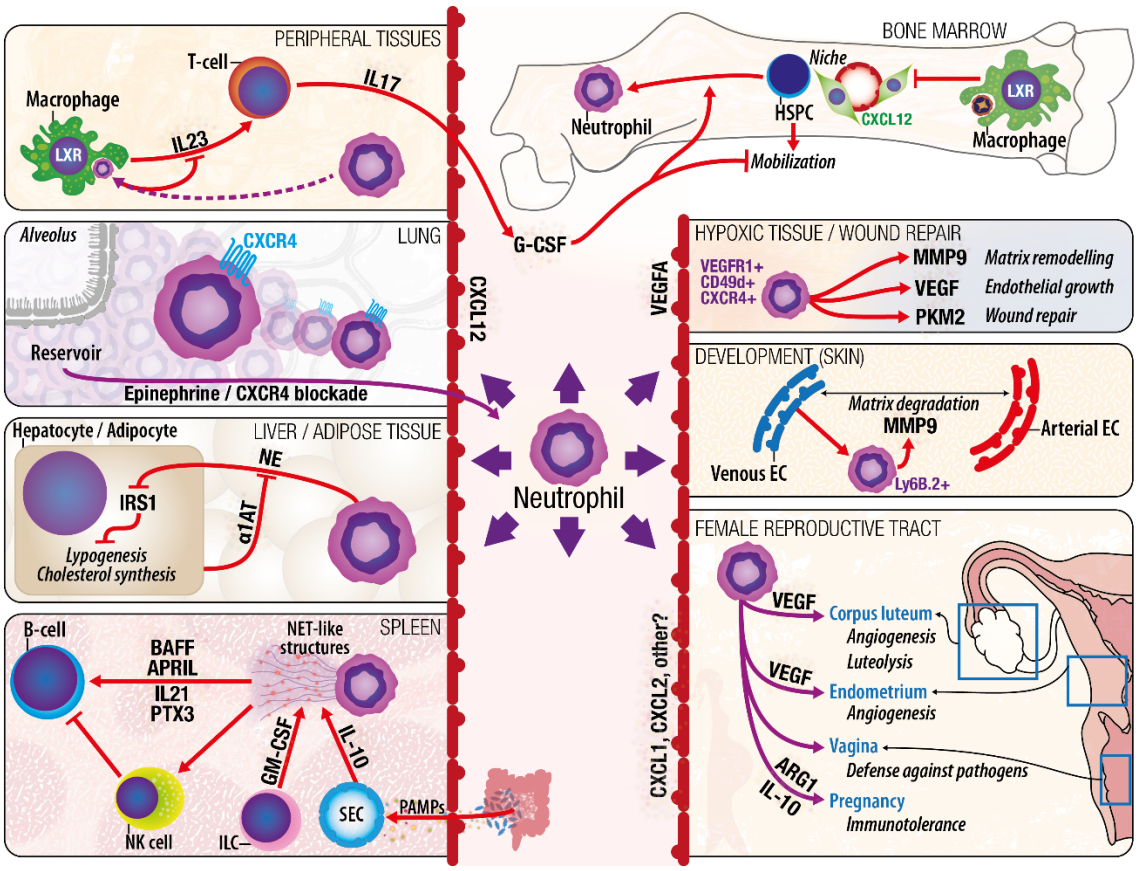

Figure 2. Neutrophils in tissue homeostasis.
After a short lifetime in the circulation, neutrophils enter multiple tissues, in which they may perform specialized functions. Engulfment by macrophages in the bone marrow cause negative regulation of hematopoietic niche cells, resulting in niche inhibition and mobilization of hematopoietic stem and progenitor cells (HSPCs) into blood. Phagocytosis of infiltrating neutrophils by macrophages in peripheral tissues remotely regulates granulopoiesis through activation of LXR receptors and downregulation of the IL23/IL17/G-CSF axis. In the lungs, neutrophils are retained by endothelial-derived CXCL12 and form a reservoir that can be mobilized into the bloodstream by certain stimuli. In the liver and adipose tissue, neutrophil-derived elastase (NE) regulates lipid metabolism through IRS1 cleavage, a process that is antagonized by the elastase inhibitor α1-antitrypsin (α1AT). Neutrophils in the spleen form NET-like structures and secrete cytokines that promote B cell maturation and antibody production. VGEFR1+ CD49d+ CXCR4+ neutrophils support angiogenesis in hypoxic tissues through production of MMP9, VEGF and PKM2. In embryonic life, the venular endothelium instructs immature neutrophils to secrete MMP9 to enable arterial alignment in the skin. In the female reproductive tract neutrophils regulate multiple processes: after ovulation, recruited neutrophils promote angiogenesis to support growth of the endometrium and corpus luteum. Along the menstrual cycle, neutrophil recruitment is differentially regulated by sex hormones to protect the vaginal lumen from infections while allowing survival of the sperm. During pregnancy, neutrophils favor an immunosuppressive state to protect the fetus.

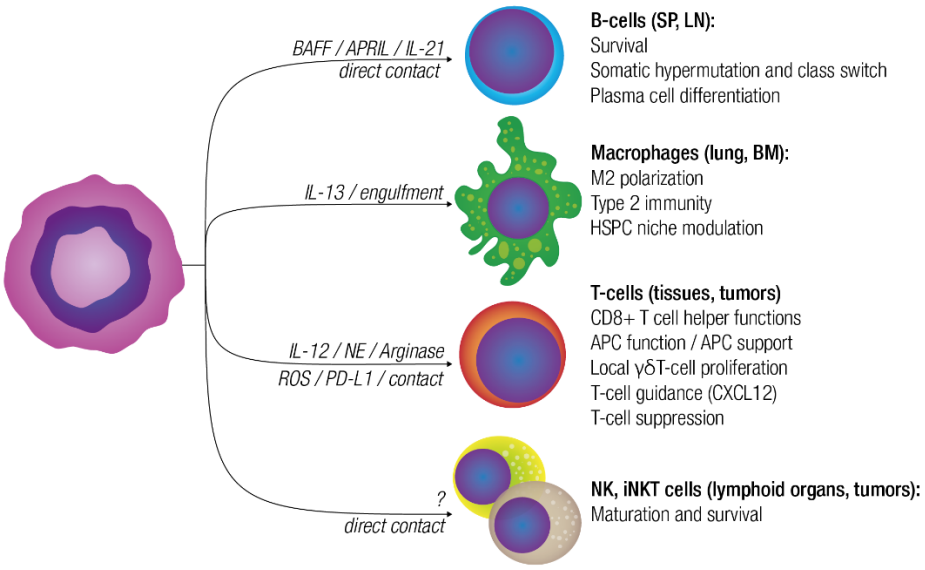

Figure 3. Neutrophils as immune helpers.
Although classically considered endpoint effector cells, neutrophils have the capacity to modulate other immune cells. Neutrophils produce factors in the marginal zone of the spleen (SP) or in lymph nodes (LN) that induce the survival and functional maturation of B cells. During helminth infections in the lung they induce long-term reprogramming of macrophages through IL-13 to elicit anti-parasitic functions, while in the bone marrow (BM) they are engulfed by macrophages to regulate the niche for hematopoietic stem and progenitor cells (HSPC). In inflamed tissues or in tumors, neutrophils guide the migration of or suppress T cell function to favor viral clearance or tumor growth, respectively. The survival and functional maturation of natural killer cells (NK) in tissues and tumors is also regulated by neutrophils through poorly defined signals, which may require cell-cell contact. APC, antigen-presenting cells; ROS, reactive oxygen species; NE, neutrophil elastase.

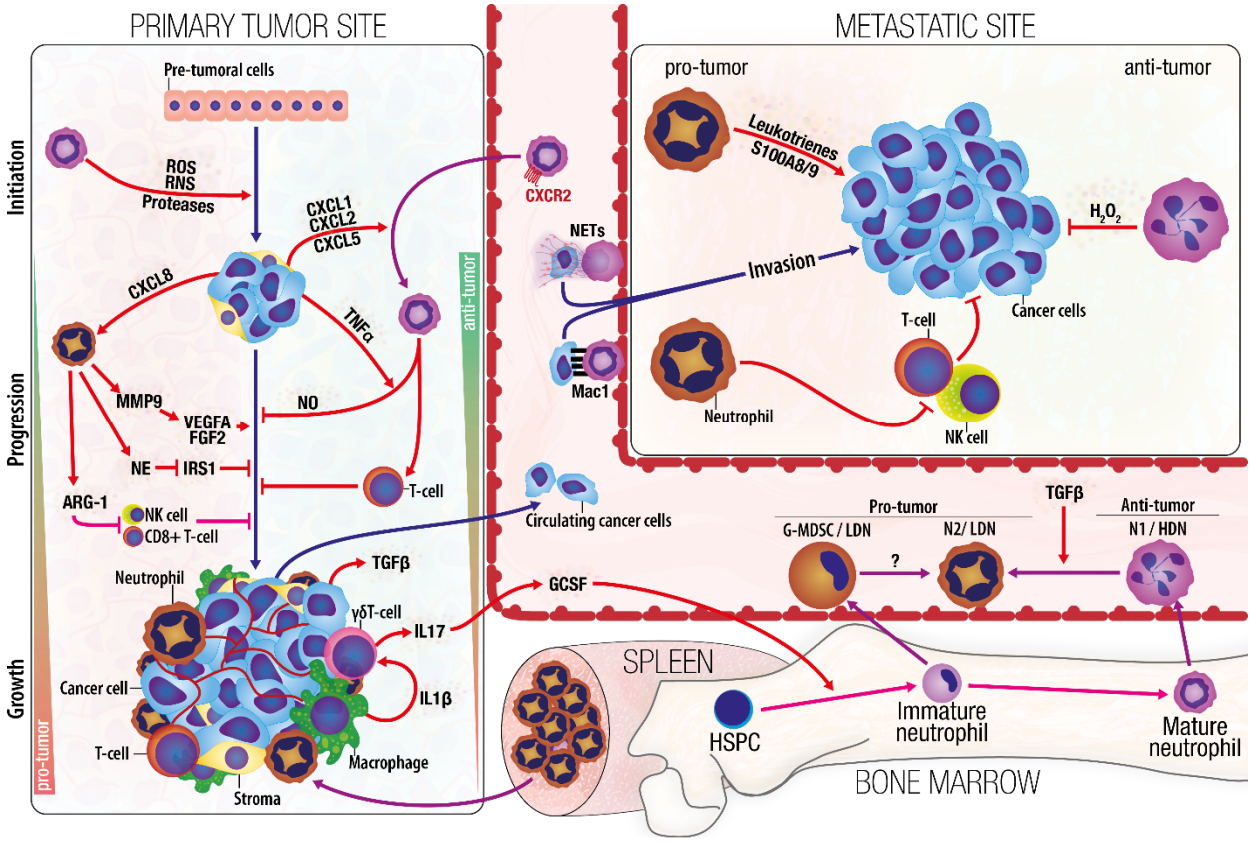

Figure 4. Neutrophils in cancer.
Neutrophils influence the tumor environment and cancer progression through multiple mechanisms. At the primary tumor site (left box), activated neutrophils can induce genetic damage or signaling in pre-tumoral cells through reactive oxygen species (ROS), reactive nitrogen species (RNS) and proteases, thereby promoting tumorigenesis (Initiation). In primary tumors, neutrophils can prevent tumor progression by activating cytotoxic immunity or nitric oxide (NO) production. As the tumor progresses, neutrophils become predominantly pro-tumorigenic: transfer of elastase (NE) activates proliferation within tumor cells; arginase-1 (ARG1) suppress CD8+ T cells- and NK cells-responses; and release of MMP9 activates VEGFA and FGF2 to support angiogenesis. As the tumor grows, cancer cells and the supporting stromal produce tumor-supporting factors: macrophages release IL1β that induces IL17 production by intratumoral γδ T cells, resulting G-CSF-dependent expansion and recruitment of pro-tumoral neutrophils from the bone marrow or the spleen; TGF-β programs immune competent neutrophils (N1) towards an immunosuppressive (N2) state. Neutrophils also influence tumor metastasis in negative and positive ways (right box). Production of hydrogen peroxide (H_2O_2) is toxic for metastatic cells. In contrast, capture of circulating cancer cells through neutrophil-derived Mac-1 or NETs favors their entry into tissues; and inhibition of natural killer (NK) and T cells responses support the survival of metastatic cells, whose proliferation is additionally favored by neutrophil-derived leukotrienes.