Neutrophil heterogeneity: implications for homeostasis and pathogenesis

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Neutrophils are polymorphonuclear leukocytes of the phagocytic system that act as first line of host defense against invading pathogens but are also important mediators of inflammation-induced injury. In contrast to other members of the innate immune system, neutrophils are classically considered a homogenous population of terminally differentiated cells with a well-defined and highly conserved function. Indeed, their short lifespan, the absent proliferative capacity, their limited ability to produce large amounts of cytokines, and the failure to recirculate from the tissue to the bloodstream have sustained this idea. However, increasing evidence over the last decade has demonstrated an unexpected phenotypic heterogeneity and functional versatility of the neutrophil population. Far beyond their antimicrobial functions, neutrophils are emerging as decision-shapers during innate and adaptive immune responses. These emerging discoveries open a new door to understand the role of neutrophils during homeostatic but also pathogenic immune processes. Thus, this review details novel insights of neutrophil phenotypic and functional heterogeneity during homeostasis and disease. (*Blood*. 2016; 127(18):2173-2181)

One neutrophil or many?

Myeloid and lymphoid members of the hematopoietic system are composed of subsets with distinct phenotypic and functional characteristics. As an example, a wide variety of monocyte[1] and macrophage[2] subpopulations has been identified that display a unique transcriptional profile defining their function during homeostasis and immune responses.[1] The diversity of tissue macrophages can partly be explained by their differential embryonic origin.[3] Beyond ontogeny, macrophage heterogeneity is dictated by environmental signals inducing differentiation or activation.[4] The recognition of external cues by macrophages triggers different patterns of epigenetic modifications, gene expression, and protein synthesis and instructs them to exert a specific function in homeostasis[4] or inflammation.[5] Such activation has been modeled as 2 opposing phenotypic states with different functional properties: M1 macrophage polarization stimulates Th1 effector and microbicidal responses, and M2 macrophage polarization fosters a Th2 and reparative response. Multiple studies have identified these phenotypes in different pathological situations and demonstrated their importance in disease outcome.[2] The dichotomous view of macrophage activation, however, is shifting toward a more plastic understanding of macrophage polarization where macrophages can exhibit multiple phenotypic and functional signatures combining the responses to different environmental stimuli that may vary in a spatiotemporal fashion. This new concept has recently been exemplified in tissue-resident[4] or tumor-associated macrophages.[6] Thus, heterogeneity and plasticity of leukocyte subsets integrate ontogenic and environmental imprints.

In contrast to other leukocyte populations, the idea of neutrophil heterogeneity has received less attention, likely based on the traditional view of unique properties of neutrophils: Limited lifespan, reduced transcriptional activity, and inability to return to the circulation after migrating to extravascular tissue. Hence, we here review emerging evidence challenging these classical concepts of neutrophil biology and further discuss how this may set the foundation for neutrophil heterogeneity.

An adaptable lifespan

Although the lifespan of circulating neutrophils is classically thought to be short,[7] this view was recently challenged by a study estimating the lifespan of human neutrophils to be 5.4 days.[8] Hence, this longer lifespan of neutrophils may set the basis for neutrophils to undergo phenotypic and functional changes. To further understand neutrophil kinetics, assessment of neutrophil lifespan within tissues is of importance (Figure 1). At sites of inflammation, neutrophil lifespan is increased through inhibition of cell apoptosis, an effect triggered by cytokines, pathogen-associated molecular patterns (PAMPs), damage-associated molecular pattern molecules (DAMPs), or environmental factors.[9,10] Indeed, in chronic inflammation, neutrophil lifespan is abnormally prolonged, thereby worsening disease prognosis. Extended neutrophil lifespan through decreased apoptosis is observed in patients with asthma[11] with acute coronary syndrome[12] and results in increased disease severity. Lifespan extension through prosurvival signals produced under inflammatory conditions may also increase the capacity of neutrophils to undergo phenotypic and functional changes and account for neutrophil heterogeneity. For example, oxygen deprivation in tissue during inflammation drives hypoxia-inducible factor–dependent activation of neutrophil prosurvival pathways[13,14] and directly impacts on the bactericidal activity of neutrophils.[13] Similarly, in the low oxygen and nutrient environment of cystic fibrosis airways, neutrophils undergo metabolic reprogramming,[15] trigger prosurvival mechanisms,[16] and acquire distinct phenotypic and functional properties (supplemental Discussion 1, available on the *Blood* Web site).

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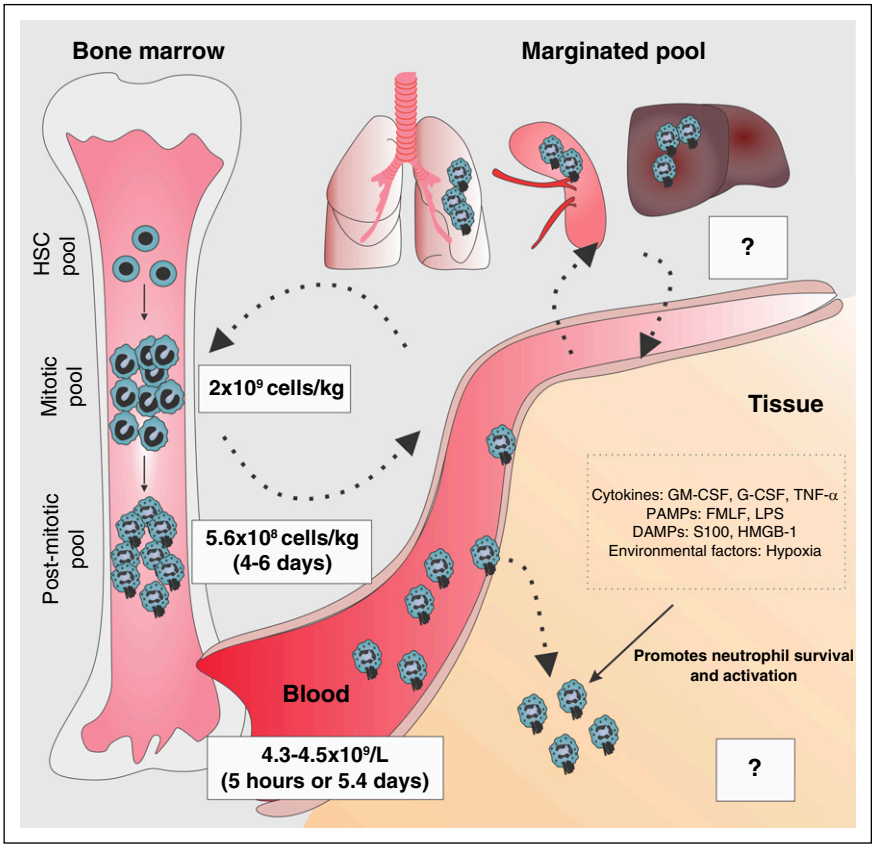

Figure 1. Integration of factors determining neutrophil lifespan. The bone marrow neutrophil lineage is composed of 3 compartments: (1) the hematopoietic stem cell (HSC) pool, (2) the mitotic pool that is comprised of myeloblasts, promyelocytes, and myelocytes, and (3) the postmitotic pool that is comprised of metamyelocytes, band cells, and mature neutrophils. The size of the mitotic and postmitotic pool is estimated at 4.36×10^9 and 8.8×10^9 cells/kg, respectively. After 4 to 6 days in the bone marrow, neutrophils are released to the bloodstream ($4.3\text{-}4.5 \times 10^9$ cells/L). The estimated period of time where neutrophils circulate is controversial. Although some studies suggest that the circulating lifespan is around 5 hours, alternative experimental calculation raises neutrophil lifespan up to 5.4 days, as reported in different studies. Neutrophils are also located in the "marginated pools," vascular pools located in the lungs, spleen, and liver where the turnover time is still unclear. After neutrophils infiltrate tissues during inflammation, interaction with tissue-derived signals such as cytokines, PAMPs, DAMPs, or environmental factors reduces neutrophil apoptosis and increases their lifespan to an extent that remains unknown. fMLF, formyl-methionyl-leucyl phenylalanine; HMGB1, high-mobility group box-1.

Transcriptional plasticity: numbers matter

Leukocyte differentiation toward functionally distinct subpopulations requires the ability of these cells to synthesize and release a new set of proteins in response to environmental signals. Although mature neutrophils are classically considered to have limited capacity for de novo protein synthesis,[17] recent work has explored the magnitude and mechanisms of neutrophil transcriptional[18] and translational regulation.[19] It is important to recognize that human neutrophil mRNA content is 10 to 20 times lower than that of other leukocytes,[20] which translates into a lower protein production on a per cell basis. However, because neutrophils recruited to inflamed tissues greatly outnumber other leukocytes, the overall impact of neutrophil-derived cytokines in the inflammatory response can be significant. Beyond cytokines, de novo protein synthesis in neutrophils extends to membrane receptors (eg, Fcγ receptor-1[21]), which may additionally alter neutrophil heterogeneity and function. In line with the ability of neutrophils to synthesize de novo proteins, recent studies report an active chromatin remodeling and a fine regulation of mRNA expression. On one side, neutrophil gene expression is controlled on an epigenetic level by chromatin histone modification such as methylation[22] or acetylation,[23] processes that tightly influence gene transcription. On the other side, neutrophils display a cell-specific pattern of noncoding regulatory regions,[24] elements that are involved in the regulation of gene expression. Interestingly, interleukin (IL)-6 expression by human neutrophils after exposure to Toll-like receptor (TLR)-8 ligands involved chromatin remodeling and increased activity of noncoding regulatory elements (eg, enhancers).[23] Thus, this evidence supports an inducible epigenetic and genetic regulation of neutrophil gene expression. Moreover, the association between genetic variants[24] and altered methylation profiles[25] in neutrophils associated with disease susceptibility emphasizes the importance of understanding how gene expression is controlled in neutrophils.

Collectively, these studies indicate that neutrophils are endowed with an active transcriptomic function, the disturbance of which may provoke abnormal gene expression and impact on disease outcome. Moreover, neutrophil transcriptional modulation during communication with the immune network or interaction with environmental signals may dictate the function required in a specific situation. For instance, IL-13$^+$ neutrophils prime macrophages toward an anti-inflammatory phenotype that enhances parasite clearance during nematode infection (supplemental Discussion 2). Similarly, neutrophil-derived IL-12 or IL-10 secreted by CD49d$^+$ neutrophil subsets shape macrophage function during bacterial infection (supplemental Discussion 3).

Multidirectional migration

Abluminal to luminal transmigration

During the last decade, a number of studies have reported the ability of neutrophils to return to the bloodstream after migrating to the extravascular space,[26-28] thus challenging the classical concept of unidirectional neutrophil migration. In vitro, reversely migrated neutrophils were shown to display a different phenotype (intercellular adhesion molecule-1 [ICAM-1]^{high}CXCR1^{low}) compared with circulating (ICAM-1^{low}CXCR1^{high}) or tissue-resident (ICAM-1^{low}CXCR1^{low}) neutrophils, a phenotype also found in low abundance in the circulation.[26] Interestingly, reverse-transmigrated neutrophils were not able to transmigrate again into the tissue and had a prolonged lifespan and an

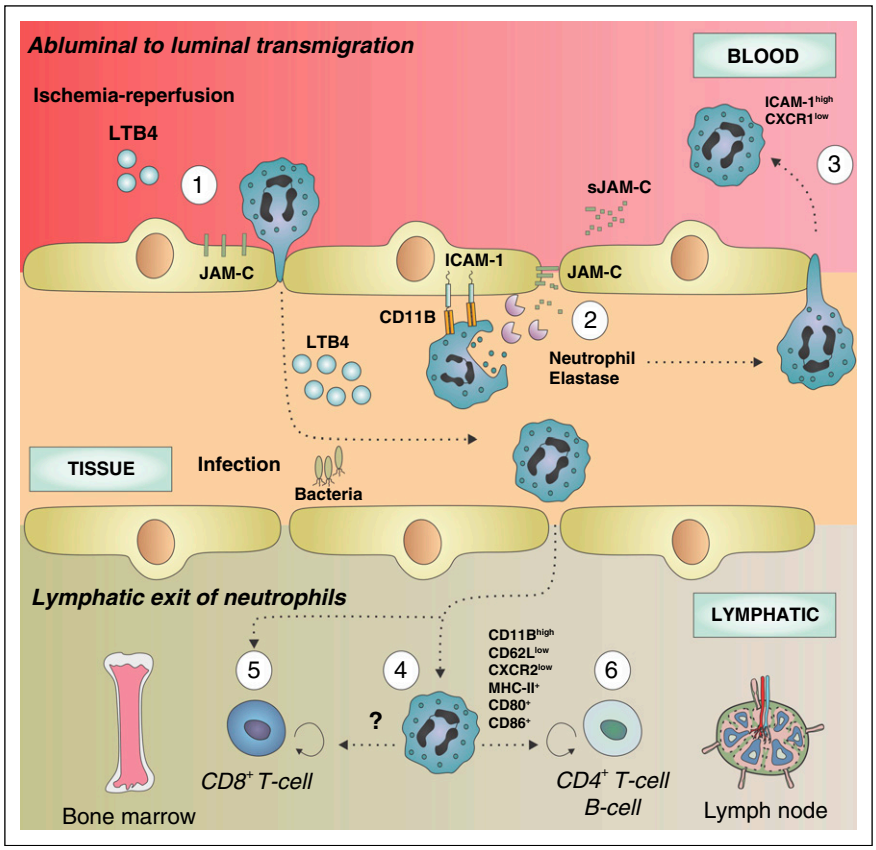

Figure 2. Mutidirectional neutrophil migration. Neutrophil reverse transmigration from the subendothelial space to the circulation occurs predominantly after ischemia/reperfusion injury. After LTB4-driven neutrophil recruitment (1), infiltrated neutrophils interact with endothelial cells via CD11b and release NE, which degrades the JAM-C (2), allowing their circulation back to the bloodstream (3). Reverse-transmigrated neutrophils exhibit a distinct phenotype characterize by ICAM-1^{high}CXCR1^{low}. During pathogen-driven inflammation, infiltrated neutrophils may also return to lymphoid organs (bone marrow or lymph node) through the circulation or the lymphatic system. Inside the lymph nodes, neutrophils display an activated phenotype (CD11B^{high}, CD62L^{low}, CXCR2^{low}) and express MHC (CH)-II and the costimulatory molecules CD80 and CD86, suggesting a newly acquired ability to present antigens (4). Furthermore, this subpopulation of neutrophils is able to modulate the adaptive immune response by promoting or repressing the T- and B-cell function in the lymph node (6). Neutrophils migrating toward the bone marrow induce CD8$^+$ T cell–dependent antiviral responses (5).

increased capacity to produce superoxide species.[26] The in vivo proof of reverse transmigration was described using intravital microscopy.[28] Although luminal to abluminal transmigration is regulated by various junctional proteins (eg, platelet endothelial cell adhesion molecule-1, VE-cadherin, and CD99), reverse transmigration predominantly depends on junctional adhesion molecule (JAM)-C.[28] Under ischemia-reperfusion, JAM-C is redistributed to a nonjunctional membrane location triggering neutrophil adhesion during luminal to abluminal migration,[29] an event that was also observed after leukotriene B4 (LTB4) treatment. On the contrary, excessive LTB4 induced presentation of neutrophil elastase on CD11b, JAM-C degradation, and subsequent reverse transmigration (Figure 2).[27] Neutrophils undergoing reverse transmigration exhibited a proinflammatory phenotype characterized by a high ICAM-1 expression.[28] Interestingly, ICAM-1^{high} neutrophils were found in distant organs after ischemia/reperfusion or LTB4 infusion, suggesting a potential implication in the systemic propagation of inflammation.[27,28] Overall, reverse transmigration prolongs neutrophil lifespan and modulates their phenotype and function, thereby contributing to neutrophil heterogeneity.

Lymphatic transit of neutrophils

An alternative way of neutrophil emigration from the tissue involves the exit through the lymphatic vessels, a route that gives neutrophils access to secondary lymphoid organs (Figure 2). During infection, mouse neutrophils are able to migrate to the lymph node through lymph and blood vessels.[30-36] The fact that neutrophils were detected carrying living bacteria from infected tissue to the lymph node underlines their migratory capacity and their potential to interact with the adaptive immune system.[30] In agreement with these observations, Hampton and colleagues recently confirmed the ability of neutrophils to migrate into the lymph nodes after skin infection.[34] Interestingly, migrated neutrophils exhibited a distinct activated phenotype (CD11B^{high}, CD62L^{low}, CXCR2^{low}), which was required for migration to the lymph nodes. Functionally, specific blocking of neutrophil migration to the lymph nodes reduced T-cell proliferation, thereby demonstrating the importance of neutrophils in regulating the adaptive response.[34] Accordingly, neutrophils were able to enter lymph nodes in a mouse model of antigen immunization and to modulate CD4$^+$ T-cell and B-cell responses.[36] Another example of neutrophil recirculation comes from the observation that neutrophils egress from virus-infected skin and infiltrate the bone marrow to interact with resident CD8$^+$ T cells resulting in enhanced antiviral responses.[32] Hence, recirculation of neutrophils to draining lymph nodes may act as an early step of antigen presentation and initiation of adaptive immune responses. In accordance with this, neutrophils recruited into lymph nodes upregulated major histocompatibility complex (MHC)-II and costimulatory molecules (CD80, CD86), which may be involved in neutrophil-dependent antigen presentation.[34] Alternatively, it has been proposed that neutrophils carrying bacteria or viruses may serve as "Trojan horses" that permit the dissemination of the infection on macrophage engulfment.[37,38] Collectively, the ability of neutrophils to egress from tissue, to reenter into the circulation, and to migrate into secondary lymphoid organs represents 1 source of neutrophil plasticity and heterogeneity.

Neutrophil heterogeneity

Recent reviews have listed various forms of neutrophil heterogeneity.[39,40] Below we discuss the most recent studies describing neutrophil heterogeneity under homeostatic and disease conditions (Table 1).[41-72]

Table 1. Neutrophil heterogeneity in homeostatic and pathological conditions

Neutrophil subset	Prevalence	Phenotypic properties	Functional properties	Homeostatic/pathological relevance	Reference
CD177^{+}	*Human*: ~50% of circulating neutrophils	CD177^{+} Proteinase 3	= Apoptosis = transmigration in human peritonitis↓ granule protein mRNA content	CD177 autoantibodies in ANCA-derived vasculitis patients	41-45
"Aged"	*Mouse*: 55 ± 14% in ZT5 (clearance from the circulation period) 8 ± 3% in Z17 (release from the circulation period)	CXCR4^{high}CD62L^{low}CD11B^{high} CD49^{high}; hypersegmented nucleus; Reduced size and granularity	= Apoptosis ↑ Phagocytosis and NETosis	Regulation of stem cell niche and circadian release of HSPCs to the circulation / Enhanced intravascular inflammation	46,47
OLFM4^{+}	*Human*: 20-25% of circulating neutrophils	OLFM4 expression in neutrophil-specific granules	= Apoptosis = Phagocytosis = Transmigration ↓ Cathepsin C activity OLFM4 + NET formation	OLFM4 autoantibodies in ANCA-derived vasculitis patients Enhanced immune response against *S aureus* and *E coli* infection in absence of OLFM4	48-52
TCR^{+}	*Human*: 3-5% of circulating neutrophils	*Human*: TCRα,β variants *Mouse*: Vα2, Vα5, Vβ1, Vβ16	Delayed apoptosis and IL-8 production on CD3/CD28 stimulation	Reduced TCR variants in aged subjects compared to young individuals	53,54
Angiogenic	*Mouse*: 2.8% *Human*: 3.2%	CD49d^{+}VEGFR1^{high}CXCR4^{high}	↑MMP9	Promotes angiogenesis in hypoxic tissue	55,56
CD62L^{dim}/CD16^{bright} CD62L^{bright}/CD16^{dim}	*Human*: 3 hours after LPS administration: CD62L^{bright}/CD16^{bright}: 60-70% CD62L^{dim}/CD16^{bright}:20-25% CD62L^{bright}/CD16^{dim}:10-15%	CD62L^{bright}; CD16^{bright}; multi-lobular nucleus CD62L^{dim}; CD16^{bright}; CD11C^{high}; CD11B^{high}; CD54^{high} ; hypersegmented nucleus CD62L^{bright}; CD16^{dim}; CD11C^{low}; CD11B^{low}; CD54^{low} ; band-form nucleus	CD62L^{dim}/CD16^{bright}: -Inhibition of T-cell proliferation through ROS release and dependent on CD11B expression	Potential implication in sepsis-related immunosuppresion	57,58
CD63^{+}	*Human:* neutrophils isolated from lung sputum	CD11B^{high} CD66^{high} CD63^{+}CD80^{+}MHC-II^{+}	↓ Glutathione activity ↑ Neutrophil elastase activity ↑ Arg1	Neutrophil elastase-dependent perpetuation of inflammation, infection and progression of cystic fibrosis airway disease	59,60
IL-13^{+}	Not defined	Ring-form nucleus; IL-5; IL-13; IL-33; insulin-like growth factor-1; Resistin-like molecule alpha/ FIZZ1; chitinase-like 3.	Priming of macrophages towards alternative or M2 phenotype	Accelerated clearance of nematodes by primed macrophages	61
CD49^{+}	*Mouse*: Sendai virus infection: 50% of BAL neutrophils MRSA infection: not defined *Human*: Non atopic patients: 1.625%; atopic patients: 6.57%	*PMN-I*: CD11B^{-}, CD49^{+}, TLR2^{+}, TLR4^{+}, TLR5^{+}, TLR8^{+}; multilobular nucleus, MPO^{high} *PMN-II*: CD11B^{+}, CD49-, TLR2^{+}, TLR4^{+}, TLR7^{+}, TLR9^{+}, ring-form nucleus; MPO^{low}	PMN-I: IL-12, CCL3, classic activation of macrophages PMN-II: IL-10, CCL2, alternative activation of macrophages	CD49d^{+} neutrophils promote virus-challenged experimental asthma CD49^{+} but not CD49^{-} neutrophils induce resistance to MRSA infection Association between CD49d^{+} neutrophils and allergic disease in humans	62-64
IL-17^{+}	*Human*: ~70% of circulating neutrophils upon IL-6 and IL-23 stimulation *Mouse*: ~17% of bone marrow neutrophils on IL-6 and IL-23 stimulation	IL-17A^{+}; IL-17ra^{+}, RORγt^{+}; dectin-2	↑ ROS production ↑ Increase fungal killing capacity	Reduction of *Aspergillus fumigatus*–mediated keratitis	65

arg1, arginase 1; BAL, bronchoalveolar lavage; MMP9, metalloproteinase-9; MPO, myeloperoxidase; MRSA, methicillin-resistant *Staphylococcus aureus*; NO, nitric oxide; pDCs, plasmacytoid dendritic cells; PMN, polymorphonuclear leukocyte; VEGF, vascular endothelial growth factor; ZT, zeitgeber time; ↑, increase; ↓, decrease; =, no change.

Table 1. (continued)

Neutrophil subset	Prevalence	Phenotypic properties	Functional properties	Homeostatic/pathological relevance	Reference
LDGs	*Human:* Healthy donors: ~17% SLE patients: 1.2-54%	Mixed population with cells with band, lobular, or myelocyte-like nuclei	↓ Phagocytosis, = MPO, =respiratory burst ↑ IFN-γ; TNF-α ↑ Endothelial cell killing capacity ↑ NET production	Promotion of SLE-associated inflammation: induction of IFN-α production by pDCs through NET release	66-68
TAN	Not defined	*N1 TAN:* Met^+; hypersegmented nuclei *N2 TAN:* Rounded nuclei	N1 TAN: ↑ tumor cell killing capacity; ↑ NO; ↑ H_2O_2; ↑ TNF-α; ↑ ICAM-1; ↓ arg1; ↓ CCL2, ↓CCL5, ↓VEGF; ↓ MMP9 N2 TAN: Rounded nuclei ↓ tumor cell killing capacity; ↓ NO; ↓ H_2O_2; ↓ TNF-α; ↓ ICAM-1; ↑ arg1; ↑ CCL2, ↑ CCL5, ↑ VEGF; ↑ MMP9 ↑ S100a8; ↑ S100a9; ↑ Prok2;	N1 and N2 TANs inhibit and promotes tumor development, respectively	69-72

arg1, arginase 1; BAL, bronchoalveolar lavage; MMP9, metalloproteinase-9; MPO, myeloperoxidase; MRSA, methicillin-resistant *Staphylococcus aureus*; NO, nitric oxide; pDCs, plasmacytoid dendritic cells; PMN, polymorphonuclear leukocyte; VEGF, vascular endothelial growth factor; ZT, zeitgeber time; ↑, increase; ↓, decrease; =, no change.

Phenotypic and functional adaption by activation

Neutrophil activation by cues derived from sites of inflammation results in exposure of distinct surface markers or de novo synthesis of cytokines, characteristics that are both commonly used to define neutrophil subsets. Neutrophil heterogeneity may thus partly be explained by a differential activation stage of neutrophil subpopulations. After interaction with endothelial selectins and chemotactic molecules neutrophil secretory vesicles are fused with the surface membrane exposing the proteins contained in these vesicles.[73-75] In this way, neutrophils activated on the endothelium quickly upregulate CD11b and CD18, thus contributing to firm adhesion.[76] In addition, tumor necrosis factor (TNF) receptors located in specific and gelatinase granules are redistributed to the cell membrane after activation.[77] Galectin-3 released by macrophages or mast cells is a potent activator of neutrophils. Its receptor, CD66, is stored in specific granules, and stimulation with lipopolysaccharide (LPS)[78] or granulocyte colony-stimulating factor (G-CSF)[79] induces the secretion of specific granules exposing CD66 and hence primes the neutrophil for a second response to galectin-3. Laminin receptor-1 is located in the specific granules and its binding to the extracellular matrix protein laminin induces the secretion of lysozyme and the production of superoxide, thereby enhancing "second-hit" responses.[80] Interestingly, circulating platelets activated by danger signals can also deliver signals through protruding domains presented by a fraction of intravascular neutrophils, a process that promotes neutrophil crawling and recruitment into inflamed tissues.[81]

Neutrophil subsets in homeostasis

CD177. In humans, CD177 glycoprotein (NB1) is exclusively expressed on the surface of neutrophils and regulates transmigration across the endothelium through the interaction with platelet endothelial cell adhesion molecule-1.[82] In humans, a fraction of $CD177^+$ neutrophils has been identified[83] coexpressing membrane-bound proteinase-3,[41] a serine protease normally located in primary granules. CD177 expression is required for surface presentation of proteinase-3,[84] which in turn facilitates the transmigration of $CD177^+$ neutrophils.[85] The variable inter- and intraindividual expression of the CD177–proteinase-3 partnership in circulating neutrophils gained importance when membrane proteinase-3 was identified as an antigen antineutrophil cytoplasmic antibody (ANCA)-dependent vasculitis.[86] Accordingly, the levels of neutrophils coexpressing proteinase-3 and CD177 are augmented in patients of ANCA-dependent vasculitis[87] and associate with increased risk of relapse.[88] However, the direct implication of $CD177^+$ or $CD177^-$ subsets in ANCA-derived vasculitis and other inflammatory diseases remains unknown. To understand the potential role of CD177, a mouse genetically deficient in this protein was generated.[89] As in humans,[82] the absence of CD177 did not affect the migratory capacity of neutrophils; however, it caused cell death in this mouse model.[89] In conclusion, although the frequency of $CD177^+$ neutrophils during inflammatory disease is consistently augmented, the functional properties of this neutrophil subset are controversial and will require further investigation.

Aged neutrophils. Neutrophil numbers are controlled by a fine balance between production, retention, mobilization, margination, and clearance. Neutrophil retention in the bone marrow is regulated by the coordinated action of CXCL12 and its receptor CXCR4, whereas neutrophil egress depends on CXCR2 and its ligands.[90,91] CXCR4 is expressed at low levels in bone marrow–resident neutrophils while almost undetectable on the surface of circulating neutrophils.[92] Interestingly, culture of human neutrophils for 20 hours triggers the

upregulation of CXCR4 expression and increases their migratory capacity toward CXCL12 and the predilection to home to the bone marrow where they are cleared.[93] In mice, this subpopulation of neutrophils, named "senescent" or "aged" neutrophils, are characterized by the upregulation of CXCR4 and CD11b and the loss of CD62L, size reduction, and hypersegmentation of the nucleus.[46] Interestingly, clearance of aged neutrophils by bone marrow–resident macrophages downregulated CXCL12 production by stromal cells and induced the mobilization of hematopoietic stem progenitor cells (HSPCs) to the circulation.[46] Moreover, numbers of circulating aged neutrophils and their clearance in the bone marrow fluctuated during the day, a process that controlled the circadian-oscillations of HSPC numbers in the blood.[46] Besides this noninflammatory function, aging predisposes overactivation of neutrophils, which increases CD11b expression and susceptibility of neutrophil extracellular trap (NET) formation.[47] Furthermore, this recent study provides evidence that neutrophil ageing is regulated by the microbiota in a TLR-dependent fashion. Interestingly, this aged subpopulation predominates in mice and humans under pathological conditions and dramatically worsens the disease outcome in a mouse model of sickle cell disease.[47] Overall, these findings illustrate that aging may be a source of neutrophil heterogeneity that predisposes to functional changes with implications in homeostasis and inflammation.

Olfactomedin-4. Olfactomedin-4 (OLFM4) is present in neutrophil-specific granules of 20% to 25% of human circulating neutrophils.[48] Although the specific function of this protein in neutrophils is still unknown, neutrophil-derived OLFM4 has been suggested to induce autoimmune responses (supplemental Discussion 4).

T-cell receptor. A repertoire of T-cell receptor (TCR) variants has unexpectedly been detected in a subset of circulating human neutrophils.[53] A detailed discussion on this subset can be found in the supplemental Discussion 5.

Angiogenic neutrophils. Under ischemia conditions, a distinct subset of neutrophils is recruited to hypoxic areas with unique phenotypic and angiogenic properties that facilitate restoration of the oxygen supply in the affected tissue[55] (supplemental Discussion 6).

Neutrophil subsets in pathologic conditions

Pathogen-mediated systemic inflammation. Systemic inflammation elicited by injury or sepsis causes overproduction and hyperreaction of neutrophils. After the initial immune response, nonresolved inflammation results in a prolonged action of neutrophils, which may cause collateral damage.[94] However, it is during this latter phase when an anti-inflammatory response is produced, resulting in immunosuppression and increasing the risk for mortality.[95] Because neutrophilia is still occurring during this phase of the disease, Pillay and colleagues aimed to examine specific alterations in the phenotype and function of neutrophils that may explain the observed immunosuppression.[57] Analysis of circulating neutrophils in LPS-challenged healthy volunteers identified fully competent mature $CD16^{bright}$ neutrophils that were accompanied by the release of banded $CD16^{dim}$ neutrophils with increased reactive oxygen species (ROS) production but impaired antimicrobial function.[57] Consequent analysis of LPS-driven neutrophil heterogeneity by the same group revealed the existence of a novel subset of mature, hypersegmented human neutrophils with immunosuppressive activity.[58] This subset was defined by the surface markers $CD11c^{bright}CD62L^{dim}CD11b^{bright}CD16^{bright}$ and was able to suppress T-cell proliferation through the release of ROS and required the expression of CD11b. The ability to suppress T-cell proliferation and responses has also been described for myeloid-derived suppressor cells (MDSCs), a leukocytic population identified in different pathologies such as infection or cancer.[96] This MDSC population is composed of a monocytic and granulocytic subpopulation with the ability to suppress T-cell proliferation and activation in a ROS- and Arginase I–dependent fashion. Although the granulocytic counterparts of this population (G-MDSC) exhibit phenotypic and functional differences compared with those observed by Pillay and colleagues,[96] both share this immunosuppressive activity. The lack of a consensus when phenotyping G-MDSCs[96] makes it difficult to discern whether these 2 populations belong to a unique heterogeneous population with immunosuppressive abilities.

In pathogen-mediated inflammation, a number of other neutrophil subsets have been described. Refer to the supplemental Discussion for information on $CD63^+$, IL-13^+, $CD49^+$, and IL-17^+ neutrophil subsets (supplemental Discussions 1, 2, 3, and 7).

Systemic lupus erythematosus. Initially an abnormal population of neutrophils isolated from patients of systemic lupus erythematosus (SLE) and other autoimmune disorders was characterized by displaying different density properties.[97] The functional characterization of this subpopulation of neutrophils, referred to as low-density neutrophils (LDGs), revealed a proinflammatory, activated phenotype with exacerbated production of type I interferons (IFNs) and reduced phagocytic capacity.[66] Human LDGs were also shown to be highly susceptible to form NETs in the absence of stimulation, which was responsible for the enhanced endothelial cell (EC) toxicity displayed by this subset.[67] Indeed, anti-ribonucleoprotein antibodies developed in SLE patients induced NET release by neutrophils causing a potent activation of dendritic cells and production of IFN-α.[68,98] Self-DNA and antimicrobial peptides present in the NET structures led to the generation of autoantibodies triggering a positive feedback loop that amplified the inflammatory response. Of note, LDGs have also been identified in other physiological or pathogenic immune alterations such as cancer,[99] sepsis,[100] pregnancy,[101] diabetes,[102] or HIV infection.[103] Interestingly, these LDG subpopulations share the ability to suppress the immune response, a characteristic that contrasts the proinflammatory phenotype of SLE-associated neutrophils.

Cancer. During tumor-driven inflammation, ≥2 different subpopulations of neutrophils have been described that exert antagonist functions. Fridlender et al demonstrated in mice that tumors were infiltrated by tumor-associated neutrophils (TANs) characterized by a hypersegmented nucleus, increased production of proinflammatory cytokines, and enhanced tumor cell killing capacity.[69] In contrast to this antitumoral population, referred to as N1, an alternative population of TANs (N2) was identified that displayed an immature phenotype and increased arginase activity and promoted tumor growth. TANs exhibit clear phenotypic and functional parallels with M1 and M2 macrophage polarization patterns. For instance, N2 neutrophils and M2 macrophages, both of which favor tumor growth, share elevated arginase activity, which is responsible for inhibition of T-cell proliferation by depletion of L-arginine.[104] The "polarization" of TANs from a protumoral toward an antitumoral phenotype was attributed to the action of the anti-inflammatory cytokine transforming growth factor (TGF)-β.[69] Interesting in this context is that the primary tumor hijacked the IL-17/G-CSF axis to promote the mobilization of protumoral neutrophils.[70] It is presently unclear whether these reported populations of protumoral neutrophils share a common origin and function. On the other hand, IFN-β has been involved in the activation of mouse and human neutrophils toward an antitumor phenotype, as demonstrated by genetically deficient mouse models[71,105] and IFN therapies.[105] Similarly, Finisguerra and colleagues recently demonstrated the importance of *Met,* a proto-oncogene whose expression in mouse and human neutrophils is essential in the transmigration, cytotoxic, and protumoral capacity of these cells.[72]

As mentioned before, MDSCs are a separate population of cells with monocytic or granulocytic phenotypes that appear in tumor-bearing mice and cancer patients and are characterized by their ability to suppress T-cell proliferation and activity.[96] Transcriptomic analysis of TANs compared with G-MDSCs and naïve neutrophils suggested that these 3 populations are distinct, with a surprisingly close proximity between G-MDSCs and naïve neutrophils compared with the TAN subpopulation.[106] Hence, it is conceivable that the N1 antitumoral and N2 protumoral phenotypes described by Fridlender and others originated from naïve neutrophils or G-MDSCs, respectively. However, because naïve neutrophils were isolated from tumor-free mice, a more detailed study would be required to define the functional differences between suppressive (G-MDSCs) or non-suppressive (naïve) neutrophils isolated from tumor-bearing mice. Unfortunately, the only method to reliably distinguish G-MDSCs from normal neutrophils depends on the analysis of their suppressive capacity, a technical handicap that impedes the proper isolation of both populations without compromising their function during manipulation. In this regard, advantage should be taken of the density properties attributed to G-MDSCs that resemble those exhibited by LDGs. Sagiv et al demonstrated that tumor-associated mouse and human LDGs are a heterogeneous population composed of mature and immature neutrophils with suppressive capacity.[99] Interestingly, the mature members of this population originated from high-density neutrophils, which acquire immunosuppressive functions after exposure to TGF-β. Thus, this study provides a plausible explanation as to the origin of suppressive, mature neutrophils (N2 TAN). On the other hand, the authors suggested that the immature LDGs originate from immature neutrophils that are released from the bone marrow before completing their maturation. Because LDGs isolated from SLE patients do not exhibit immunosuppressive capacity, it is reasonable to considerer that the acquisition of low-density properties and the immunosuppressive capacity are independent processes that occur after neutrophil activation to thus far unknown environmental signals. Interestingly, in vitro activation of circulating neutrophils isolated from healthy donors induced the acquisition of the suppressive capacity and low-density properties probably after degranulation.[107] In line with these results, LDGs[101,103] and G-MDSCs[107] express high levels of CD66b, a receptor mobilized from the granules to the surface on cell activation. However, additional studies are needed to further clarify the exact origin of these cells and the external signals that confer their immunosuppressive capacities.

Conclusions

As technical handicaps are being solved by the generation of novel techniques and animal models, a wide range of new and fascinating functions attributed to neutrophils is being discovered. The classical view of neutrophils does not accommodate all aspects of neutrophil biology, and thus the previously ascribed functions of neutrophils seem incomplete. The recently described prolonged lifespan of neutrophils, their ability to de novo synthesize cytokines, and the capacity to recirculate through different tissues and organs extend their repertoire of immunomodulatory functions by interacting and modulating the immune response exerted by both the innate and adaptive system. Moreover, these recently identified properties of neutrophils align with the increasing evidence for the existence of neutrophil subpopulations with distinct phenotypic and functional characteristics. It is indeed this renovated view of neutrophils as a plastic cell that may partly explain neutrophil heterogeneity. Understanding neutrophil heterogeneity may be instrumental to develop novel therapies that specifically target pathogenic neutrophil subsets without compromising immunity.

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Authorship

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References

1. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol.* 2014; 14(6):392-404.
2. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol.* 2011;11(11):723-737.
3. Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature.* 2015;518(7540): 547-551.
4. Lavin Y, Winter D, Blecher-Gonen R, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell.* 2014;159(6):1312-1326.
5. Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity.* 2014;41(1):14-20.
6. Kratochvill F, Neale G, Haverkamp JM, et al. TNF counterbalances the emergence of M2 tumor macrophages. *Cell Reports.* 2015;12(11): 1902-1914.
7. Cartwright GE, Athens JW, Wintrobe MM. The kinetics of granulopoiesis in normal man. *Blood.* 1964;24:780-803.
8. Pillay J, den Braber I, Vrisekoop N, et al. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. *Blood.* 2010;116(4):625-627.
9. Geering B, Stoeckle C, Conus S, Simon HU. Living and dying for inflammation: neutrophils, eosinophils, basophils. *Trends Immunol.* 2013; 34(8):398-409.
10. Sundqvist M, Wekell P, Osla V, et al. Increased intracellular oxygen radical production in neutrophils

during febrile episodes of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome. *Arthritis Rheum.* 2013;65(11):2971-2983.

11. Uddin M, Nong G, Ward J, et al. Prosurvival activity for airway neutrophils in severe asthma. *Thorax.* 2010;65(8):684-689.
12. Garlichs CD, Eskafi S, Cicha I, et al. Delay of neutrophil apoptosis in acute coronary syndromes. *J Leukoc Biol.* 2004;75(5):828-835.
13. Peyssonnaux C, Datta V, Cramer T, et al. HIF-1alpha expression regulates the bactericidal capacity of phagocytes. *J Clin Invest.* 2005; 115(7):1806-1815.
14. Thompson AA, Elks PM, Marriott HM, et al. Hypoxia-inducible factor 2α regulates key neutrophil functions in humans, mice, and zebrafish. *Blood.* 2014;123(3):366-376.
15. Laval J, Touhami J, Herzenberg LA, et al. Metabolic adaptation of neutrophils in cystic fibrosis airways involves distinct shifts in nutrient transporter expression. *J Immunol.* 2013; 190(12):6043-6050.
16. Makam M, Diaz D, Laval J, et al. Activation of critical, host-induced, metabolic and stress pathways marks neutrophil entry into cystic fibrosis lungs. *Proc Natl Acad Sci USA.* 2009; 106(14):5779-5783.
17. Cassatella MA. The production of cytokines by polymorphonuclear neutrophils. *Immunol Today.* 1995;16(1):21-26.
18. Tecchio C, Micheletti A, Cassatella MA. Neutrophil-derived cytokines: facts beyond expression. *Front Immunol.* 2014;5:508.
19. Yost CC, Denis MM, Lindemann S, et al. Activated polymorphonuclear leukocytes rapidly synthesize retinoic acid receptor-alpha: a mechanism for translational control of transcriptional events. *J Exp Med.* 2004;200(5): 671-680.
20. Scapini P, Calzetti F, Cassatella MA. On the detection of neutrophil-derived vascular endothelial growth factor (VEGF). *J Immunol Methods.* 1999;232(1-2):121-129.
21. Cassatella MA, Flynn RM, Amezaga MA, Bazzoni F, Vicentini F, Trinchieri G. Interferon gamma induces in human neutrophils and macrophages expression of the mRNA for the high affinity receptor for monomeric IgG (Fc gamma R-I or CD64). *Biochem Biophys Res Commun.* 1990;170(2):582-588.
22. Zilbauer M, Rayner TF, Clark C, et al. Genome-wide methylation analyses of primary human leukocyte subsets identifies functionally important cell-type-specific hypomethylated regions. *Blood.* 2013;122(25):e52-e60.
23. Zimmermann M, Aguilera FB, Castellucci M, et al. Chromatin remodelling and autocrine TNFα are required for optimal interleukin-6 expression in activated human neutrophils. *Nat Commun.* 2015;6:6061.
24. Naranbhai V, Fairfax BP, Makino S, et al. Genomic modulators of gene expression in human neutrophils. *Nat Commun.* 2015;6:7545.
25. Coit P, Yalavarthi S, Ognenovski M, et al. Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. *J Autoimmun.* 2015;58:59-66.
26. Buckley CD, Ross EA, McGettrick HM, et al. Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration. *J Leukoc Biol.* 2006;79(2):303-311.
27. Colom B, Bodkin JV, Beyrau M, et al. Leukotriene B4-Neutrophil Elastase Axis Drives Neutrophil Reverse Transendothelial Cell Migration In Vivo. *Immunity.* 2015;42(6): 1075-1086.
28. Woodfin A, Voisin MB, Beyrau M, et al. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. *Nat Immunol.* 2011;12(8): 761-769.
29. Scheiermann C, Colom B, Meda P, et al. Junctional adhesion molecule-C mediates leukocyte infiltration in response to ischemia reperfusion injury. *Arterioscler Thromb Vasc Biol.* 2009;29(10):1509-1515.
30. Abadie V, Badell E, Douillard P, et al. Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood.* 2005;106(5):1843-1850.
31. Chtanova T, Schaeffer M, Han SJ, et al. Dynamics of neutrophil migration in lymph nodes during infection. *Immunity.* 2008;29(3):487-496.
32. Duffy D, Perrin H, Abadie V, et al. Neutrophils transport antigen from the dermis to the bone marrow, initiating a source of memory CD8+ T cells. *Immunity.* 2012;37(5):917-929.
33. Gorlino CV, Ranocchia RP, Harman MF, et al. Neutrophils exhibit differential requirements for homing molecules in their lymphatic and blood trafficking into draining lymph nodes. *J Immunol.* 2014;193(4):1966-1974.
34. Hampton HR, Bailey J, Tomura M, Brink R, Chtanova T. Microbe-dependent lymphatic migration of neutrophils modulates lymphocyte proliferation in lymph nodes. *Nat Commun.* 2015; 6:7139.
35. Yang CW, Strong BS, Miller MJ, Unanue ER. Neutrophils influence the level of antigen presentation during the immune response to protein antigens in adjuvants. *J Immunol.* 2010; 185(5):2927-2934.
36. Yang CW, Unanue ER. Neutrophils control the magnitude and spread of the immune response in a thromboxane A2-mediated process. *J Exp Med.* 2013;210(2):375-387.
37. Coombes JL, Charsar BA, Han SJ, et al. Motile invaded neutrophils in the small intestine of Toxoplasma gondii-infected mice reveal a potential mechanism for parasite spread. *Proc Natl Acad Sci USA.* 2013;110(21):E1913-E1922.
38. Peters NC, Egen JG, Secundino N, et al. In vivo imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. *Science (New York).* 2008;321(5891):970-974.
39. Beyrau M, Bodkin JV, Nourshargh S. Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. *Open Biol.* 2012;2(11):120134.
40. Kruger P, Saffarzadeh M, Weber AN, et al. Neutrophils: between host defence, immune modulation, and tissue injury. *PLoS Pathog.* 2015;11(3):e1004651.
41. Bauer S, Abdgawad M, Gunnarsson L, Segelmark M, Tapper H, Hellmark T. Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils. *J Leukoc Biol.* 2007;81(2):458-464.
42. Abdgawad M, Pettersson Å, Gunnarsson L, et al. Decreased neutrophil apoptosis in quiescent ANCA-associated systemic vasculitis. *PLoS One.* 2012;7(3):e32439.
43. Hu N, Mora-Jensen H, Theilgaard-Mönch K, et al. Differential expression of granulopoiesis related genes in neutrophil subsets distinguished by membrane expression of CD177. *PLoS One.* 2014;9(6):e99671.
44. Stroncek DF. Neutrophil-specific antigen HNA-2a, NB1 glycoprotein, and CD177. *Curr Opin Hematol.* 2007;14(6):688-693.
45. Wang L, Ge S, Agustian A, Hiss M, Haller H, von Vietinghoff S. Surface receptor CD177/NB1 does not confer a recruitment advantage to neutrophilic granulocytes during human peritonitis. *Eur J Haematol.* 2013;90(5):436-437.
46. Casanova-Acebes M, Pitaval C, Weiss LA, et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell.* 2013;153(5): 1025-1035.
47. Zhang D, Chen G, Manwani D, et al. Neutrophil ageing is regulated by the microbiome. *Nature.* 2015;525(7570):528-532.
48. Clemmensen SN, Bohr CT, Rørvig S, et al. Olfactomedin 4 defines a subset of human neutrophils. *J Leukoc Biol.* 2012;91(3):495-500.
49. Amirbeagi F, Thulin P, Pullerits R, et al. Olfactomedin-4 autoantibodies give unusual c-ANCA staining patterns with reactivity to a subpopulation of neutrophils. *J Leukoc Biol.* 2015;97(1):181-189.
50. Liu W, Yan M, Liu Y, McLeish KR, Coleman WG Jr, Rodgers GP. Olfactomedin 4 inhibits cathepsin C-mediated protease activities, thereby modulating neutrophil killing of Staphylococcus aureus and Escherichia coli in mice. *J Immunol.* 2012;189(5):2460-2467.
51. Liu W, Yan M, Sugui JA, et al. Olfm4 deletion enhances defense against Staphylococcus aureus in chronic granulomatous disease. *J Clin Invest.* 2013;123(9):3751-3755.
52. Welin A, Amirbeagi F, Christenson K, et al. The human neutrophil subsets defined by the presence or absence of OLFM4 both transmigrate into tissue in vivo and give rise to distinct NETs in vitro. *PLoS One.* 2013;8(7): e69575.
53. Puellmann K, Kaminski WE, Vogel M, et al. A variable immunoreceptor in a subpopulation of human neutrophils. *Proc Natl Acad Sci USA.* 2006;103(39):14441-14446.
54. Fuchs T, Püellmann K, Scharfenstein O, et al. The neutrophil recombinatorial TCR-like immune receptor is expressed across the entire human life span but repertoire diversity declines in old age. *Biochem Biophys Res Commun.* 2012; 419(2):309-315.
55. Christoffersson G, Vågesjö E, Vandooren J, et al. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood.* 2012;120(23):4653-4662.
56. Massena S, Christoffersson G, Vågesjö E, et al. Identification and characterization of VEGF-A-responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. *Blood.* 2015;126(17):2016-2026.
57. Pillay J, Ramakers BP, Kamp VM, et al. Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J Leukoc Biol.* 2010;88(1): 211-220.
58. Pillay J, Kamp VM, van Hoffen E, et al. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest.* 2012;122(1):327-336.
59. Ingersoll SA, Laval J, Forrest OA, et al. Mature cystic fibrosis airway neutrophils suppress T cell function: evidence for a role of arginase 1 but not programmed death-ligand 1. *J Immunol.* 2015; 194(11):5520-5528.
60. Tirouvanziam R, Gernez Y, Conrad CK, et al. Profound functional and signaling changes in viable inflammatory neutrophils homing to cystic fibrosis airways. *Proc Natl Acad Sci USA.* 2008; 105(11):4335-4339.
61. Chen F, Wu W, Millman A, et al. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nat Immunol.* 2014;15(10):938-946.
62. Cheung DS, Ehlenbach SJ, Kitchens RT, et al. Cutting edge: CD49d+ neutrophils induce

FcepsilonRI expression on lung dendritic cells in a mouse model of postviral asthma. *J Immunol.* 2010;185(9):4983-4987.

63. Sigua JA, Buelow B, Cheung DS, et al. CD49d-expressing neutrophils differentiate atopic from nonatopic individuals. *J Allergy Clin Immunol.* 2014;133(3):901-904.
64. Tsuda Y, Takahashi H, Kobayashi M, Hanafusa T, Herndon DN, Suzuki F. Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant Staphylococcus aureus. *Immunity.* 2004;21(2):215-226.
65. Taylor PR, Roy S, Leal SM Jr, et al. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORγt and dectin-2. *Nat Immunol.* 2014;15(2):143-151.
66. Denny MF, Yalavarthi S, Zhao W, et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. *J Immunol.* 2010;184(6):3284-3297.
67. Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J Immunol.* 2011;187(1):538-552.
68. Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci Transl Med.* 2011;3(73): 73ra20.
69. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell.* 2009;16(3):183-194.
70. Coffelt SB, Kersten K, Doornebal CW, et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature.* 2015;522(7556):345-348.
71. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J Clin Invest.* 2010;120(4):1151-1164.
72. Finisguerra V, Di Conza G, Di Matteo M, et al. MET is required for the recruitment of anti-tumoural neutrophils. *Nature.* 2015;522(7556): 349-353.
73. Borregaard N, Kjeldsen L, Rygaard K, et al. Stimulus-dependent secretion of plasma proteins from human neutrophils. *J Clin Invest.* 1992;90(1):86-96.
74. Hidalgo A, Peired AJ, Wild MK, Vestweber D, Frenette PS. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. *Immunity.* 2007;26(4):477-489.
75. Zarbock A, Lowell CA, Ley K. Spleen tyrosine kinase Syk is necessary for E-selectin-induced alpha(L)beta(2) integrin-mediated rolling on intercellular adhesion molecule-1. *Immunity.* 2007;26(6):773-783.
76. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* 2013;13(3): 159-175.
77. Porteu F, Nathan CF. Mobilizable intracellular pool of p55 (type I) tumor necrosis factor receptors in human neutrophils. *J Leukoc Biol.* 1992;52(1):122-124.
78. Almkvist J, Fäldt J, Dahlgren C, Leffler H, Karlsson A. Lipopolysaccharide-induced gelatinase granule mobilization primes neutrophils for activation by galectin-3 and formylmethionyl-Leu-Phe. *Infect Immun.* 2001; 69(2):832-837.
79. de Haas M, Kerst JM, van der Schoot CE, et al. Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neutrophils. *Blood.* 1994;84(11):3885-3894.
80. Yoon PS, Boxer LA, Mayo LA, Yang AY, Wicha MS. Human neutrophil laminin receptors: activation-dependent receptor expression. *J Immunol.* 1987;138(1):259-265.
81. Sreeramkumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science (New York).* 2014; 346(6214):1234-1238.
82. Sachs UJ, Andrei-Selmer CL, Maniar A, et al. The neutrophil-specific antigen CD177 is a counter-receptor for platelet endothelial cell adhesion molecule-1 (CD31). *J Biol Chem.* 2007; 282(32):23603-23612.
83. Verheugt FW, von dem Borne AE, van Noord-Bokhorst JC, van Elven EH, Engelfriet CP. Serological, immunochemical and immuoncytological properties of granulocyte antibodies. *Vox Sang.* 1978;35(5):294-303.
84. von Vietinghoff S, Tunnemann G, Eulenberg C, et al. NB1 mediates surface expression of the ANCA antigen proteinase 3 on human neutrophils. *Blood.* 2007;109(10):4487-4493.
85. Kuckleburg CJ, Tilkens SB, Santoso S, Newman PJ. Proteinase 3 contributes to transendothelial migration of NB1-positive neutrophils. *J Immunol.* 2012;188(5):2419-2426.
86. Jennette JC, Hoidal JR, Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood.* 1990;75(11):2263-2264.
87. Hu N, Westra J, Huitema MG, et al. Coexpression of CD177 and membrane proteinase 3 on neutrophils in antineutrophil cytoplasmic autoantibody-associated systemic vasculitis: anti-proteinase 3-mediated neutrophil activation is independent of the role of CD177-expressing neutrophils. *Arthritis Rheum.* 2009; 60(5):1548-1557.
88. Rarok AA, Stegeman CA, Limburg PC, Kallenberg CG. Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. *J Am Soc Nephrol.* 2002;13(9):2232-2238.
89. Xie Q, Klesney-Tait J, Keck K, et al. Characterization of a novel mouse model with genetic deletion of CD177. *Protein Cell.* 2015; 6(2):117-126.
90. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest.* 2010;120(7):2423-2431.
91. Köhler A, De Filippo K, Hasenberg M, et al. G-CSF-mediated thrombopoietin release triggers neutrophil motility and mobilization from bone marrow via induction of Cxcr2 ligands. *Blood.* 2011;117(16):4349-4357.
92. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity.* 2003;19(4):583-593.
93. Nagase H, Miyamasu M, Yamaguchi M, et al. Cytokine-mediated regulation of CXCR4 expression in human neutrophils. *J Leukoc Biol.* 2002;71(4):711-717.
94. Ortega-Gómez A, Perretti M, Soehnlein O. Resolution of inflammation: an integrated view. *EMBO Mol Med.* 2013;5(5):661-674.
95. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol.* 2013;13(12):862-874.
96. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* 2012;12(4):253-268.
97. Hacbarth E, Kajdacsy-Balla A. Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. *Arthritis Rheum.* 1986;29(11): 1334-1342.
98. Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med.* 2011;3(73):73ra19.
99. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Reports.* 2015; 10(4):562-573.
100. Janols H, Bergenfelz C, Allaoui R, et al. A high frequency of MDSCs in sepsis patients, with the granulocytic subtype dominating in gram-positive cases. *J Leukoc Biol.* 2014;96(5): 685-693.
101. Ssemaganda A, Kindinger L, Bergin P, et al. Characterization of neutrophil subsets in healthy human pregnancies. *PLoS One.* 2014;9(2): e85696.
102. Wong SL, Demers M, Martinod K, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med.* 2015;21(7): 815-819.
103. Cloke T, Munder M, Taylor G, Müller I, Kropf P. Characterization of a novel population of low-density granulocytes associated with disease severity in HIV-1 infection. *PLoS One.* 2012; 7(11):e48939.
104. Fletcher M, Ramirez ME, Sierra RA, et al. l-Arginine depletion blunts antitumor T-cell responses by inducing myeloid-derived suppressor cells. *Cancer Res.* 2015;75(2): 275-283.
105. Andzinski L, Kasnitz N, Stahnke S, et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int J Cancer.* 2016;138(8):1982-1993.
106. Fridlender ZG, Sun J, Mishalian I, et al. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PLoS One.* 2012;7(2):e31524.
107. Rodriguez PC, Ernstoff MS, Hernandez C, et al. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res.* 2009;69(4):1553-1560.