The Neutrophil life cycle

Andrés Hidalgo[1,2,*], Edwin R Chilvers[3*], Charlotte Summers[4*], Leo Koenderman[5*]

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

[2] Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximillians-Universität München and German Centre for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Munich, Germany

[3] National Heart and Lung Institute, Imperial College London, UK

[4] Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK

[5] Laboratory of Translational Immunology, Department of Respiratory Medicine, University Medical Centre Utrecht, Utrecht, Netherlands

* Corresponding author

Acknowledgements

The work in the authors laboratories is funded by (ERC) Medical Research Council, Wellcome Trust, GlaxoSmithKline, MedImmune, the NIHR Cambridge Biomedical Research Centre, British Heart Foundation, National Institute for Health, Cambridge NIHR Bioledical Research Centre, the MCIU (Ministerio de Ciencia, Innovación y Universidades), the Pro-CNIC Foundation, the Dutch Science Agenda (NWA) and the Netherlands Organization for Scientific Research (NWO). The CNIC is a Severo Ochoa Center of Excellence (MEIC award SEV-2015-0505).

Glossary

CMP, or common myeloid progenitors are a type of hematopoietic progenitors that give rise to all myeloid-lineage cells in adult hematopoiesis; **CGD**, chronic granulomatous disease is an immunodeficiency characterized by mutations in genes needed for the generation of reactive oxygen species in granulocytes; **Chédiak-Higashi syndrome** is caused by deficiency in a gene required for lysosomal trafficking and phagocytosis that results in immune-deficiency and albinism; **Lazy pool,** is a subset of neutrophil progenitors that rarely divide in the steady-state but activate upon acute demand for granulopoiesis; **CyTOF** or mass cytometry is a technique that combines mass spectrometry and flow cytometry thus allowing multiparametric (>30) assessment of cell markers; **Neutrophil progenitors,** are a group of hematopoietic cells that are already committed to the neutrophil lineage by successive proliferation and/or differentiation; **Rapid mobilizable pool**, is a population of neutrophils in the bone marrow that are rapidly mobilized into the circulation during stress conditions; **Neutrophil rejuvenation** refers to the appearance of immature forms of neutrophils in the blood after release in the bloodstream; **Granulocyte-MDSC**, or granulocytic myeloid-derived suppressor cells is a subset of neutrophils with T cell-suppressive activity; **Conveyor belt-like model**, a model of granulopoiesis in which immature, but not proliferative, neutrophils give rise to a progressively mature neutrophil; **Cytospin** a preparation of cells that have been centrifuged on a slide for staining and morphological evaluation; **Marginated pool,** a population of intravascular neutrophils that is adhered to the endothelial lining and is not free flowing in the bloodstream; **Priming**, an intermediate activation state of neutrophils that involves active intracellular signaling; **Pulse-chase experiment**, an approach typically based on metabolic labeling of cells to follow their dynamics in live animals; **Endothelial selectins**, are two receptors (E- and P-selectins) present on endothelial cells that enable leukocyte rolling under flow conditions; **Rolling-defective neutrophils,** are neutrophils that lack the glycoprotein ligands that engage selectins during the rolling process; **Granulopoiesis**, the process of generation of neutrophils; **Hematopoietic stem and progenitor cells (HPSC)** are a rare population of hematopoietic cells that can give rise to all blood lineages; **Leukocyte adhesion deficiency**, a group of genetic disorders that affect the capacity of leukocytes to roll or adhere on the vascular endothelium; **Intravascular crawling**, is a type of cell migration on the endothelial surface; **Neutrophil re-programming**, is a theoretical phenomenon whereby neutrophils change their phenotype and function; **Hepatic marginated pool,** is the group of leukocytes found within the liver microvasculature; **Kupffer cells,** macrophages of the liver; **Granule proteins** is a group of enzymes and anti-microbial proteins found within cytoplasmic granules; **NETs**, or neutrophil extracellular traps are DNA-based structures that are released by activated neutrophils and have microbicidal and pro-thrombotic properties.
Leo, CARS and SIRS need to be introduced here

Abstract

Neutrophils are an essential part of the innate immune system that forms the first line of defense against invading micro-organisms. Contrary to the consensus on the physiological importance of these cells, active debate still exists regarding their life-cycle. Neutrophils first differentiate in the bone marrow from a common myeloid progenitor (CMP) into mature cells. The size of the neutrophil pool outside the bone marrow determines the rate of production of these cells, which is gauged by macrophages via the IL23/IL-17/G-CSF axis. Once mobilized from the BM, neutrophils redistribute into many tissues in homeostasis and upon immune challenge with poorly understood kinetics. At the end of their physiological life, neutrophils are cleared by macrophages both in the BM, liver, spleen and other distant sites. This review describes the dynamic distribution of neutrophils across tissues in health and disease, and emphasizes the differences between humans and model organisms. It also highlights gaps in knowledge that should be fulfilled in the near future if we intend to exploit the unique features of neutrophils in the clinic.

Although there is consensus in recognizing neutrophils as an essential part of the innate immune response, active debate still exists regarding their life-cycle. Neutrophils first differentiate in the bone marrow through a series of progenitor intermediaries before entering the peripheral blood, in a process that gauges the extramedullary pool size via the IL23/IL-17/G-CSF axis. Once believed to be directly eliminated in the marrow, liver and spleen after circulating for less than one day, neutrophils are now known to redistribute into multiple tissues with poorly understood kinetics. This review provides an update on the dynamic distribution of neutrophils across tissues in health and disease, and emphasizes differences between humans and model organisms. We further highlight issues to be addressed to exploit the neutrophil's unique features in the clinic.

Highlights box

- Neutrophils are produced by committed progenitors in the bone marrow
- The lifetime of neutrophils remains controversial
- Neutrophils are found in most healthy tissues at varying numbers
- The mechanisms of neutrophil extravasation into tissues differ in homeostasis and disease
- Manipulation of the neutrophil life-cycle is a promising strategy for the treatment of inflammatory diseases

- Differences among species may reflect the remarkable sensitivity of neutrophils to environmental conditions.

1. **The neutrophil as a double-edged sword in health and disease.**

Neutrophils (also named polymorphonuclear leukocytes) are phagocytes that play an essential role in defending the host against invading pathogens, particularly bacteria and fungi (Kolaczkowska and Kubes, 2013; Scapini et al., 2016). The killing of these organisms in phagosomes is mediated by 1) fusion with lysosomes (granules) liberating cytotoxic proteins/peptides/enzymes into the phagolysosome (Cowland and Borregaard, 2016), and 2) activation of a membrane bound NADPH-oxidase producing superoxide anions (O_2^-) that in turn are metabolized into hydrogen peroxide (H_2O_2) and other reactive oxygen species (Babior et al., 1973). The cells employ these mechanisms both inside the phagolysosome as well as outside the cell. In the latter process the fusion of granules (degranulation) and activation of NADPH-oxidase localize at the plasma membrane (Roos et al., 2003; Segal, 2005). The importance of these processes is illustrated by the severe immune deficiencies that are associated with impaired killing mechanisms in neutrophils, such as those found in **chronic granulomatous disease** (genetic defect in the NADPH-oxidase) (Curnutte et al., 1975) and **Chédiak-Higashi syndrome** (granule deficiency) (Kaplan et al., 2008) .
Apart from their essential roles in immune homeostasis, neutrophils are involved in the pathogenesis of many inflammatory diseases ranging from acute inflammation in trauma or ischemic patients, to chronic inflammation found in diseases such as COPD (Hellebrekers et al., 2018; Leliefeld et al., 2016). All these clinical conditions are associated with dysregulated migration, activation and survival of neutrophils.
Despite the importance of neutrophils in innate immune responses and their well-recognized deleterious role in inflammatory diseases, surprisingly little is known regarding their life span(s) both in time and place, as well as in health and disease (Lahoz-Beneytez et al., 2016; Pillay et al., 2011). Likewise, it is unclear whether heterogeneous populations exist with distinct temporal and anatomical properties (Hellebrekers et al., 2018). It is clear, however, that targeting the full neutrophil compartment in inflammatory diseases can cause more problems than solutions. On the other hand, if neutrophils could be targeted via disease-specific mechanisms, while leaving the immune and homeostatic functions intact, such intervention would hold enormous promise in the treatment of inflammatory diseases, which currently represent a global epidemic in the aging population. To achieve this, it is

mandatory to understand the basic rules guiding the life cycle of neutrophils in health and disease. This review focusses on both.

2. Neutrophil formation and release from the bone marrow

The mammalian mitotic neutrophil pool

The neutrophil originates from myeloid lineage progenitor cells (common myeloid progenitors) located within the bone marrow and extramedullary tissues including the spleen. During the initial differentiation steps the myeloid progenitors (myeloblasts) retain their propensity to differentiate into both the monocyte/macrophage lineages and the neutrophil lineage as well as the other myeloid cells, namely eosinophils and basophils. This common differentiation ends with the last progenitor that can differentiate into both linages, the granulocyte macrophage progenitor or GMP (Evrard et al., 2018; Kawamura et al., 2017; Kim et al., 2017; Manz et al., 2002; Ramírez and Mendoza, 2018; Zhu et al., 2018). Hereafter, the differentiation of neutrophils and monocytes (and other myeloid cells) bifurcates from a metastable bipotent progenitor (in mice) (Olsson et al., 2016) and the first progenitor that is 'neutrophil-committed' is the neutrophil pro-myelocyte (Cowland and Borregaard, 1999). This cell-type in humans can be recognized by having a round nucleus and a relatively dark cytoplasm (see **Figure I**) and is able to divide; this is the first cell of the so-called mitotic neutrophil pool. The number of divisions in the pro-myelocyte stage in man is unclear as the cell can either proliferate or differentiate into the next stage, the myelocyte (Cowland and Borregaard, 1999; Ramírez and Mendoza, 2018). This cell can be recognized by having a round nucleus with an initial dent and less dark cytoplasm. It is the last cell in the neutrophil lineage that can proliferate, and represents the last cell in the mitotic pool (see **Figure I**).

Several early studies indicated that human myelocytes (Dresch et al., 1986; Mary, 1985) and possibly pro-myelocytes (Dresch et al., 1986) might be heterogeneous in their propensity to divide. It was initially proposed that up to 50% of these cells proliferate only very slowly and are residing in a 'so-called' **lazy pool** (Dresch et al., 1986; Mary, 1985). It is thought that these cells do not contribute greatly to neutropoiesis in homeostasis yet provide a means to mobilize neutrophils quickly as/when required by accelerated or 'emergency' proliferation and differentiation (Manz and Boettcher, 2014). The presence of a putative lazy pool is of key importance for the interpretation of kinetic data obtained by cellular modelling (see below (Lahoz-Beneytez et al., 2016)).

Recently, several studies have used mass cytometry (**CyTOF**) and single-cell RNA sequencing (scRNA-seq) to re-examine the proliferation and differentiation of the neutrophil

lineage and have identified (in both mouse and human bone marrow) the presence of neutrophil progenitor cells referred to as **neutrophil progenitors** (preNeu, NeuP or NEP), which appear to be committed, unipotent and early-stage neutrophil progenitors as shown in adoptive transfer assays (Evrard et al., 2018; Kim et al., 2017; Zhu et al., 2018) . However, these studies did not 'bench-mark' these cells with the known pro-myelocyte and myelocyte stages as described above. Therefore, it remains uncertain whether these progenitors are new/unique or reflect all, or part of, the known (pro-) myelocyte pools.

The post-mitotic neutrophil pool

Following the myelocyte stage the neutrophil progenitors lose their capacity to divide and enter the so-called 'post-mitotic pool' particularly studied in human cells (Cronkite et al., 1959; Dancey et al., 1976; Fliedner et al., 1964; Steinbach et al., 1979). This represents the beginning of a true maturation program starting with meta-myelocytes that in humans are recognized by a kidney shaped nucleus and clear cytoplasm. These cells in turn mature into banded cells with horseshoe-shaped nuclei and again clear cytoplasm (see **Figure I**). These cells are not found in the peripheral blood in homeostasis, but can be identified in the circulation during periods of acute infection or inflammation as the so-called 'left-shift' in the neutrophil population first described by Arneth (Arneth, 1904). The post-mitotic pool takes around 5-6 days from the last division of the myelocyte to the transition of the banded cell into the mature neutrophil in humans (see **Figure I** and (Cronkite et al., 1959; Fliedner et al., 1964; Lahoz-Beneytez et al., 2016; Steinbach et al., 1979)). This time is significantly shorter (2-3 days) in rodents (Terashima et al., 1996)

The mature neutrophil pool

Most studies undertaken on neutrophil differentiation to-date consider that the mature neutrophil in the bone marrow represents the end of the post-mitotic stage. These studies imply that neutrophils undertake terminal differentiation in the bone marrow before being liberated into the peripheral blood (see Tak et al., 2013). Some authors suggest that part of the large number of bone marrow neutrophils ($7x10^9$/kg body weight) are mobilized as a "**rapid mobilizable pool**" during periods of inflammatory stress (Joyce and Boggs, 1979; Steele et al., 1987), however it is uncertain what the fate of these cells then is under homeostasis as apoptosis in the neutrophil pool in the bone marrow is a rare event. In

support of this concept, a recent study (Grassi et al., 2018) suggested that the largest difference in the transcriptome during differentiation of human neutrophils in the bone marrow is found during transition from the bone marrow mature neutrophil to neutrophils in the peripheral blood. However, an important caveat of this study is the fact that the bone marrow samples were from different donors to the blood cells, and that the isolation procedures differed. This leaves open the possibility that the differences in transcriptomes were in fact caused by inter-donor differences and/or *ex vivo* manipulation of these human cells.

There are further concerns regarding the concept that terminal differentiation of *mature* neutrophils is completed in the bone marrow before mobilization to the peripheral blood, as mobilization of these cells during acute inflammation should then be associated by **'rejuvenation'** in the blood compartment. However, this was not found by (Tak et al., 2017) who using metabolic labeling in humans *in vivo* showed that mature neutrophils mobilized during acute experimental inflammation exhibit the same kinetics as mature cells before challenge implying the same age post labeling. Hence it is possible that the mature neutrophil compartment behaves as a single compartment in full exchange between the blood, bone marrow and possibly other tissue sites such as spleen. This implies that neutrophilia and left shifts seen during inflammation can be explained by a mere shift in relative sizes of the pools residing in bone marrow and peripheral blood. This shift in cell numbers can quickly normalize by remigration of blood cells back to the bone marrow pool upon regaining homeostasis (Løvås et al., 1996; Martin et al., 2003). This interpretation, however, awaits experimental support.

Extramedullary differentiation

A fascinating issue when considering the life cycle of neutrophils is the possibility of terminal differentiation occurring outside the bone marrow. This concept is still hypothetical but is supported by several lines of evidence, including the presence of immature progenitors trafficking throughout multiple tissues in mice (Massberg et al., 2007). It provides a rationale as to why progenitors are mobilized into the peripheral blood to be alternatively imprinted by extramedullary sites. Not much is known about putative underlying mechanisms but neutrophil progenitors have been found in the spleen (Jhunjhunwala et al., 2016). It is tempting to speculate that tissue-induced, and possibly tissue-selective, alternative imprinting can result in the generation of neutrophil subsets with alternative functions, as discussed below for the mouse. Several studies imply that **neutrophil-myeloid derived suppressor**

cells (granulocyte-MDSC) differentiate in the spleen (Jordan et al., 2017). This issue will be discussed in more detail later in this review.

Cellular markers of the different neutrophil differentiation stages in humans

The description of the myeloid lineage described above is not qualitative and, until recently, based largely on visual microscopic analysis. A rather historic but landmark study (Donohue et al., 1958) described that the absolute number of promyelocytes/myelocytes, metamyelocytes, banded neutrophils and mature cells in the bone marrow is remarkably stable, suggesting a '**conveyor belt-like' model** (Cartwright et al., 1964). Another important finding from these early studies is that the total bone marrow pool of neutrophils is 6-8 times larger than the total peripheral blood pool (Cartwright et al., 1964; Dancey et al., 1976; Donohue et al., 1958).
Newer technologies based on flow cytometry have confirmed the heterogeneity of neutrophils within the bone marrow, but to date this has not been aligned with the corresponding morphology of these populations (Matarraz et al., 2011). A similar type of analysis can be seen in **Figure I**, which shows that the entire differentiation pathway of human neutrophils can be captured using the expression of three receptors: Mac-1 (CD11b), L-selectin (CD62L) and FcγRIII (CD16). Flow-sorting of the different populations and subsequent analysis of the resulting **cytospin** preparations demonstrates that it is possible to identify and isolate the different maturing forms of neutrophils in the bone marrow and the peripheral blood.

This flow technology-based cell phenotyping also allows for a more accurate quantification of the cell numbers within the different differentiation stages in the bone marrow. However, the existing studies do not completely align (Donohue et al., 1958; Matarraz et al., 2011). This might reflect the fact that bone marrow aspirates are not completely representative of the total cell content of the bone marrow due to hemodilution (Bain, 2001) and/or a more sturdy association of progenitors with the bone marrow stromal niche, which serves to trap the dividing cells in the stroma. It is of utmost importance to obtain experimental data to determine the absolute numbers of neutrophils and neutrophil precursors in bone marrow as they are the basis for future models describing differentiation and kinetics of the neutrophil compartment (see below).

3. The kinetics of circulating neutrophils

The intravascular neutrophil pools

Mature neutrophils are present in the vasculature in two pools: a free-flowing intravascular blood pool and a blood pool residing in certain tissues. This latter pool is generally referred to as the '**marginated pool'**. Early studies suggested that marginated neutrophils are in complete equilibrium with the free-flowing cells and, therefore neutrophils from either pool were indistinguishable (Athens et al., 1961). The major sites for marginated neutrophils is the liver, spleen and bone marrow itself (Ussov et al., 1995), with debate existing as to their presence in the lungs, which may be specific only for certain species such as primates, mice and dogs (Devi et al., 2013; Doerschuk et al., 1993; Price and Dale, 1977).

The kinetics of circulating neutrophils and neutrophil precursors remains somewhat uncertain. In 1929 Weisskotten carried out experiments to determine the kinetics of neutrophils in the peripheral blood of rabbits using a toxin, benzol, which specifically targets cycling cells (Weiskotten, 1930). This work concluded that the half-life (t½) of circulating neutrophils in the rabbit was 3-4 days. However, this study fell into oblivion as more recent labeling studies did not support this relatively long half-life (see below).

Kinetics of neutrophils in the peripheral blood determined by reinfusion of *ex vivo* labeled neutrophils

Important experiments performed in the fifties and sixties, and confirmed more recently, have led to the commonly cited belief that circulating neutrophils are short-lived cells with a t½ of 7-9 hours (Cartwright et al., 1964; Dancey et al., 1976). In these experiments *ex vivo* labeled autologous neutrophils were infused into volunteers and the disappearance rate of label in the blood interpreted as representative of the circulatory half-life of these cells. This conclusion was supported by metabolic labeling in mice with D_2O showing t½ of around 9 -18 hrs (Basu et al., 2002; Pillay et al., 2010a). The interpretation of the human data has been challenged by authors who have argued that *ex vivo* manipulation of neutrophils might have changed the homing characteristics of these cells and affect their behavior in peripheral blood (see Tak et al., 2013). While deliberate priming of neutrophils both *ex vivo* and *in vivo* clearly impacts on the distribution of these cells within the vasculature, with most neutrophils homing to the lung (Summers et al., 2014; Tam et al., 1992), the above data have been

consistently reproduced using newer techniques that induce minimal-to-no detectable cell **priming** (e.g. Farahi et al., 2012). Hence while priming undoubtedly has a profound impact on the behavior of neutrophils *in vivo* (Pillay et al., 2010b; Vogt et al., 2018), the effect of priming and activation *per se* on the intravascular (circulating, marginated and intra-vascular entrapment e.g. in the pulmonary capillary network) half-life of neutrophils has yet to be determined, and much of the above data still stands.

Kinetics of neutrophils in the peripheral blood determined by *in vivo* labeling with radioactive or stable isotopes

To circumvent the difficulties of *ex vivo* cell manipulation several studies have applied *in vivo* labeling methodologies to track and trace the kinetics of neutrophils. Several labels have been used including ^{3}H-thymidine (Cronkite et al., 1959), ^{3}H-DFP and ^{32}P-DFP (Cartwright et al., 1964). These studies have produced a slightly more finessed dataset that support both a short as well as a relatively long neutrophil lifespan. A short life span is supported by the quick disappearance of label in a logarithmic fashion (Cartwright et al., 1964), however this assumes that the majority of the neutrophil compartment is present in the peripheral blood. As discussed above (Dancey et al., 1976; Donohue et al., 1958) the majority of the neutrophil compartment resides outside the bloodstream and is likely in complete exchange. Therefore, the disappearance rate of the label can also be explained by a redistribution of the cells into the whole neutrophil compartment that takes several hours. Indeed, in a rat model where labeled neutrophils are re-infused its takes several hours for neutrophils to end up in the bone marrow (Løvås et al., 1996). Of note, this population of cells that 'disappear' from the circulating bloodstream can be mobilized again as has been shown in calves challenged with corticosteroids, implying bone marrow margination rather than formal uptake in a tissue compartment (Vincent, 1974). However, this hypothesis awaits more experimental support. The data obtained in the study by Cartwright and colleagues applying ^{32}P-DFP *in vivo* can also be interpreted as supporting a longer half-life for neutrophils as the pulse labeling with ^{32}P-DFP led to *stable* labeling of blood neutrophils for 11 days whereas the post-mitotic time was around 5-6 days (Cartwright et al., 1964, and see below). This would be in support for the neutrophil lifespan results of Pillay and colleagues (Pillay et al., 2011; see below). The experiments applying ^{32}P-DFP are difficult to repeat/reproduce because of ethical constraints.

Fortunately, new technology can re-evaluate these studies by *in vivo* labeling with the stable isotope deuterium (^{2}H) in the form of $^{2}H_2O$ or ^{2}H-6,6-glucose (Macallan et al., 1998). Under

these conditions deuterium is built into the ribose moiety of the DNA of cycling cells including the cells of the mitotic pool of neutrophils progenitors. It allows a '**pulse-chase'** type of experiment by following the enrichment of ^{2}H in the DNA. were the first to apply this technology for the analysis of the kinetics of neutrophils in peripheral blood and concluded that the lifespan of human neutrophils in peripheral blood is around 5 days and murine neutrophils of around 18 hrs (Pillay et al., 2011). However, this finding for human cells was challenged by several authors (Lahoz-Beneytez et al., 2016; Tofts et al., 2011) arguing that the slowness in disappearance of label from the blood could be explained by a 'slow neutrophil compartment' in the bone marrow and a 'fast compartment' in the peripheral blood. This view was supported by (Lahoz-Beneytez et al., 2016) in studies using short term labeling with ^{2}H-glucose, which also concluded that neutrophils have a short half-life in peripheral blood. However, these latter conclusions were based on the assumptions that all (pro-) myelocytes divide equally, that neutrophils in the bone marrow and blood do not belong to a homogenous pool, that neutrophils do not return to the bone marrow, and that there is no lazy-pool of myelocytes. These assumptions are in contrast with data showing that rat and human (pro)myelocytes that are dividing keep dividing with a cycle time of around 14 hrs (Constable and Blackett, 1972; Stryckmans et al., 1966), that neutrophils can migrate back to the bone marrow (Løvås et al., 1996; Martin et al., 2003), the presence of a so-called 'lazy neutrophil pool' (Dresch et al., 1986; Mary, 1985), and data that support the concept that neutrophils in bone marrow and blood belong to the same kinetic pool (Tak et al., 2017).

In fact, even these published data can be used to support a significantly longer half-life of neutrophils in the peripheral blood as the *R*-value of 5.5 that leads to an equally good fit of the data supports a t½ of 2.6 days (lifespan of 3.7 days). It is, however, debatable that a model based on ordinary differential equations is the best choice for describing neutrophil kinetics as the data are not supportive of a model where all (pro-) myelocytes have an equal chance to divide. It seems more likely that only a part of the (pro-) myelocyte pool is dividing with a narrow division time supporting a conveyor belt type of differentiation such as suggested before (Cartwright et al.,1964) rather than a model based on chance.

4. Neutrophil fate and function within tissue compartments

Dynamics of neutrophils in blood and tissues

Mouse and human neutrophils newly released into the bloodstream are endowed with distinct phenotypic properties in that they gradually change over time following circadian oscillations and, at least in the mouse, these phenotypic changes parallel changes in their transcriptional and migratory properties (Adrover et al., 2019; Adrover et al., 2016) (see **figure II**). A major functional pathway affected by (circadian) time is rearrangement of the actin cytoskeleton, loss of surface microvilli and subsequent reduction in the capacity of murine neutrophils to roll on **endothelial selectins**. Ultimately, these changes result in a reduced ability to migrate to inflamed tissues over time (Adrover et al., 2019). While the mechanisms of migration are discussed in more detail in other reviews from this collection, it is important to note here that **rolling-defective neutrophils** can still adhere to unstimulated vessels in the dermal microcirculation (and possibly in other tissues) in a selectin-independent manner (Adrover et al., 2019), through mechanisms possibly similar to those identified in patrolling monocytes (Auffray et al., 2007). These features may explain the efficient entry of neutrophils from blood into naïve tissues, including skin, liver, intestine or bone marrow (Casanova-Acebes et al., 2018) Although similar patterns of recruitment of neutrophils into human tissues have not yet been evaluated, the similar circadian properties and kinetics of neutrophils have been reported in human blood (Adrover et al., 2019) suggesting that multi-organ infiltration in the steady-state might be a conserved feature across species. In addition, indirect demonstration that neutrophils infiltrate tissues as part of their natural life cycle comes from studies in mice defective in genes needed for neutrophil adhesion and elimination, which develop severe alterations in **granulopoiesis** and trafficking of **hematopoietic stem and progenitor cells** (HSPC) even under homeostatic conditions (Hong et al., 2012; Stark et al., 2005). Similar hematopoietic alterations found in patients bearing **leukocyte adhesion deficiency** (LAD) mutations (van de Vijver et al., 2013) suggest that a similar regulatory loop involving neutrophil migration into naïve tissues may also operate in humans. Mechanistically, this regulation is mediated by transcriptional repression of IL23 in tissue-resident phagocytes as they take up senescent neutrophils (A-Gonzalez et al., 2017; Hong et al., 2012; Stark et al., 2005). These studies in the mouse have prompted a renewed interest in defining the dynamics and fate of neutrophils in healthy tissues, beyond their lifetime in the circulation.

Retention and function of neutrophils in the lung microvascular bed

While the dynamics of neutrophil entry into naïve or challenged tissues is increasingly well-appreciated in the mouse (Casanova-Acebes et al., 2018), whether and how this occurs in human tissues remains largely unknown. Current knowledge in humans largely relies on

comparative studies only in certain organs, such as the lung and the spleen. Intravital microscopy studies in the murine lung microvasculature have revealed a substantial number of neutrophils within the network of small capillary vessels that could be rapidly mobilized with the CXCR4 antagonist plerixafor in both mice and primates (Devi et al., 2013) and are actively crawling on small pulmonary capillaries of mice (Yipp et al., 2017). Nonetheless, conflicting studies in humans and mice debate whether CXCR4 is indeed a retention signal for neutrophils in the lungs (Liu et al., 2015) and further work is required. This margination of circulating neutrophils and **intra-vascular crawling** in murine lungs dramatically increase upon exposure to endotoxin or live bacteria (Yipp et al., 2017). Although the size of the intra-vascular marginated neutrophil pool in humans is thought to be much smaller than the one shown in the studies in mice (Summers et al., 2010), similar changes in surface markers and in the number of circulating neutrophils is seen after in vivo treatment with endotoxin- (Yipp et al., 2017) or platelet-activating factor- (Tam et al., 1992). This, together with the rapid *in vitro* adhesion of LPS-stimulated human neutrophils to primary pulmonary endothelial cells (Yipp et al., 2017), suggests that similar dynamics and intravascular behavior might take place in the human lung. In keeping with this notion, humanized sickle-cell disease (SCD) mice display frequent interactions of neutrophils with platelets within the lungs, with formation of microemboli that trigger vaso-occlusive crises that recapitulate those occurring in SCD patients (Bennewitz et al., 2017). These series of findings suggest the presence of an abundant population of neutrophils in the resting murine pulmonary microcirculation that can serve as a pool for rapid mobilization(Devi et al., 2013), are important for local anti-microbial responses, but can potentially elicit acute pulmonary injury. Additional studies in the mouse have shown that the lung may also provide a site of neutrophil re-programming that enables their return to the BM for final elimination (Wang et al., 2017), while in turn pulmonary neutrophils can instruct transcriptional programs in the mouse lungs that influence metastatic invasion (Casanova-Acebes et al., 2018).

Retention and function of neutrophils in the spleen

The spleen also represents a tissue in which the function of neutrophils has been studied in considerable detail both in resting and diseased scenarios. Studies of human spleens demonstrated the presence of at least two populations of neutrophils in the perifollicular zone which induce IgM secretion and Ig-class switch in marginal zone B cells through the secretion of BAFF, APRIL, IL21 and pentraxin 3 (Chorny et al., 2016; Puga et al., 2012). Notably, these neutrophil populations in the human spleen were found to be induced post-natally by local signals such as IL10 and GM-CSF and involved in inducing anti-microbial

immunoglobulins in a T cell-independent manner (Puga et al., 2012). These specialized functions of neutrophils in the spleen could not be reproduced in an independent study (Nagelkerke et al., 2014) and, in the mouse, a B-helper phenotype has been reported only in the context of chronic lymphocytic leukemia (Gätjen et al., 2016). Kubes and colleagues demonstrated the presence of additional neutrophil populations in the red pulp of the mouse spleen that clear bacteria from the surface of macrophages, and an additional immature population that expands upon infection *Streptococcus pneumoniae* (Deniset et al., 2017). Thus, resident (and possibly recruited and reprogrammed) neutrophils are endowed with distinct antimicrobial functions in the spleen. Of note, while there is clear evidence for neutrophil accumulation and destruction in the spleen in humans, where approximately 30% of all circulating neutrophils end up, evidence for the spleen acting as a major site of destruction of endogenous neutrophils in mice is lacking (Saverymuttu et al., 1985).

Uptake and function of neutrophils in the liver

The liver is an organ of active accumulation and destruction of neutrophils. Studies in humans demonstrated rapid accumulation of infused, radio-labelled neutrophils in the liver (the '**hepatic marginated pool'**) together with intense uptake at latter times implying that this organ is also an important site for the homeostatic destruction of neutrophils (Saverymuttu et al., 1985; Szczepura et al., 2011). In the rat liver, apoptotic (TUNEL+) neutrophils can be detectable in the steady-state but their number escalates dramatically following LPS treatment. **Kupffer cells** that line the hepatic sinusoids actively phagocytose phosphatidyl serine (PS)-positive neutrophils and depletion of Kupffer cells re-routes neutrophils to other tissues, where they may contribute to organ damage (Shi et al., 2001). These findings agree with the observed accumulation of exogenously infused mouse neutrophils mostly in the bone marrow and liver. In these studies, the immature neutrophils preferentially homed back to the bone marrow and these could be re-mobilized to sites of infection (Suratt et al., 2001). Importantly, the liver is also a key tissue for the elimination of circulating bacteria (Jenne and Kubes, 2013), and phagocytic neutrophils which are then taken up by Kupffer cells contribute to dampening and resolving hepatic inflammation once the pathogens have been cleared (Holub et al., 2009). Thus, the liver represents a primary site of neutrophil elimination, however other possible functions within this organ remain to be explored.

Migration and function of neutrophils in the bone marrow

In addition to being the main site of production, the bone marrow is also a site of active neutrophil clearance. Studies using mice in parabiosis demonstrated that senescent or aged neutrophils return to the marrow upon completion of their life cycle in blood through a mainly CXCR4-dependent mechanism (Casanova-Acebes et al., 2013; Martin et al., 2003; Wang et al., 2017), and there is evidence for active recycling in the human marrow as well (Szczepura et al., 2011). Whether this scenario maps exactly to humans is yet to be determined as circulating human neutrophils express trace levels of cell surface CXCR4 and studies using *ex vivo* radiolabeled neutrophils suggest that these cells are removed randomly rather than in an age-dependent manner (Saverymuttu et al., 1985). However, various roles of neutrophils in the marrow are beginning to emerge, at least in the mouse. For example, aged neutrophils that return to the BM after circulating in blood are phagocytosed by medullary macrophages (Furze and Rankin, 2008), resulting in suppression of CXCL12-producing niche cells, temporal inhibition of niche functions and circadian release of HSPC into the circulation (Casanova-Acebes et al., 2013). Because similar variations in circulating HSPC numbers occur in humans (Lucas et al., 2008), it is possible that they perform similar suppressive functions.

An important but still poorly-defined issue when considering the fate of neutrophils is their actual lifetime within different tissues before final destruction, as this has not been yet rigorously measured. Our own preliminary evidences in the mouse suggest dwell times of less than one day in the steady-state, which may still be sufficient to influence many aspects of tissue physiology through the release of cytokines, **granule proteins**, or even **NETs** as reported in the human spleen (Puga et al., 2012). In other contexts, for example the murine lymph node, our preliminary work suggest much longer tissue residency times . Importantly, these lifetimes can be markedly extended in the context of inflammation, as multiple cytokines and bacterial products extend the survival of human and murine neutrophils, at least in part, through regulation of the anti-apoptotic factor Mcl-1 (Colotta et al., 1992; Moulding et al., 1998). Thus, essential features of the neutrophil life cycle, namely their lifespan in different tissues and under inflammatory states, remain to date poorly defined and this should be an important task for the coming years.

5. The Yin and Yang in neutrophil targeted therapies. It is noteworthy that several important clinical conditions are associated with either hyper- or hypo-activation of the neutrophil compartment. Chronic inflammatory diseases such as chronic obstructive pulmonary disease (Oudijk et al., 2005) as well as acute inflammatory conditions such as systemic inflammatory response syndrome (SIRS) (Hazeldine et al., 2017; Hietbrink et al.,

2011) are typically associated with hyper-activation of neutrophils . Under these conditions it is expected that inhibition of neutrophils will be beneficial for the patient. A similar inhibition of neutrophils might be suited for cancer patients where activated neutrophils play a role in suppression of anti-tumoral immunity (Fridlender et al., 2009; Zhou et al., 2018). On the other hand, insufficient activation of neutrophils in clinical conditions such as found in the compensatory anti-inflammatory response syndrome (CARS) in patients with trauma or major operations calls for activation of these cells to prevent development of severe infectious complications such as sepsis (Adib-Conquy and Cavaillon, 2009; Mortaz et al., 2018). It is clear that timing of such therapies is of essence as both hypo- and hyper-activation of neutrophils can co-exist in patients suffering from acute inflammatory conditions (Mortaz et al., 2018; Osuchowski et al., 2014). A clear example are multi-trauma patients where SIRS is dominant during the first days after trauma and a dominant CARS can develop after several days (Hietbrink et al., 2013).

Clinician Corner Box

- Neutrophils are among the principal effectors of the innate immune response and are instrumental in the first line of defense against invading microbes
- Whilst there may be important differences between human neutrophils and those of other mammalian species, much has been learned from studies in transgenic animal models
- The production, circulation and clearance of neutrophils is altered by inflammatory stimuli such as those encountered in acute conditions such as bacteremia, and chronic conditions such as chronic obstructive pulmonary disease
- Therapies precisely targeted at the deleterious effects of neutrophils, whilst leaving beneficial ones intact, will be required for successful manipulation of these cells in the clinic

5. Concluding remarks

The variety of kinetics and functions described for neutrophils are consistent with the emerging view that these cells are multifaceted. At least part of the neutrophil pool is essential in host defense against invading micro-organisms and is crucial for a successful immune response. On the negative side, neutrophils are involved in the pathogenesis of a plethora of inflammatory diseases, and can additionally suppress anti-tumor responses.

Thus, it is now clear that neutrophil may become important targets of future therapies to suppress hyper-inflammation as well as hypo-inflammation dependent on the nature of the disease. Elucidation of the precise mechanisms underlying these antagonistic functions will allow development of new therapies targeting one arm of the neutrophil functions while sparing their beneficial functions (see Outstanding questions). In particular, lessons learned from understanding the lifecycle of neutrophils in different states and tissues can translate into clinical benefit; for example, manipulation of the life-cycle of neutrophils may allow extending the duration of their beneficial functions, while blocking the development or detrimental functions of these cells under pathological scenarios. As we continue to better understand the dynamics and physiological facets of neutrophils, we hope that new therapeutic strategies will appear that harness the unique features of these cells.

Outstanding questions box

- What are the real transit times of neutrophils in blood, bone marrow and tissues?
- What is the evolutionary basis for the diurnal behavior of neutrophils?
- What are the mechanisms and tissues of neutrophil clearance in mouse and man?
- Can the life cycle neutrophils be reprogrammed or manipulated for the clinic?
- What is the role of the large marginated pools of neutrophils that reside under physiological conditions in the liver and the spleen?
- How do viruses and bacteria subvert the life cycle of neutrophils?

6. References

A-Gonzalez, N., Quintana, J. A., García-Silva, S., Mazariegos, M., González de la Aleja, A., Nicolás-Ávila, J. A., et al. (2017). Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J. Exp. Med.* 214, 1281–1296. doi:10.1084/jem.20161375.

Adib-Conquy, M., and Cavaillon, J. M. (2009). Compensatory anti-inflammatory response syndrome. *Thromb. Haemost.* 101, 36–47. doi:10.1160/TH08-07-0421.

Adrover, J.M. Del Fresno, C. Crainiciuc, G. Cuartero, M.I. Casanova-Acebes, M. Weiss, L.A. Huerga-Encabo, H. Silvestre-Roig, C. Rossaint, J. Cossío, I. Lechuga-Vieco, A.V. García-Prieto, J. Gómez-Parrizas, M. Quintana, J.A. Ballesteros, I. Martin-Salamanc, A. (2019). A Neutrophil Timer Coordinates Immune Defense and Vascular Protection Title. *Immunity* epub ahead. doi:doi: 10.1016/j.immuni.2019.01.002.

Adrover, J. M., Nicolás-Ávila, J. A., and Hidalgo, A. (2016). Aging: A Temporal Dimension for Neutrophils. *Trends Immunol.* 37, 334–345. doi:10.1016/j.it.2016.03.005.

Arneth, J. (1904). Die neutrophilen Leukozyten bei Infektionskrankheiten. *Dtsch med Wochenschr* 30, 92–94.

Auffray, C., Fogg, D., Garfa, M., Elain, G., Join-Lambert, O., Kayal, S., et al. (2007). Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science (80-.).* 317, 666–670. doi:10.1126/science.1142883.

Babior, B. M., KiPNEs, R. S., and Cumvu, J. T. (1973). THE PRODUCTION BY LEUKOCYTES OF SUPEROXIDE, A POTENTIAL BACTERICIDAL AGENT. *Biol. Def. Mech.* 52, 741–744. doi:10.1172/JCI107236.

Bain, B. J. (2001). Bone marrow aspiration. *J. Clin. Pathol.* 54, 657–663. doi:10.1136/jcp.54.9.657.

Basu, S., Hodgson, G., Katz, M., and Dunn, A. R. (2002). Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. *Blood* 100, 854–861. doi:10.1182/blood.V100.3.854.

Bennewitz, M. F., Jimenez, M. A., Vats, R., Tutuncuoglu, E., Jonassaint, J., Kato, G. J., et al. (2017). Lung vaso-occlusion in sickle cell disease mediated by arteriolar neutrophil-platelet microemboli. *JCI Insight* 2. doi:10.1172/jci.insight.89761.

Biermann, H., Pietz, B., Dreier, R., Schmid, K. W., Sorg, C., and Sunderkötter, C. (1999). Murine leukocytes with ring-shaped nuclei include granulocytes, monocytes, and their precursors. *J. Leukoc. Biol.* 65, 217–231. doi:10.1002/jlb.65.2.217.

Bolliger, A. P., Everds, N. E., Zimmerman, K. L., Moore, D. M., Smith, S. A., and Barnhart, K. F. (2011). “Hematology of laboratory animals,” in *Schalm’s Veterinary Hematology*,

852–887. doi:10.1007/s13398-014-0173-7.2.

Cartwright, G. E., Athens, J. W., Wintrobe, M. M. (1964). The kinetics of granulopoiesis in normal man. *Blood* 24, 780–803. doi:http://dx.doi.org.proxy.library.uu.nl/.

Casanova-Acebes, M., Nicolás-Ávila, J. A., Li, J. L., García-Silva, S., Balachander, A., Rubio-Ponce, A., et al. (2018). Neutrophils instruct homeostatic and pathological states in naive tissues. *J. Exp. Med.* 215, 2778–2795. doi:10.1084/jem.20181468.

Casanova-Acebes, M., Pitaval, C., Weiss, L. A., Nombela-Arrieta, C., Chèvre, R., A-González, N., et al. (2013). XRhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell* 153. doi:10.1016/j.cell.2013.04.040.

Chorny, A., Casas-Recasens, S., Sintes, J., Shan, M., Polentarutti, N., García-Escudero, R., et al. (2016). The soluble pattern recognition receptor PTX3 links humoral innate and adaptive immune responses by helping marginal zone B cells. *J. Exp. Med.* 213, 2167–2185. doi:10.1084/jem.20150282.

Colotta, F., Re, F., Polentarutti, N., Sozzani, S., and Mantovani, A. (1992). Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80, 2012–20. doi:1382715.

Constable, T. B., and Blackett, N. M. (1972). THE CELL POPULATION KINETICS OF NEUTROPHILIC CELLS. *Cell Prolif.* 5, 289–302. doi:10.1111/j.1365-2184.1972.tb00367.x.

Cowland, J. B., and Borregaard, N. (1999). Isolation of neutrophil precursors from bone marrow for biochemical and transcriptional analysis. *J. Immunol. Methods* 232, 191–200. doi:10.1016/S0022-1759(99)00176-3.

Cowland, J. B., and Borregaard, N. (2016). Granulopoiesis and granules of human neutrophils. *Immunol. Rev.* 273, 11–28. doi:10.1111/imr.12440.

Cronkite, E. P., Fliedner, T. M., Bond, V. P., Rubini, J. R., Brecher, G., and Quastler, H. (1959). DYNAMICS OF HEMOPOIETIC PROLIFERATION IN MAN AND MICE STUDIED BY H3-THYMIDINE INCORPORATION INTO DNA. *Ann. N. Y. Acad. Sci.* 77, 803–820. doi:10.1111/j.1749-6632.1959.tb36943.x.

Curnutte, J. T., Kipnes, R. S., and Babior, B. M. (1975). Defect in Pyridine Nucleotide Dependent Superoxide Production by a Particulate Fraction from the Granulocytes of Patients with Chronic Granulomatous Disease. *N. Engl. J. Med.* 293, 628–632. doi:10.1056/NEJM197509252931303.

Dancey, J. T., Deubelbeiss, K. A., and Harker andFinch, L. A. C. A. (1976). Neutrophil kinetics in man. *J. Clin. Invest.* 58, 705–715. doi:10.1172/JCI108517.

Deniset, J. F., Surewaard, B. G., Lee, W.-Y., and Kubes, P. (2017). Splenic Ly6G ^{high} mature and Ly6G ^{int} immature neutrophils contribute to eradication of *S. pneumoniae*. *J. Exp.*

Med. 214, 1333–1350. doi:10.1084/jem.20161621.

Devi, S., Wang, Y., Chew, W. K., Lima, R., A-González, N., Mattar, C. N. Z., et al. (2013). Neutrophil mobilization via plerixafor-mediated CXCR4 inhibition arises from lung demargination and blockade of neutrophil homing to the bone marrow. *J. Exp. Med.* 210, 2321–2336. doi:10.1084/jem.20130056.

Doerschuk, C. M., Beyers, N., Coxson, H. O., Wiggs, B., and Hogg, J. C. (1993). Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung. *J. Appl. Physiol.* 74, 3040–3045. doi:10.1152/jappl.1993.74.6.3040.

DONOHUE, D. M., REIFF, R. H., HANSON, M. L., BETSON, Y., and FINCH, C. A. (1958). Quantitative measurement of the erythrocytic and granulocytic cells of the marrow and blood. *J. Clin. Invest.* 37, 1571–1576. doi:10.1172/JCI103750.

Dresch, C., Troccoli, G., and Mary, J. Y. (1986). Growth Fraction of Myelocytes In Normal Human Granulopoiesis. *Cell Prolif.* 19, 11–22. doi:10.1111/j.1365-2184.1986.tb00711.x.

Ekpenyong, A. E., Toepfner, N., Fiddler, C., Herbig, M., Li, W., Cojoc, G., et al. (2017). Mechanical deformation induces depolarization of neutrophils. *Sci. Adv.* 3. doi:10.1126/sciadv.1602536.

Eruslanov, E. B., Singhal, S., and Albelda, S. M. (2017). Mouse versus Human Neutrophils in Cancer: A Major Knowledge Gap. *Trends in Cancer* 3, 149–160. doi:10.1016/j.trecan.2016.12.006.

Evrard, M., Kwok, I. W. H., Chong, S. Z., Teng, K. W. W., Becht, E., Chen, J., et al. (2018). Developmental Analysis of Bone Marrow Neutrophils Reveals Populations Specialized in Expansion, Trafficking, and Effector Functions. *Immunity* 48, 364–379.e8. doi:10.1016/j.immuni.2018.02.002.

FLIEDNER, T. M., CRONKITE, E. P., KILLMANN, S. A., and BOND, V. P. (1964). GRANULOCYTOPOIESIS. II. EMERGENCE AND PATTERN OF LABELING OF NEUTROPHILIC GRANULOCYTES IN HUMANS. *Blood* 24, 683–700.

Fridlender, Z. G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., et al. (2009). Polarization of Tumor-Associated Neutrophil Phenotype by TGF-β: “N1” versus “N2” TAN. *Cancer Cell* 16, 183–194. doi:10.1016/j.ccr.2009.06.017.

Furze, R. C., and Rankin, S. M. (2008). Neutrophil mobilization and clearance in the bone marrow. *Immunology* 125, 281–288. doi:10.1111/j.1365-2567.2008.02950.x.

Gao, J. L., and Murphy, P. M. (1993). Species and subtype variants of the N-formyl peptide chemotactic receptor reveal multiple important functional domains. *J. Biol. Chem.* 268, 25395–25401.

Gätjen, M., Brand, F., Grau, M., Gerlach, K., Kettritz, R., Westermann, J., et al. (2016).

Splenic marginal zone granulocytes acquire an accentuated neutrophil B-cell helper phenotype in chronic lymphocytic leukemia. *Cancer Res.* 76, 5253–5265. doi:10.1158/0008-5472.CAN-15-3486.

Gillis, C. M., Jönsson, F., Mancardi, D. A., Tu, N., Beutier, H., Van Rooijen, N., et al. (2017). Mechanisms of anaphylaxis in human low-affinity IgG receptor locus knock-in mice. *J. Allergy Clin. Immunol.* 139, 1253–1265.e14. doi:10.1016/j.jaci.2016.06.058.

Grassi, L., Pourfarzad, F., Ullrich, S., Merkel, A., Were, F., Carrillo-de-Santa-Pau, E., et al. (2018). Dynamics of Transcription Regulation in Human Bone Marrow Myeloid Differentiation to Mature Blood Neutrophils. *Cell Rep.* 24, 2784–2794. doi:10.1016/j.celrep.2018.08.018.

Hazeldine, J., Naumann, D. N., Toman, E., Davies, D., Bishop, J. R. B., Su, Z., et al. (2017). Prehospital immune responses and development of multiple organ dysfunction syndrome following traumatic injury: A prospective cohort study. *PLoS Med.* 14. doi:10.1371/journal.pmed.1002338.

Hellebrekers, P., Vrisekoop, N., and Koenderman, L. (2018). Neutrophil phenotypes in health and disease. *Eur. J. Clin. Invest.* 48. doi:10.1111/eci.12943.

Hietbrink, F., Koenderman, L., Althuizen, M., Pillay, J., Kamp, V., and Leenen, L. P. H. (2013). Kinetics of the innate immune response after trauma: Implications for the development of late onset sepsis. *Shock* 40. doi:10.1097/SHK.0b013e318295a40a.

Hietbrink, F., Koenderman, L., and Leenen, L. P. H. (2011). Intramedullary nailing of the femur and the systemic activation of monocytes and neutrophils. *World J. Emerg. Surg.* 6. doi:10.1186/1749-7922-6-34.

Holub, M., Cheng, C.-W., Mott, S., Wintermeyer, P., van Rooijen, N., and Gregory, S. H. (2009). Neutrophils Sequestered in the Liver Suppress the Proinflammatory Response of Kupffer Cells to Systemic Bacterial Infection. *J. Immunol.* 183, 3309–3316. doi:10.4049/jimmunol.0803041.

Hong, C., Kidani, Y., A-Gonzalez, N., Phung, T., Ito, A., Rong, X., et al. (2012). Coordinate regulation of neutrophil homeostasis by liver X receptors in mice. *J. Clin. Invest.* 122, 337–347. doi:10.1172/JCI58393.

Jenne, C. N., and Kubes, P. (2013). Immune surveillance by the liver. *Nat. Immunol.* 14, 996–1006. doi:10.1038/ni.2691.

Jhunjhunwala, S., Alvarez, D., Aresta-DaSilva, S., Tang, K., Tang, B. C., Greiner, D. L., et al. (2016). Splenic progenitors aid in maintaining high neutrophil numbers at sites of sterile chronic inflammation. *J. Leukoc. Biol.* 100, 253–260. doi:10.1189/jlb.1HI0615-248RR.

Jordan, K. R., Kapoor, P., Spongberg, E., Tobin, R. P., Gao, D., Borges, V. F., et al. (2017). Immunosuppressive myeloid-derived suppressor cells are increased in splenocytes from

cancer patients. *Cancer Immunol. Immunother.* 66, 503–513. doi:10.1007/s00262-016-1953-z.

Joyce, R. A., and Boggs, D. R. (1979). Visualizing the marrow granulocyte reserve. *J. Lab. Clin. Med.* 93, 101–110. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med1&NEWS=N&AN=759519.

Kaplan, J., De Domenico, I., and Ward, D. M. (2008). Chediak-Higashi syndrome. [Miscellaneous Article]. *Curr. Opin. Hematol.* 15, 22–29.

Kawamura, S., Onai, N., Miya, F., Sato, T., Tsunoda, T., Kurabayashi, K., et al. (2017). Identification of a Human Clonogenic Progenitor with Strict Monocyte Differentiation Potential: A Counterpart of Mouse cMoPs. *Immunity* 46, 835–848.e4. doi:10.1016/j.immuni.2017.04.019.

Kim, M. H., Yang, D., Kim, M., Kim, S. Y., Kim, D., and Kang, S. J. (2017). A late-lineage murine neutrophil precursor population exhibits dynamic changes during demand-adapted granulopoiesis. *Sci. Rep.* 7. doi:10.1038/srep39804.

Kolaczkowska, E., and Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 13, 159–175. doi:10.1038/nri3399.

Lahoz-Beneytez, J., Elemans, M., Zhang, Y., Ahmed, R., Salam, A., Block, M., et al. (2016). Human neutrophil kinetics: Modeling of stable isotope labeling data supports short blood neutrophil half-lives. *Blood* 127, 3431–3438. doi:10.1182/blood-2016-03-700336.

Leliefeld, P. H. C., Wessels, C. M., Leenen, L. P. H., Koenderman, L., and Pillay, J. (2016). The role of neutrophils in immune dysfunction during severe inflammation. *Crit. Care* 20. doi:10.1186/s13054-016-1250-4.

Liu, Q., Li, Z., Gao, J. L., Wan, W., Ganesan, S., Mcdermott, D. H., et al. (2015). CXCR4 antagonist AMD3100 redistributes leukocytes from primary immune organs to secondary immune organs, lung, and blood in mice. *Eur. J. Immunol.* 45, 1855–1867. doi:10.1002/eji.201445245.

Løvås, K., Knudsen, E., Iversen, P. O., and Benestad, H. B. (1996). Sequestration patterns of transfused rat neutrophilic granulocytes under normal and inflammatory conditions. *Eur J Haematol* 56, 221–229.

Lucas, D., Battista, M., Shi, P. A., Isola, L., and Frenette, P. S. (2008). Mobilized Hematopoietic Stem Cell Yield Depends on Species-Specific Circadian Timing. *Cell Stem Cell* 3, 364–366. doi:10.1016/j.stem.2008.09.004.

Macallan, D. C., Fullerton, C. A., Neese, R. A., Haddock, K., Park, S. S., and Hellerstein, M. K. (1998). Measurement of cell proliferation by labeling of DNA with stable isotope-labeled glucose: Studies in vitro, in animals, and in humans. *Proc. Natl. Acad. Sci.* 95,

708–713. doi:10.1073/pnas.95.2.708.

Manz, M. G., and Boettcher, S. (2014). Emergency granulopoiesis. *Nat. Rev. Immunol.* 14, 302–314. doi:10.1038/nri3660.

Manz, M. G., Miyamoto, T., Akashi, K., and Weissman, I. L. (2002). Prospective isolation of human clonogenic common myeloid progenitors. *Proc. Natl. Acad. Sci.* 99, 11872–11877. doi:10.1073/pnas.172384399.

Martin, C., Burdon, P. C. E., Bridger, G., Gutierrez-Ramos, J. C., Williams, T. J., and Rankin, S. M. (2003). Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* 19, 583–593. doi:10.1016/S1074-7613(03)00263-2.

Mary, J. Y. (1985). Normal human granulopoiesis revisited. II. Bone marrow data. *Biomed. Pharmacother.* 39, 66–77. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3893558.

Massberg, S., Schaerli, P., Knezevic-Maramica, I., Köllnberger, M., Tubo, N., Moseman, E. A., et al. (2007). Immunosurveillance by Hematopoietic Progenitor Cells Trafficking through Blood, Lymph, and Peripheral Tissues. *Cell* 131, 994–1008. doi:10.1016/j.cell.2007.09.047.

Matarraz, S., Fernandez, C., Albors, M., Teodosio, C., López, A., Jara-Acevedo, M., et al. (2011). Cell-cycle distribution of different cell compartments in normal versus reactive bone marrow: A frame of reference for the study of dysplastic hematopoiesis. *Cytom. Part B - Clin. Cytom.* 80 B, 354–361. doi:10.1002/cyto.b.20605.

Mortaz, E., Alipoor, S. D., Adcock, I. M., Mumby, S., and Koenderman, L. (2018). Update on neutrophil function in severe inflammation. *Front. Immunol.* 9. doi:10.3389/fimmu.2018.02171.

Moulding, D., Quayle, J., Hart, C., and Edwards, S. (1998). Mcl-1 expression in human neutrophils: Regulation by cytokines and correlation with cell survival. *Blood* 90, 2495–2502. doi:10.1021/pr0701512.

Nagelkerke, S. Q., Aan De Kerk, D. J., Jansen, M. H., Van Den Berg, T. K., and Kuijpers, T. W. (2014). Failure to detect functional neutrophil B Helper cells in the human spleen. *PLoS One* 9. doi:10.1371/journal.pone.0088377.

Olsson, A., Venkatasubramanian, M., Chaudhri, V. K., Aronow, B. J., Salomonis, N., Singh, H., et al. (2016). Single-cell analysis of mixed-lineage states leading to a binary cell fate choice. *Nature* 537, 698–702. doi:10.1038/nature19348.

Osuchowski, M. F., Welch, K., Siddiqui, J., and Remick, D. G. (2014). Circulating Cytokine/Inhibitor Profiles Reshape the Understanding of the SIRS/CARS Continuum in Sepsis and Predict Mortality. *J. Immunol.* 177, 1967–1974. doi:10.4049/jimmunol.177.3.1967.

Oudijk, E.-J. D., Nijhuis, E. H. J., Zwank, M. D., Van De Graaf, E. A., Mager, H. J., Coffer, P. J., et al. (2005). Systemic inflammation in COPD visualised by gene profiling in peripheral blood neutrophils. *Thorax* 60. doi:10.1136/thx.2004.034009.

Perussia, B. (1983). Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. *J. Exp. Med.* 158, 1092–1113. doi:10.1084/jem.158.4.1092.

Pillay, J., Den Braber, I., Vrisekoop, N., Kwast, L. M., De Boer, R. J., Borghans, J. A. M., et al. (2010a). In vivo labeling with ^{2}H<inf>2</inf>O reveals a human neutrophil lifespan of 5.4 days. *Blood* 116. doi:10.1182/blood-2010-01-259028.

Pillay, J., Den Braber, I., Vrisekoop, N., Kwast, L. M., De Boer, R. J., Borghans, J. A. M., et al. (2011). The in vivo half-life of human neutrophils. *Blood* 117. doi:10.1182/blood-2010-11-317875.

Pillay, J., Ramakers, B. P., Kamp, V. M., Loi, A. L. T., Lam, S. W., Hietbrink, F., et al. (2010b). Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J. Leukoc. Biol.* 88. doi:10.1189/jlb.1209793.

Price, T. H., and Dale, D. C. (1977). Neutrophil preservation. The effect of short-term storage on in vivo kinetics. *J. Clin. Invest.* 59, 475–480. doi:10.1172/JCI108662.

Puga, I., Cols, M., Barra, C. M., He, B., Cassis, L., Gentile, M., et al. (2012). B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat. Immunol.* 13, 170–180. doi:10.1038/ni.2194.

Ramírez, C., and Mendoza, L. (2018). Phenotypic stability and plasticity in GMP-derived cells as determined by their underlying regulatory network. *Bioinformatics* 34, 1174–1182. doi:10.1093/bioinformatics/btx736.

Reljic, R. (2006). In search of the elusive mouse macrophage Fc-alpha receptor. *Immunol. Lett.* 107, 80–81. doi:10.1016/j.imlet.2006.04.014.

Risso, A. (2000). Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. *J Leukoc Biol* 68, 785–792. doi:10.1189/jlb.68.6.785.

Roos, D., Van Bruggen, R., and Meischl, C. (2003). Oxidative killing of microbes by neutrophils. *Microbes Infect.* 5, 1307–1315. doi:10.1016/j.micinf.2003.09.009.

Rose, S., Misharin, A., and Perlman, H. (2012). A novel Ly6C/Ly6G-based strategy to analyze the mouse splenic myeloid compartment. *Cytom. Part A* 81 A, 343–350. doi:10.1002/cyto.a.22012.

Saverymuttu, S. H., Peters, A. M., Keshavarzian, A., Reavy, H. J., and Lavender, J. P. (1985). The kinetics of 111Indium distribution following injection of 111Indium labelled autologous granulocytes in man. *Br. J. Haematol.* 61, 675–685. doi:10.1111/j.1365-2141.1985.tb02882.x.

Scapini, P., Marini, O., Tecchio, C., and Cassatella, M. A. (2016). Human neutrophils in the saga of cellular heterogeneity: insights and open questions. *Immunol. Rev.* 273, 48–60. doi:10.1111/imr.12448.

Segal, A. W. (2005). HOW NEUTROPHILS KILL MICROBES. *Annu. Rev. Immunol.* 23, 197–223. doi:10.1146/annurev.immunol.23.021704.115653.

Shi, J., Gilbert, G. E., Kokubo, Y., and Ohashi, T. (2001). Role of the liver in regulating numbers of circulating neutrophils. *Blood* 98, 1226–1230. doi:10.1182/blood.V98.4.1226.

Stark, M. A., Huo, Y., Burcin, T. L., Morris, M. A., Olson, T. S., and Ley, K. (2005). Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* 22, 285–294. doi:10.1016/j.immuni.2005.01.011.

Steele, R. W., Steele, C. R., Pilkington, N. S., and Charlton, R. K. (1987). Functional capacity of marginated and bone marrow reserve granulocytes. *Infect. Immun.* 55, 2359–2363.

Steinbach, K. H., Schick, P., Trepel, F., Raffler, H., Döhrmann, J., Heilgeist, G., et al. (1979). Estimation of kinetic parameters of neutrophilic, eosinophilic, and basophilic granulocytes in human blood. *Blut* 39, 27–38. doi:10.1007/BF01008072.

Stryckmans, P., Cronkite, E. P., Fache, J., Fliedner, T. M., and Ramos, J. (1966). Deoxyribonucleic acid synthesis time of erythropoietic and granulopoietic cells in human beings. *Nature* 211, 717–720. doi:10.1097/BRS.0b013e31817c64d5.

Summers, C., Rankin, S. M., Condliffe, A. M., Singh, N., Peters, A. M., and Chilvers, E. R. (2010). Neutrophil kinetics in health and disease. *Trends Immunol.* 31, 318–324. doi:10.1016/j.it.2010.05.006.

Summers, C., Singh, N. R., White, J. F., Mackenzie, I. M., Johnston, A., Solanki, C., et al. (2014). Pulmonary retention of primed neutrophils: A novel protective host response, which is impaired in the acute respiratory distress syndrome. *Thorax* 69, 623–629. doi:10.1136/thoraxjnl-2013-204742.

Suratt, B. T., Young, S. K., Lieber, J., Nick, J. a, Henson, P. M., and Worthen, G. S. (2001). Neutrophil maturation and activation determine anatomic site of clearance from circulation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 281, L913–L921. doi:10.1152/ajplung.2001.281.4.L913.

Szczepura, K. R., Ruparelia, P., Solanki, C. K., Balan, K., Newbold, P., Summers, C., et al. (2011). Measuring whole-body neutrophil redistribution using a dedicated whole-body counter and ultra-low doses of 111Indium. *Eur. J. Clin. Invest.* 41, 77–83. doi:10.1111/j.1365-2362.2010.02382.x.

Tak, T., Tesselaar, K., Pillay, J., Borghans, J. A. M., and Koenderman, L. (2013). What's your age again? Determination of human neutrophil half-lives revisited. *J. Leukoc. Biol.*

94. doi:10.1189/jlb.1112571.

Tak, T., Wijten, P., Heeres, M., Pickkers, P., Scholten, A., Heck, A. J. R., et al. (2017). Human CD62L<inf>dim</inf>neutrophils identified as a separate subset by proteome profiling and in vivo pulse-chase labeling. *Blood* 129. doi:10.1182/blood-2016-07-727669.

Tam, F. W., Clague, J., Dixon, C. M., Stuttle, a W., Henderson, B. L., Peters, a M., et al. (1992). Inhaled platelet-activating factor causes pulmonary neutrophil sequestration in normal humans. *Am. Rev. Respir. Dis.* 146, 1003–8. doi:10.1164/ajrccm/146.4.1003.

Terashima, T., Wiggs, B., English, D., Hogg, J. C., and van Eeden, S. F. (1996). Polymorphonuclear leukocyte transit times in bone marrow during streptococcal pneumonia. *Am J Physiol* 271, L587-92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8897906.

Tofts, P. S., Chevassut, T., Cutajar, M., Dowell, N. G., and Peters, A. M. (2011). Doubts concerning the recently reported human neutrophil lifespan of 5.4 days. *Blood* 117, 6050–6052. doi:10.1182/blood-2010-10-310532.

van de Vijver, E., van den Berg, T. K., and Kuijpers, T. W. (2013). Leukocyte Adhesion Deficiencies. *Hematol. Oncol. Clin. North Am.* 27, 101–116. doi:10.1016/j.hoc.2012.10.001.

Vincent PC, Chanana AD, Cronkite EP, Joel DD (1974). The intravascular survival of neutrophils labeled in vivo. *Blood* 43, 371–377.

Vogt, K. L., Summers, C., Chilvers, E. R., and Condliffe, A. M. (2018). Priming and de-priming of neutrophil responses in vitro and in vivo. *Eur. J. Clin. Invest.* 48. doi:10.1111/eci.12967.

Wang, J., Hossain, M., Thanabalasuriar, A., Gunzer, M., Meininger, C., and Kubes, P. (2017). Visualizing the function and fate of neutrophils in sterile injury and repair. *Science (80-.).* 358, 111–116. doi:10.1126/science.aam9690.

Weiskotten, H. G. (1930). The Normal Life Span of the Neutrophile (Amphophile) Leucocyte (Rabbit): The Action of Benzol IXNo Title. *Am J Pathol.* 6, 183–190.

Yipp, B. G., Kim, J. H., Lima, R., Zbytnuik, L. D., Petri, B., Swanlund, N., et al. (2017). The lung is a host defense niche for immediate neutrophil-mediated vascular protection. *Sci. Immunol.* 2, eaam8929. doi:10.1126/sciimmunol.aam8929.

Zhou, J., Nefedova, Y., Lei, A., and Gabrilovich, D. (2018). Neutrophils and PMN-MDSC: Their biological role and interaction with stromal cells. *Semin. Immunol.* 35, 19–28. doi:10.1016/j.smim.2017.12.004.

Zhu, Y. P., Padgett, L., Dinh, H. Q., Marcovecchio, P., Blatchley, A., Wu, R., et al. (2018). Identification of an Early Unipotent Neutrophil Progenitor with Pro-tumoral Activity in

Mouse and Human Bone Marrow. *Cell Rep.* 24, 2329–2341.e8. doi:10.1016/j.celrep.2018.07.097.

Table I: Relevant differences between murine and human immune systems

Property	**Murine neutrophils**	**Human neutrophils**	**Reference**
Percentage of neutrophils in peripheral blood	10-25%	50-70%	(Bolliger et al., 2011)
Neutrophil size	8.64 ± 0.14 μM	10.39 ± 0.19 μM	(Ekpenyong et al., 2017)
Nuclear morphology	Ring-like	Segmented	(Biermann et al., 1999)
Neutrophil granule contents	Defensins absent; low expression of BPI, MPO, β-Glucaronidase, lysozyme, alkaline phosphatase, and Arginase-1	Defensins present; high expression of BPI, MPO, β-Glucaronidase, lysozyme, alkaline phosphatase, and Arginase-1	(Risso, 2000)
Chemokine expression	CCL6, CCL9, CXCL15, CCL12 found in mice, but not humans	CXCR1, CXCL8, CXCL7, CXCL11, CCL13. CCL14, CCL15, CCL18, CCL23, CCL24/CCL26 found in humans, but not mice	(Eruslanov et al., 2017)
Neutrophil antigen expression	Express Gr-1 and Ly-6G	Absent	(Rose et al., 2012)
Different Fc receptor expression	Do not express FcαRI Do not express FcγRI	Express FcαRI Inducible expression of FcγRI	(Gillis et al., 2017; Perussia, 1983; Reljic, 2006)
Affinity of fMLF receptor	Low	High	(Gao and Murphy, 1993)

Figures

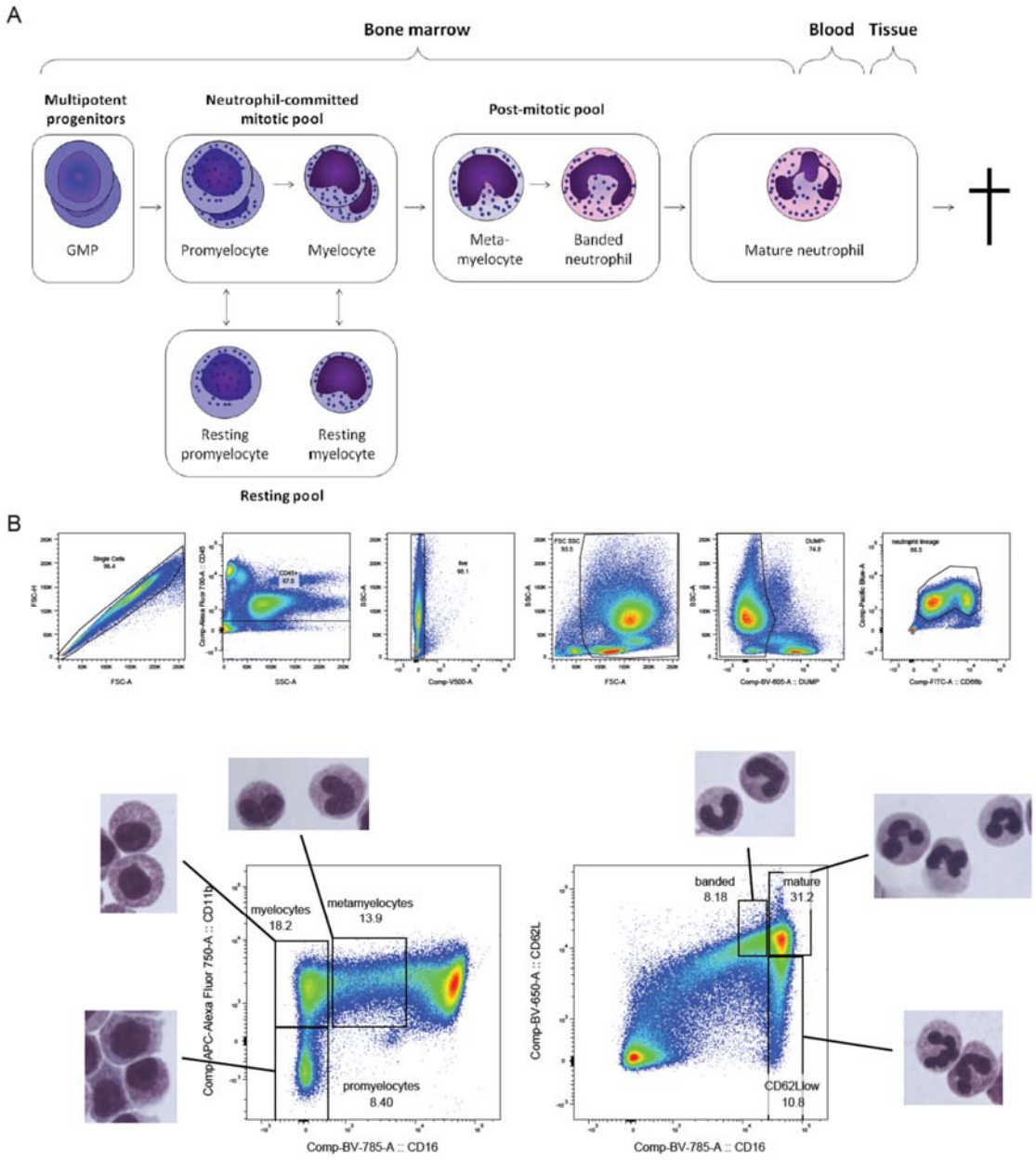

Figure 1: Differentiation of the neutrophil compartment in the bone marrow

A. Model for neutrophil differentiation in the bone marrow. In this model differentiation of neutrophil and its progenitors behaves like a conveyor belt as firstly suggested by Cartwright et al. (Cartwright, G. E., Athens, J. W., Wintrobe, 1964). Neutrophil committed differentiation

starts with the neutrophilic promyelocyte and myelocyte that both have the propensity to divide and as such are part of the mitotic pool. Hereafter, the cells stop dividing and mature into metamyelocytes and band neutrophils both of which are not found in the peripheral blood (i.e. post-mitotic pool). Hereafter, the cells enter the mature state in which they are in exchange between bone marrow, blood and other tissues.
B. Differences in marker expression of differentiating neutrophils in bone marrow. The neutrophil lineage in the bone marrow can be visualized and sorted on scatter characteristics and the expression of three markers: Mac-1 (CD11b), FcγRIII (CD16B) and L-selectin (CD62L). Cytospins are shown of the cells sorted from the indicated gates.

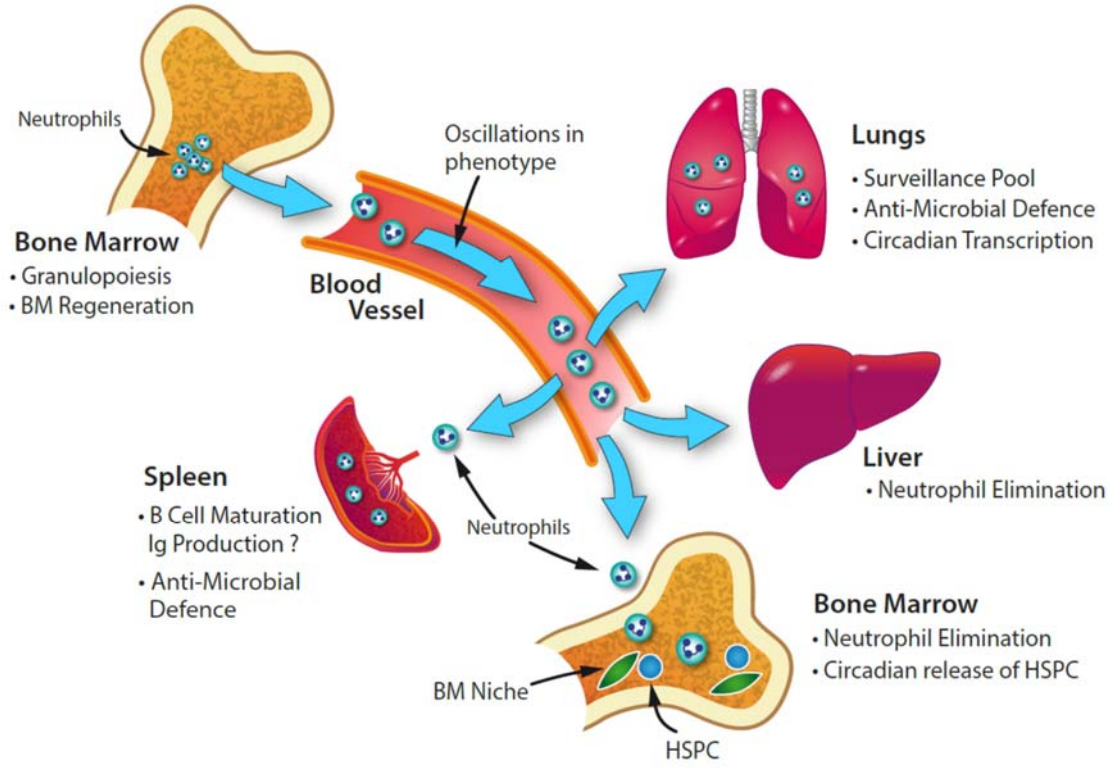

Figure 2: Neutrophil fate within tissue compartments

Neutrophils produced in the BM and released into the circulation can enter multiple tissues after a process of diurnal aging (oscillations in phenotype), even in the absence of inflammatory stimuli. In these tissues they play prominent roles related to anti-microbial defense, immune cell maturation, regulation of stem cell niches, or are ultimately eliminated. For example, while in the BM granulopoiesis and vascular regeneration may represent relevant functions before they are released into blood, neutrophils acquire the capacity to regulate the circadian release of hematopoietic stem and progenitor clles (HSPC) when they return to the marrow for elimination. Similarly, specialized functions in the spleen for B cell maturation or in the lungs for immune surveillance suggest a wealth of functions tailored to each tissue as detailed in the text.