INNATE IMMUNITY

Neutrophils: New insights and open questions

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Neutrophils are the first line of defense against bacteria and fungi and help combat parasites and viruses. They are necessary for mammalian life, and their failure to recover after myeloablation is fatal. Neutrophils are short-lived, effective killing machines. Their life span is significantly extended under infectious and inflammatory conditions. Neutrophils take their cues directly from the infectious organism, from tissue macrophages and other elements of the immune system. Here, we review how neutrophils traffic to sites of infection or tissue injury, how they trap and kill bacteria, how they shape innate and adaptive immune responses, and the pathophysiology of monogenic neutrophil disorders.

Neutrophils are the most abundant leukocytes in human blood. In the process of killing infectious microbes, neutrophils can generate enormous collateral damage. Thus, neutrophil recruitment and activation are regulated at multiple levels (Fig. 1). This Review is focused on recent discoveries and unresolved issues in neutrophil biology. We aim to emphasize physiologically important mechanisms and clinically relevant findings. We will not fully review phagocytosis mechanisms and the role of neutrophils in autoimmune diseases and cancer because there are excellent recent reviews on these subjects.

MECHANISMS MEDIATING NEUTROPHIL RECRUITMENT TO SITES OF INFECTION OR TISSUE INJURY

Neutrophil pools in blood and elsewhere

In the adult mammal, neutrophils are produced in the bone marrow and released at a steady rate under homeostatic conditions (*1*). Differentiation from hematopoietic stem cells to common myeloid progenitor cells to lineage-committed progenitors that mature into neutrophils takes more than 10 days. Several transcription factors—including PU.1, CCAAT/enhancer binding protein α (C/EBPα), growth factor independence 1 (GFI1), and C/EBPε—are necessary for neutrophil maturation during steady-state granulopoiesis. With the recent advent of high-dimensional technologies, the ability to identify previously unknown hematopoietic progenitors has increased. Recently, three groups have identified neutrophil progenitors that show unipotency for neutrophils in both mice and humans (*1*–*3*). Ng and colleagues (*2*) identified a proliferative neutrophil precursor (preNeu) in mouse bone marrow that is short lived and rapidly differentiates into mature $Ly6G^+CXCR2^+$ neutrophils. The transcription factor C/EBPε regulates the development of this early neutrophil progenitor. In mixed chimeric bone marrow studies in mice, loss of CEBP/ε caused loss of both the newly identified neutrophil progenitor as well as all immature and mature neutrophil populations from bone marrow. These studies suggest that C/EBPε is a key part of the master transcriptional control pathway that defines neutrophil-exclusive commitment under homeostatic conditions. These investigators also identified a counterpart of preNeu in human bone marrow that is $CD66b^+CD117^-CD34^-$ (*2*). Zhu *et al.* (*3*) identified a heterogeneous early neutrophil progenitor (hNeP) in human bone marrow that is likely located upstream of the precursor identified by Ng's group in that it is $CD66b^+CD117^+$ and can be fractionated into $CD34^+$ and $CD34^-$ subsets. Kang and colleagues (*1*) identified a late-lineage neutrophil progenitor in mouse bone marrow. This progenitor is also unipotent for neutrophils but is likely located downstream of the early progenitor identified by Ng and colleagues in the neutrophil developmental tree. These new progenitors and perhaps additional ones shed new light on the concept of the granulocyte-monocyte progenitor because it relates to neutrophil development. Understanding how these previously unidentified neutrophil progenitors modulate disease will be important for new therapeutic approaches to combat diseases such as cancer, in which neutrophils play a critical role.

Although estimates suggest that humans make about 1 billion neutrophils per day per kilogram of body weight, this can increase to 10 billion during infections. It is accepted now that neutrophils can live longer than 24 hours in tissues, especially in inflammatory milieus, and some have estimated their life span to be as long as 7 days, with their extended survival mediated in part by cytokine-activated endothelial cells. Neutrophil life span in tissues is thought to be extended two- to threefold over blood neutrophils (*4*). A pool of neutrophils is present in the lung under steady-state conditions in which its retention is thought to be mediated by CXCR4, and its release is proposed to respond to infection or injury [reviewed in (*5*)]. Neutrophils traffic to epithelial surfaces and some tissues under homeostatic conditions, but neither the mechanisms nor the regulation of this process are known. The regulation of blood neutrophil numbers seems to be, in part, dependent on the number of apoptotic neutrophils that are phagocytosed by tissue dendritic cells (DCs) and macrophages. Phagocytosis of apoptotic neutrophils reduced the production of interleukin-23 (IL-23), which reduced production of IL-17 by certain nonconventional lymphocytes, including γδ T cells, leading to less granulocyte colony-stimulating factor (G-CSF) and reduced neutrophil production. Conversely, blocking neutrophil entry into tissues led to less phagocytosis; increased IL-23, IL-17, and G-CSF; and more neutrophil production (*6*). The concentration of neutrophils

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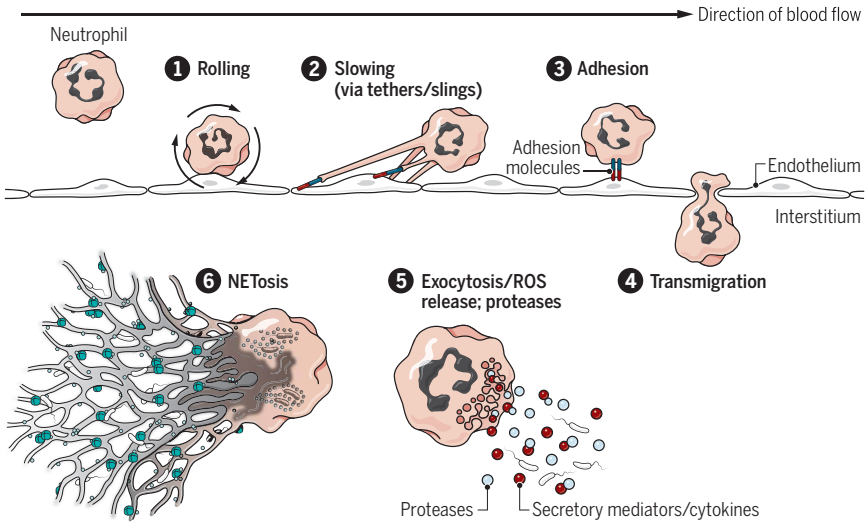

Fig. 1. Neutrophils in infection and inflammation. In response to infection or inflammation, circulating neutrophils display surface molecules that facilitate their interaction with the activated endothelium. (**1** and **2**) The process of rolling, mediated by L-selectin and PSGL-1, a ligand for P-selectin, induces β_2 integrin extension, which slows down the rolling process, a mechanism also mediated by the formation of tethers and slings. (**3**) The process is followed by firm adhesion of neutrophils to the activated endothelium, mediated by adhesion molecules, including β_2 integrins. (**4**) Neutrophil transmigration through the endothelium and the basal membrane requires the mobilization of intracellular vesicles. Once in the interstitial space, neutrophils follow chemotactic gradients formed by pathogen-derived molecules or inflammatory mediators. (**5** and **6**) Neutrophils display a battery of defense mechanisms that include the internalization of pathogens for intracellular killing, the release of proteases and ROS that generate a hostile environment and contribute to the microbicidal function of these cells, and the formation of NETs composed of chromatin and secretory mediators that help trap bacteria. During inflammation and infection, neutrophils release mediators that contribute to shaping the subsequent immune response by modulating adaptive immune cell function.

in the blood varies by more than twofold during the course of each day (*7*). The microbiome also affects neutrophil numbers by increasing the number of aged neutrophils through a Toll-like receptor (TLR) and MyD88-dependent mechanism (*8*). These findings beg the question how different microbiomes might alter neutrophil number and function.

Receptors and adhesion molecules in neutrophil arrest

Neutrophils reach their destination through the blood system. They achieve this by expressing chemokine receptors, receptors for lipid mediators such as leukotriene B4, complement factors such as C5a, and bacterial products such as *N*-formyl-methionyl-leucyl-phenylalanine (*9*). Neutrophils express several integrin adhesion receptors of the β2 and β1 families. The β_2 integrins LFA-1 ($\alpha_L\beta_2$) and Mac-1 ($\alpha_M\beta_2$) are functionally most important in mediating neutrophil slow rolling, arrest, transendothelial migration, phagocytosis, and respiratory burst [production of superoxide anion by the nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) oxidase]. Mac-1 and $\alpha_X\beta_2$ (CD11c/CD18) have extremely broad ligand specificities, allowing neutrophils to adhere to degraded extracellular matrix or even to plastic, glass, and components of medical devices (*10*). Neutrophils express L-selectin and ligands for P- and E-selectins (*11*), which are involved in mediating leukocyte rolling.

Neutrophil arrest mediated by inside-out integrin activation

Inside-out integrin activation is a key event in neutrophil recruitment. Neutrophils accumulate signals while rolling on P-selectin, which leads to β_2 integrin extension, but not conversion to the high-affinity state. According to the switchblade model of integrin activation, extension precedes acquisition of the high-affinity conformation of the α_L A (also known as I) domain, which contains the ligand-binding site. Recently, this view has been challenged by the observation that rolling human neutrophils show clusters of extended β_2 integrins (expected), clusters of high-affinity bent β_2 integrins (unexpected), and clusters of β_2 integrins that are both extended and in a high-affinity conformation (expected; these integrins can bind ligand in trans). The bent high-affinity β_2 integrins interact with intercellular adhesion molecules (ICAMs) on the neutrophil surface in cis, which strongly inhibits neutrophil adhesion and aggregation. This same study also suggests that chemokine receptor signaling may only trigger the high-affinity integrin conformation but not extension (*12*). These unexpected findings revive the "deadbolt" model of integrin activation, which predicts the existence of high-affinity bent integrins. The deadbolt model is supported by site-directed mutagenesis studies in leukocyte-like cell lines, but more work is needed to fully understand inside-out integrin activation in neutrophils.

Leukocyte adhesion deficiencies

That neutrophil integrins are medically relevant is starkly demonstrated by leukocyte adhesion deficiency type I (LAD-I) (*13*). Patients with LAD-I have mutations in *ITGB2*, the gene encoding the β_2 chain (CD18) of leukocyte integrins, and show a characteristic lack of umbilical cord healing, resulting in delayed separation (Table 1). As children and adults, they suffer from severe recurrent bacterial infections, periodontitis, and often ulcerative inflammation. LAD-II, in which neutrophils cannot produce selectin ligands because of a defect in fucose transport caused by mutations in *GFTP* (*14*), also shows a severe neutrophil phenotype. LAD-III is characterized by functional null mutations of the *FERMT3* gene encoding kindlin-3 (*15*). LAD-III children suffer from severe neutrophil adhesion defects and recurrent bleeding because of an attendant defect in platelet integrin ($\alpha_{IIb}\beta_3$) activation (Table 1).

Neutrophil chemotaxis

The neutrophil receptors for chemoattractants (*9*) are all G protein coupled. CXCR2 is one of the most important chemokine receptors in mouse neutrophils (binds CXCL1, CXCL2, CXCL5, CXCL6, and CXCL7); in human neutrophils, CXCR1 is also involved in recognizing CXCL8. In addition, the receptors for the bacteria- and mitochondria-derived formyl peptides (FPR1 and FPR2) and for the lipid mediator leukotriene B4 (LTB4) play important roles in neutrophil recruitment in both human and mouse. Neutrophils also express CCR1, CCR2, CCR3, CCR5, CXCR3, and CXCR4, which broadens their responsiveness to chemokines (*9*). Some of these receptors are expressed

in neutrophil granules and come to the plasma membrane upon degranulation. Concurrent analysis of neutrophils in the bone marrow, blood, and joint in an arthritis model found that CXCR2 is increased on neutrophils as they migrate from the bone marrow into the blood and then into tissue, whereas expression of CCR1, BLT1, and C5aR was not affected (*16*). Thus, selective mechanisms, from protein synthesis to degranulation, regulate receptor up-regulation during migration. It has now become clear that the receptor coupling can determine the resulting function. CXCR2 couples through both Gαi2 and Gαi3 associated with various Gβγ subunits. Coupling through Gαi2 specifically promotes β_2 integrin activation and arrest of rolling neutrophils. Coupling through Gαi3 is required for chemotactic neutrophil migration to CXC chemokines but not arrest. Gαi2 but not Gαi3 is necessary for interstitial chemotaxis (*17*). The mobilization of neutrophils from the bone marrow to circulation is also a process that involves neutrophil chemotaxis, and it is now clear that neutrophil release is negatively regulated by CXCR4, whereas CXCR2 promotes neutrophil mobilization (*18*).

Swarming

Neutrophils show a strong tendency for collective swarming, a self-organized migration mechanism that requires communication among the swarming neutrophils, leading to neutrophil accumulation and the formation of neutrophil clusters (*17*). The initial steps in swarming are independent of integrin-mediated adhesion. Interestingly, individual knockout of most known chemoattractant receptors fails to affect interstitial chemotaxis, suggesting that these receptors have overlapping functions in swarming. However, neutrophils that lack the high-affinity receptor for LTB4 have impaired recruitment during the late phases of the swarming response (*17*), an integrin-dependent process. LTB4 is not the only possible relay mechanisms used by neutrophils; a recent study shows that migrating neutrophils leave chemokine-enriched (CXCL12) fragments as trails that mediate the recruitment of other immune cells (*19*). Whether this mechanism is important for swarming is currently unknown. Several reports showed swarming of neutrophils to sterile injury, regulated by formylated peptides, LTB4, chemokines, and complement (*17*, *20*).

Sterile injury

In sterile injury, neutrophils enter the site of injury but encounter no pathogens. Numerous groups have suggested that they enter to help clear debris, but the evidence for this is limited. Intravascular danger signals induce neutrophil recruitment to sites of focal tissue necrosis in vivo (*20*), but in extreme cases, neutrophil infiltration also leads to tissue necrosis (*21*). Recent evidence suggests that neutrophil infiltration is critically important for revascularization of damaged tissue (*22*). There is some evidence that neutrophils can reverse-migrate back out of the tissue into the vasculature (*23*).

Tethers and slings

Neutrophils are the model cells used to develop the now classical leukocyte adhesion cascade (*24*). Neutrophils can adhere to activated endothelium even in the presence of very high shear stress. This adhesion is thought to be enabled by four molecular and cellular properties of neutrophils: (i) Selectins and probably integrins form catch bonds that become stronger as force is applied (*25*); (ii) neutrophils are very pliable cells, with plenty of ruffles (microvilli) and excess membrane, which allows them to deform and "hug the wall" (*26*); (iii) neutrophils form long, thin tethers (Fig. 1) (*27*) that balance the drag force and the torque; and (iv) tethers detach and swing around, landing in front of the rolling neutrophils as slings, and can provide a self-adhesive substrate (*28*). Tethers end in anchoring plates that contain P-selectin glycoprotein ligand-1 (PSGL-1) (*28*), extended and partially activated β_2 integrins (*12*), and cytoskeletal molecules. It is unknown how tethers are connected to the cortical cytoskeleton. Interestingly, the molecular program that allows the formation of tethers and slings is inducible as observed in CD4 T cells after differentiation (*28*).

Open questions

Central open questions in neutrophil biology relate to the molecular cues that define the recruitment of these cells to different tissues with diverse architectures and molecular dynamics. Whether neutrophil recruitment during infection and inflammation are mediated by different mechanisms from those that regulate the recruitment of neutrophils involved in tissue homeostasis needs further elucidation. Neutrophil adhesion follows the selectin-integrin cascade model in many organs, including skin, connective tissue, skeletal muscle, and the intestinal wall. However, in liver sinusoids, neutrophil recruitment is selectin independent and requires CD44 on the neutrophil and hyaluronan on the endothelial cells (*20*, *29*). Neutrophil recruitment to the lung is selectin independent and occurs at the capillary level, and the role of β_2 integrins varies with the infection or stimulus. In large veins, neutrophil adhesion is linked to thrombosis (*30*). The mechanism of neutrophil recruitment to the arterial wall is only partially understood (*31*) and requires further investigation. Whether tissue-specific recruitment patterns could help generate therapeutic strategies is still speculative. For instance, because CD44 is involved in neutrophil recruitment to the liver, but not elsewhere, strategies that target CD44 or its ligand hyaluronan (*29*) might offer a way to specifically target the liver. Another molecule, vascular adhesion protein-1 (VAP-1), a cell-surface amine oxidase and neutrophil adhesion molecule, is also involved in liver inflammation and fibrosis (*32*).

MECHANISMS OF HOST PROTECTION AND INFLAMMATION

Degranulation

To execute a rapid and precise response to infections, neutrophils rely on preformed molecules stored in a variety of intracellular granules. Granule proteins regulate adhesion, transmigration, phagocytosis, and neutrophil extracellular trap (NET) formation. The secretory proteins also constitute some of the most toxic, readily releasable factors produced by the human body. Thus, neutrophil degranulation, although important for controlling infections, can induce potent proinflammatory responses.

Neutrophil secretory organelles include azurophilic (primary), specific (secondary), and gelatinase (tertiary) granules (*33*) and the endocytic vesicles multivesicular bodies (MVBs) (*34*) and secretory vesicles (*35*). Secretory vesicles are rapidly mobilized in response to weak stimulation to initiate the neutrophil response by the up-regulation of adhesion molecules and chemotactic receptors, including Mac-1 and CXCR2 (*35*), thus linking degranulation with neutrophil recruitment. Secondary and tertiary granules are mobilized in response to increasingly stronger stimuli and contain the formyl-peptide receptor (FPR1), gelatinase B (matrix metalloproteinase-9) and the antimicrobial peptide cathelicidin. Cytochrome b_{558}, the membrane-associated subunit of the NADPH oxidase is also present in these granules. The NADPH oxidase is an enzymatic complex responsible

Table 1. Monogenic diseases that affect neutrophils. AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

Gene Protein	Disease name	Inheritance pattern	Molecular mechanisms
Disorders with primarily neutropenia			
ELANE **Neutrophil elastase**	Congenital neutropenia or cyclic neutropenia	AD or somatic	Maturation arrest, premature apoptosis Unfolded protein response, ER stress
JAGN1 **Jagunal homolog 1**	Congenital neutropenia	AR	Differentiation defect, premature apoptosis ER secretory pathway
CSF3R **Colony stimulating factor receptor**	Congenital neutropenia	AR and AD	Bone marrow production and release signaling defect
GFI1 **Growth factor independence 1**	Congenital neutropenia	AD	Myeloid cell differentiation Transcription repressor
G6PC3 **Glucose-6-phosphatase**	Congenital neutropenia	AR	Bone marrow retention, apoptosis ER stress, glycosylation defect
HAX1 **HS1-associated protein X1**	Kostmann syndrome	AR	Bone marrow production and release G-CSF signaling defect
Multisystemic syndromes with neutropenia			
AK2 **Adenylate kinase 2**	Reticular dysgenesis	AR	Differentiation defect Mitochondrial metabolism dysfunction
RMRP **RNAase mitochondrial RNA processing**	Cartilage hair hypoplasia	AR	Bone marrow dysfunction Preribosomal RNA processing
SBDS **Schwachman-Bodian-Diamond syndrome protein**	Schwachman-Bodian-Diamond syndrome	AR	Differentiation defect, premature apoptosis Ribosome biogenesis
DNM2 **Dynamin 2**	Charcot-Marie-Tooth disease	AD	Membrane trafficking, microtubules
TAZ1 **Tafazzin**	Barth syndrome	XLR	Mitochondrial membrane dynamics
G6PT **Glucose-6-phosphate transporter**	Glycogen storage disease type 1b	AR	Premature apoptosis ER stress, mitochondrial dysfunction
Immunodeficiency syndromes with neutropenia			
BTK **Bruton's tyrosine kinase**	X-linked agammaglobulinemia	XLR	Chemotaxis defect, reactive oxygen defect
WAS **Wiskott-Aldrich syndrome**	X-linked neutropenia Wiskott-Aldrich syndrome	XLR	Decreased proliferation, increased apoptosis Actin polymerization
CD40L **CD40 ligand**	Hyper-IgM syndrome	XLR	Adhesion and transmigration
CXCR4 **Chemokine receptor CXCR4**	WHIM (warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis)	AD	Bone marrow and tissue homing abnormality Defective chemokine receptor function
STK4 **Serine/threonine kinase 4**	STK4 deficiency	AR	Increased apoptosis Mitochondrial dysfunction
GINS1 **Go-ichi-ni-san complex subunit 1**	GINS1 deficiency	AR	Impaired cell cycle Defective DNA repair
Neutrophil dysfunction disorders			
ITGB2 **Leukocyte integrin β_2 chain**	LAD-I	AR	Neutrophil adhesion/migration defects Adhesion molecule deficiency
GFTP **GDP fucose transporter**	LAD-II	AR	Selectin deficiency Defect in fucose transport

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Gene Protein	Disease name	Inheritance pattern	Molecular mechanisms
FERMT3 **Kindlin-3**	LAD-III	AR	Neutrophil adhesion defect
CYBB **Cytochrome b-245β; gp91phox**	Chronic granulomatous disease	XLR	Defective oxidative burst NADPH oxidase enzyme defect
CYBA **Cytochrome b-245α; p22phox**	Chronic granulomatous disease	AR	Defective oxidative burst NADPH oxidase enzyme defect
NCF1 **Neutrophil cytosolic factor-1; p47phox**	Chronic granulomatous disease	AR	Defective oxidative burst NADPH oxidase enzyme defect
NCF2 **Neutrophil cytosolic factor-2; p67phox**	Chronic granulomatous disease	AR	Defective oxidative burst NADPH oxidase enzyme defect
NCF4 **Neutrophil cytosolic factor-4; p40phox**	Chronic granulomatous disease	AR	Defective oxidative burst NADPH oxidase enzyme defect
G6PD **Glucose-6-phosphate dehydrogenase**	G6PD deficiency	AR	Defective oxidative burst, NETosis defect Enzyme deficiency
RAC2 **Ras-related C3 botulinum toxin 3**	Neutrophil immunodeficiency syndrome	AR	GTPase deficiency, defective oxidative burst Secretory and phagocytosis defect
MYD88 **Myeloid differentiation 88**	MyD88 deficiency	AR	Neutrophil aging TLR and IL-1R signaling defect
IRAK4 **Interleukin-1 receptor associated kinase 4**	IRAK4 deficiency	AR	Defective migration and phagocytosis Impaired TLR and IL-1 receptor responses
Secretory lysosome/granule defects			
LYST **Lysosomal trafficking regulator**	Chediak-Higashi syndrome	AR	Neutrophil signaling defect Abnormal lysosome and melanosome trafficking
RAB27A **RAB27a**	Griscelli syndrome type 2	AR	Reduced mature neutrophils Membrane trafficking/phagosome secretion defect
UNC13D **MUNC13-4**	Familial hemophagocytic lymphohistiocytosis type 3	AR	Neutropenia, vesicular trafficking, and secretion defects
STXBP2 **Syntaxin binding protein 2 (MUNC18-2)**	Familial hemophagocytic lymphohistiocytosis type 5	AR	Secretion and bactericidal defects
WDR1 **Actin-interacting protein 1 (Aip1)**	WDR1 deficiency	AR	Mild neutropenia, impaired chemotaxis Normal bacterial killing and increased oxidative burst
MLK1 **Megakaryoblastic leukemia 1 (MKL1)**	MLK1 deficiency	AR	Decreased phagocytosis and impaired migration
AP3B1 **Adaptor-related protein complex β1**	Hermansky-Pudlack syndrome	AR	Reduced mature neutrophils Abnormal vesicular trafficking of proteins
LAMTOR2 **Late endosomal/lysosomal adaptor, MAPK and MTOR activator 2**	Immunodeficiency due to defect in MAPBP-interacting protein (p14 deficiency)	AR	Abnormal neutrophil maturation and function Late endosome biogenesis
CEBPE **CCAAT enhancer binding protein ε**	Specific granule deficiency	AR	Neutrophil chemotaxis defect Abnormal or absent granule formation

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Gene Protein	Disease name	Inheritance pattern	Molecular mechanisms
Autoinflammatory disorders			
NLPR3 **Cryopyrin**	Cryopyrin-associated periodic syndromes (CAPS)	AD or somatic	Neutrophil homeostasis dysregulation Inflammasome mediated IL-1β release/ cell death
MEFV **Pyrin**	Familial Mediterranean fever	AR or rarely AD	Neutrophil chemotaxis and phagocytosis defect Enhanced apoptosis and altered adhesion Hyperactive inflammasome-mediated IL-1β release Alterations in F-actin dynamics
MVK **Mevalonate kinase**	Mevalonic aciduria and hyper-IgD syndrome	AR	Pyrin inflammasome activation due to RhoA inactivation and compromised phosphatidylinositol 3-kinase activity secondary to prenylation defect
TNFRSF1A **Tumor necrosis factor receptor–1A**	Tumor necrosis factor receptor–associated periodic syndrome	AD	Neutrophil apoptosis resistance TNFR signaling or shedding defect
NOD2 **Nucleotide oligomerization domain 2**	Blau syndrome	AD	Increased ocular neutrophil rolling and adherence Activation of nuclear factor κB/ IL-1B–mediated inflammation
IL1RN **Interleukin-1 receptor antagonist**	Deficiency of IL-1 receptor antagonist	AR	Neutrophil mobilization and activation Unregulated IL-1 receptor activation
CD2BP1 **CD2 binding protein 1**	Pyogenic arthritis pyoderma gangrenosum and acne (PAPA)	AD	Neutrophil mobilization and activation Defective actin dynamics
PSMB8 **Proteasome subunit β type 8**	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)	AR	Neutrophil mobilization and activation IFN dysregulation
TMEM173 **Stimulator of interferon genes (STING)**	STING-associated vasculopathy with onset in infancy (SAVI)	AD	Neutrophil mobilization and activation IFN dysregulation
Other disorders			
DNASE1 **Deoxyribonuclease 1**	Monogenic systemic lupus erythematosus	AR	Defective NET degradation Increased ROS production IFN dysregulation
DNASE1L3 **Deoxyribonuclease 1L3**	Monogenic systemic lupus erythematosus	AD	Defective NET degradation Increased ROS production IFN dysregulation
CFTR **Cystic fibrosis transmembrane regulator**	Cystic fibrosis	AR	Delayed neutrophil apoptosis Impaired MPO activity

for the rapid conversion of molecular oxygen into superoxide anion at the expense of NADPH and is composed of the membrane-associated subunits $p22^{phox}$ and $gp91^{phox}$ that form the flavocytochrome b_{558}, the cytosolic factors $p47^{phox}$ and $p67^{phox}$, and the accessory small guanosine triphosphatase (GTPase) Rac2. Deficiency of any of the components of NADPH oxidase is associated with recurrent life-threatening bacterial and fungal infections and by the formation of inflammatory granulomas as observed in chronic granulomatous disease (CGD) (*36*). Secretion of the most toxic cargoes from azurophilic granules requires sensitization through priming, a process that mediates the amplification of the oxidative or the secretory responses by sequential exposure of neutrophils to a first agonist (primer) that induces molecular changes that enhance the cellular response to a second stimulus (agonist) (*37*). Several inflammatory mediators and

pathogen-associated molecular patterns are known neutrophil priming agents (*37*). Different from other neutrophil-mediated proinflammatory processes, priming is considered to be reversible and can be deactivated as part of the process termed "depriming." Azurophilic granule secretion is also induced through contact-dependent stimulation mediated by β_2 integrins or activation by immune complexes (*38*). Beneficial effects of neutrophil exocytosis include extracellular bacterial killing, as suggested for periodontal disease–associated pathogens (*39*). However, under pathological conditions, these toxic cargoes are secreted into the circulation, leading to endothelial dysfunction and systemic inflammation (*40*). For example, the atherosclerosis biomarker (*41*) myeloperoxidase (MPO) generates hypochlorite, a potent oxidant capable of both killing microorganisms and inducing tissue damage. MPO has nitric oxide oxidase activity and impairs endothelial function. Elastase, cathepsin G, and proteinase 3 are azurophilic granule serine proteases with broad substrate specificity that regulate the inflammatory response through the processing of the extracellular matrix, cytokines, chemokines, and receptors (*42*). Tissue damage through the uncontrolled release of proteolytic enzymes is associated with pathological conditions, including metabolic syndrome, fibrosis, systemic inflammatory response syndrome, sepsis, physical trauma, and cancer progression.

Vesicular trafficking, small GTPases, and effectors

Because the mobilization of secretory vesicles and tertiary granules is important for the initial neutrophil response but exacerbated specific and azurophilic granule exocytosis induces inflammation, the identification of granule-specific mechanisms of secretion is of central importance. Vesicular trafficking and exocytosis are regulated by small GTPases and their interacting effector molecules, which define the identity, responsiveness, and functional heterogeneity of neutrophil granules (Fig. 2) (*43*). The small GTPase Rab27a (*43*) regulates degranulation of tertiary, specific, and a subpopulation of azurophilic granules, whereas azurophilic granules that lack Rab27a engage in phagosomal maturation but not in secretion (Fig. 2) (*43*, *44*). How the different sets of Rab27a-positive secretory organelles can undergo differential exocytosis is still an open question. A possible scenario is that granules recruit different Rab effector molecules, a mechanism that may require granule-specific scaffold proteins. Although 11 Rab27a effectors have been described, only 4 (JFC1, Munc13-4, exophilin-5, and Slp3) have been identified in neutrophils, with the functions of Slp3 and exophilin-5 still unknown. Munc13-4, a docking mediator and fusion sensor (Fig. 2) (*44*), whose function is counteracted by the protein kinase STK24 (*45*), is necessary for the secretion of all neutrophil granules. By contrast, Rac2 (*46*) and JFC1 (*44*) are two independent selective regulators of azurophilic granules in human neutrophils. JFC1 binds Gem-interacting protein (GMIP), which induces inactivation of granule-associated RhoA and the depolymerization of actin around granules to facilitate their movement through cortical actin (*47*). The rapid granule movement through cortical actin suggests that additional actin-depolymerizing molecules may play a substantial role. In addition, the contribution of the exocytosis regulators Rab3 and the octameric protein complex exocyst, present in neutrophil secretory organelles (*33*), to selective degranulation requires further analysis. Last, effector promiscuity may help explain why deletion of some of these molecules induces deeper function impairment than others. Munc13-4 not only regulates exocytosis but also controls late and recycling endosome function (*48*) by mechanisms that involve binding to the SNARE (soluble *N*-ethylmaleimide–sensitive factor attachment protein receptors) syntaxin 7 and to Rab11, respectively.

Cross-talk between vesicular trafficking and migration has started to be elucidated but needs further analysis. Thus, the down-regulation of secretion regulators decreases neutrophilic tissue infiltration (*49*). This is, in part, explained by the role of secretion in the up-regulation of adhesion proteins as demonstrated in macrophage-stimulating-1–dependent transmigration studies (*50*). It has been suggested that chemotaxis is controlled by an exocytosis-mediated mechanism that includes the localized secretion of proteases in a Rab27a-dependent manner to induce uropod detachment. A recent report challenged this view by proposing that Rab27a mediates the secretion of LTB4-containing exosomes from MVBs to facilitate neutrophil relay during chemotaxis (*51*). Whether localized protease and LTB4 secretion are mutually exclusive or complementary mechanisms, and how LTB4 is released from or presented to its receptor by exosomes, remains elusive. Last, whether vesicular trafficking contributes to the polarization of Ras GTPases and their regulatory proteins [guanine-exchange factors (GEFs) and GTPase-activating proteins] during chemotaxis and migration is also an open question that needs further analysis. Various signaling pathways triggered by PSGL-1 and chemokine receptors initiate integrin activation through inside-out signaling, and CalDAG-GEFI, p-REX, and Vav-1 have been identified as possible GEFs (*52*). It is unclear how these regulatory proteins arrive at the site of activation in a rolling cell or a polarized, migrating cell.

Monogenic diseases in degranulation

Genetic defects in Rab27a and Munc13-4 are associated with the human immunodeficiencies Griscelli syndrome type 2 and familial hemophagocytic lymphohistiocytosis (FHL) type 3, respectively (Table 1). Defects in the docking factor Munc18-2 lead to defective neutrophil exocytosis and are associated with FHL5. Patients with the deficiencies GS2, FHL3, and FHL5 suffer from recurrent viral and bacterial infections (Table 1) caused by impaired function of cytotoxic T lymphocytes, natural killer (NK) cells, and neutrophils. Homozygous mutations in *WDR1* and *MKL1*—which encode for an actin-interacting protein that regulates disassembly and for a transcriptional regulator of actin regulatory genes, respectively (*53*, *54*)—are associated with neutrophil dysfunctions, although their possible roles in exocytosis need further analysis.

Inhibitors of exocytosis

Preclinical studies have identified the first group of small-molecule, neutrophil-specific inhibitors of exocytosis (*55*). Previous studies have described peptide inhibitors of exocytosis that target myristoylated alanine-rich C kinase substrate in leukocytes and airway epithelium (*56*), as well as peptide inhibitors of neutrophil exocytosis by targeting SNAREs (*57*). Different from these peptide-based inhibitors, compounds called Nexinhibs (neutrophil exocytosis inhibitors) are small-molecule inhibitors of the Rab27a-JFC1 interaction (*55*). Nexinhibs decrease both human neutrophil exocytosis in vitro and neutrophil degranulation in vivo in mouse models of systemic inflammation, without affecting other important neutrophil innate immune responses, including phagocytosis and NET production (*55*). Furthermore, knockdown of JFC1 inhibits Rab27a-dependent exocytosis in neutrophils (*47*) but does not substantially affect secretion in cytotoxic T lymphocytes. Thus, inhibitors of the Rab27a-JFC1 interaction, although a good target for therapeutic intervention in neutrophilic inflammation, are not expected to affect cytotoxic T lymphocyte

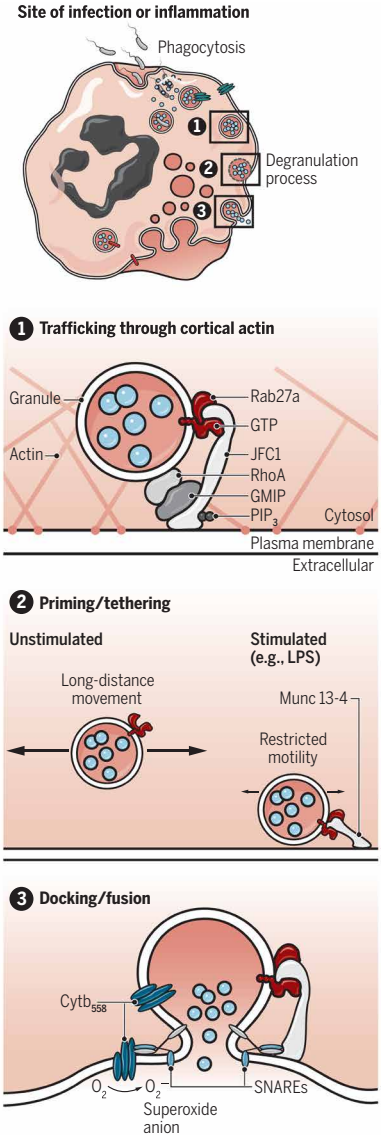

Fig. 2. Neutrophil degranulation. Neutrophils contain granules filled with enzymes, opsonins, adhesion molecules, and receptors. During infection, neutrophil granule cargoes are delivered into the phagosome to kill bacteria intracellularly (phagocytosis). In response to inflammation or infection mediators, including TLR ligands and formylated peptides, neutrophils mobilize intracellular granules and release their cargoes in a controlled and graded fashion (degranulation). The release of proteases into the extracellular milieu not only helps to kill bacteria but also damages host tissues. (**1**) At the molecular level, the secretory machinery of neutrophil granules includes the Rab27a effector JFC1, which facilitates granule trafficking through cortical actin by a mechanism that involves inhibition of RhoA by the GTPase-activating protein GMIP (*47*). (**2**) Priming of neutrophil exocytosis requires the Rab27a effector Munc13-4, which enables granule tethering by restricting their motility and increasing the likelihood of positive interactions with counter receptors at the plasma membrane (*102*). This is induced by several stimuli, including lipopolysaccharide (LPS). (**3**) Munc13-4 interacts with SNAREs, facilitating the fusion of granule membranes with the plasma membrane in stimulated neutrophils. This mechanism permits the release of granule cargoes into the extracellular milieu and the incorporation of granule membrane proteins—including the NADPH oxidase membrane-associated subunit, the cytochrome b_{558}—into the plasma membrane and triggers the production of extracellular superoxide anion (O_2^-). PIP_3, phosphatidylinositol 3,4,5-trisphosphate.

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function. The use of secretion inhibitors is proposed to be more effective than inhibitors for single proteases, an approach that, although partially successful in some clinical conditions, has been found ineffective for some proinflammatory syndromes. The substantially decreased neutrophil secretion, tissue infiltration, and neutrophil-mediated systemic inflammation induced by Nexinhibs support studies that show that neutrophil exocytosis is important in systemic inflammation (*49*) and may have applications in sepsis and cancer, in which neutrophil secretory proteins are involved.

Neutrophil extracellular traps

Beyond killing by degranulation and phagocytosis, the latter being one of the most important antimicrobial neutrophil mechanisms (*58*), neutrophils can use their chromatin to trap and kill microbes. Neutrophils evolved different mechanisms to modify their chromatin, decorate it with proteins from the cytoplasm and granules, and expel it into the extracellular space. These structures are called NETs (Fig. 1) (*59*). Usage of chromatin in host defense is evolutionarily conserved and appears in many organisms, including plants. This suggests that with the emergence of more complex genomes, chromatin evolved not only to manage the much larger amount of DNA to allow gene regulation and chromosome duplication but also to defend the organism against danger.

Mechanisms of NET formation

There are different pathways to make NETs. Most forms of NET formation require cell death in a process called NETosis, whereas other pathways may include expulsion of the nucleus without affecting viability (*60*). Intriguingly, larger microbes are more effective at inducing NETs, suggesting that NETs may be deployed when the organism is too large to be phagocytosed (*61*); however, the sensing mechanisms and signaling pathways used by neutrophils to decide whether to phagocytose or to produce NETs are currently unknown. Several pathogens and pathogen-derived molecules are efficient NET inducers [a comprehensive list of in vitro physiological inducers is provided in (*62*)]. Among physiological inducers of NET release, *Staphylococcus aureus* bacteria are powerful inducers of NET formation independent of cell death, as observed in mouse skin and in human abscesses (*60*). Other physiological NET inducers include hyphae from fungi and crystals from patients with gout. Diverse signaling cascades lead to NET formation. One well-studied pathway requires the activation of the neutrophil's NADPH oxidase that forms superoxide, which can dismutate to hydrogen peroxide, which activates a high–molecular weight protein complex formed by the azurophilic granule proteins elastase, MPO, proteinase 3, and likely other proteins called the "azurosome." This complex includes MPO, which produces hypochloric acid and allows the dissociation of the azurosome and the release of its components into the cytoplasm. One of these components is neutrophil elastase (NE), which subsequently, and very likely in concert with related enzymes, migrates into the nucleus. There, NE processes histones, allowing chromatin decondensation, the swelling of the nucleus, and eventually the release of NETs (*63*). However, other mechanisms, including NADPH oxidase–independent mechanisms, have been described, suggesting that different activators initiate specific NETosis pathways, which end up tailoring the NETs to fulfill diverse biological functions.

During NETosis, the nuclear membrane vesiculates, whereas the cytoplasmic membrane remains still intact, allowing chromatin to come in contact with cytoplasmic components. The scaffold of NETs

is composed of DNA. Mass spectrometry analysis of NETs revealed the presence of a limited protein repertoire (*64*). It is still an open question how proteins are selected to adorn the NETs (*65*), but exocytosis does not appear to be involved. Importantly, NETs are rich in histones, which are essential to organize DNA and also potent antimicrobials. Other NET-related proteins are also antimicrobials, including bactericidal permeability–increasing protein and defensins, as well as enzymes that are functional at inflammatory sites, such as proteases and divalent cation chelators that inhibit microbial growth (*64*). Proteins in NETs, in particular histones, are modified during NETosis—for example, through citrullination (*66*), which may affect their function and immunogenicity and is important in the pathogenesis of rheumatoid arthritis. In mice, NETs are degraded by DNASE1 and DNASE1L3. Genetic absence of these enzymes leads to lethal NET-induced thrombosis (*67*).

Functions and pathogenesis of NETs

NETs bind viruses, bacteria, fungi, and parasites, probably by means of electrostatic forces, preventing their spread and colonization of distant organs. Bacteria such as group A streptococci and pneumococci evolved deoxyribonucleases (DNases) as virulence factors. These microbial DNases degrade NETs, releasing the bound bacteria to colonize other organs (*68*). Interestingly, NETs are also essential in nucleating thrombi. NETs can trap platelets and red blood cells and initiate coagulation. The formation of NETs during coagulation can be pathogenic and result in diseases such as deep vein thrombosis (*67*, *69*).

Similarly, if NETs are formed inappropriately or are not promptly degraded, then they can become pathogenic because of their potential not only to initiate coagulation but also to exert toxic effects. For instance, histones are highly toxic to endothelial cells (*70*). Besides vascular disorders, a well-investigated disease in which NETs are pathogenic is systemic lupus erythematosus (SLE). In SLE, patients develop autoantibodies against DNA, histones, and neutrophil antigens, all of which are present in NETs. Neutrophils isolated from patients with SLE are prone to making NETs, whereas autoantibodies are reported to activate neutrophils to undergo NETosis, which, in turn, activate DCs to make type I interferons (IFNs), a signature of the disease (*71*). NETs are degraded by DNase1 in plasma, which is produced and secreted by the pancreas. Mutations in DNase1 and homologous nucleases are linked to inherited forms of SLE (*72*). The lack of NET degradation by serum DNases, even in patients not carrying mutations in these enzymes, is also linked to the exacerbation of the disease. Thus, NETs contribute to both disease and the generation of autoantibodies, supporting the initiation of SLE (*73*).

NETs are also implicated in numerous common noninfectious diseases (*74*) such as Alzheimer's disease, chronic obstructive pulmonary disease, diabetes, cystic fibrosis, cancer, atherosclerosis, and various forms of arthritis. The variety of pathologies in which NETs are described is not surprising given the fundamental function of neutrophils in immune reactions. Hence, understanding how NETs are formed and the function of each of the components has the potential to help treat numerous diseases.

HOW NEUTROPHILS SHAPE INNATE AND ADAPTIVE IMMUNE RESPONSES

Cytokines and other mediators

Activated neutrophils shape both innate and adaptive immune responses (*75*). They do so via multiple mechanisms: (i) by releasing preformed mediators such as alarmins; (ii) by recognizing intracellular nucleic acids of foreign origin via specific cytoplasmic pattern recognition receptors (*76*); (iii) by extruding NETs, which, in turn, activate the immune system through DNA receptors (*73*); (iv) by migrating into lymph nodes and presenting antigens to memory CD4 T cells (*77*); or (v) by producing a variety of cytokines (Fig. 3A) (*78*). Neutrophils produce, on a per-cell basis, much lower cytokine amounts than DCs, lymphocytes, or monocytes/macrophages, although there are some exceptions (such as vascular endothelial growth factor, IL-1ra, CCL19, CCL23, or B cell–activating factor) (*78*). However, during inflammation, the number of neutrophils far outweighs the number of all other leukocytes, allowing neutrophil-derived cytokines to contribute substantially to local amounts of cytokines. Extensive data support a role of neutrophil-derived cytokines not only in influencing both initiation and progression of various inflammatory, infectious, and autoimmune diseases but also in regulating hematopoiesis, angiogenesis, wound healing, and cancer growth (*75*, *78*). There are substantial differences in the cytokine repertoire produced by mouse and human neutrophils (*78*). Some neutrophil-derived cytokines have been shown to be controlled at the epigenetic level (*79*) as well as to mediate complex interactions that neutrophils engage with nonimmune cells (such as platelets and mesenchymal stem cells), innate immune cells [such as mast cells, monocytes, macrophages, DCs, and innate lymphoid cells (ILCs)], and adaptive immune cells (subpopulations of T and B cells) (Fig. 3B). Interestingly, neutrophil-centered networks can be alternatively regulated either by additional neutrophil-derived products—such as preformed inflammatory mediators (such as proteases, pentraxin-3, and alarmins), complement components, and extracellular vesicles—or by cell contact–dependent interactions. In the context of these networks, neutrophils and their partners reciprocally modulate their activation/functional status and survival (*75*). This may explain why neutrophil longevity increases several-fold in inflamed tissues. However, neutrophils are also known to undergo spontaneous or stimulus-induced apoptosis, which is essential for resolution of inflammation (*80*). Resolution of inflammation is also supported by soluble mediators released from neutrophils, such as annexin A1, proresolving lipids, scavenger molecules, and anti-inflammatory cytokines (such as transforming growth factor–β, IL-1ra, and in mouse models, IL-10 and IL-22) (*80*).

Contact-dependent mechanisms

The ability of neutrophils to positively or negatively influence innate and adaptive immune leukocytes has been shown to also occur via contact-dependent mechanisms (*75*, *81*). For instance, a β_2 integrin (CD18)–driven release of arginase 1 (*82*)—or alternatively, reactive oxygen species (ROS) (*83*), by discrete immunosuppressive neutrophil subsets—has been shown to ultimately lead to inhibition of T cell proliferation or production of IFN-γ under coculture conditions. Contact-dependent interactions that involve neutrophils and both NK cells and 6-sulfo LacNAc monocytes through CD18/ICAM-3 and CD18/ICAM-1, respectively, have been shown to potently enhance the production of IFN-γ by NK cells (*84*). Other neutrophil-centered cross-talk occurring by means of contact-dependent mechanisms and involving DCs, ILCs, and monocytes/macrophages support the concept that neutrophils form a kind of immunological synapse to provide specific and direct instructions to the target cells (*83*).

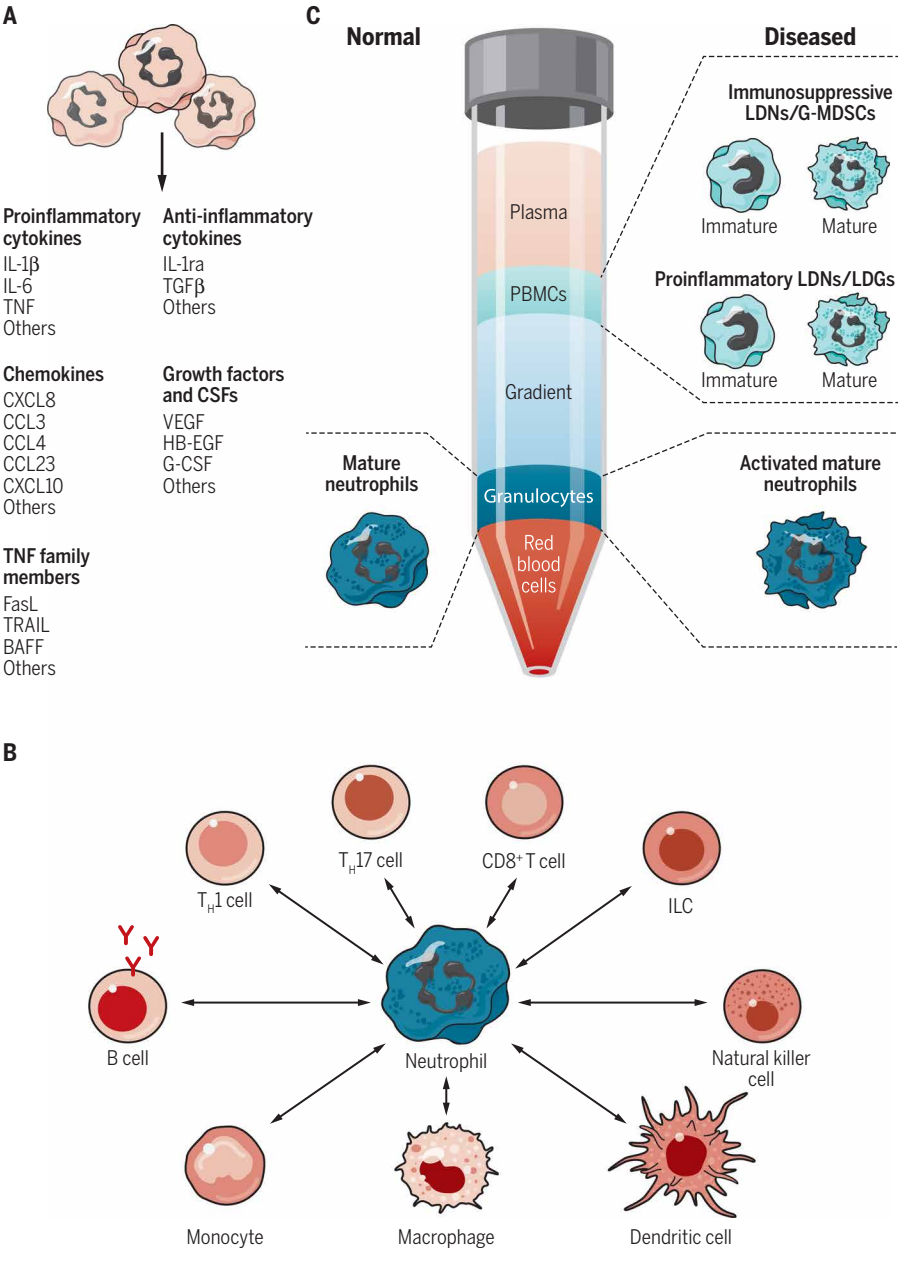

Fig. 3. Features of neutrophils in immunity. (**A**) Various cytokines, chemokines, and growth factors that neutrophils can produce and release in response to appropriate stimulation. (**B**) The various leukocyte subtypes with which neutrophils have been shown to engage in bidirectional cross-talk. (**C**) Heterogeneous populations of neutrophils can be recovered from the blood of healthy donors (normal, left) or patients with diseases (such as systemic inflammation, autoimmune diseases, and cancer) (diseased, right). After centrifugation of blood from healthy donors over density gradients, granulocytes typically sediment on top of the red cells, whereas mononuclear cells (PBMCs) localize at the interface between the plasma and the density gradient layer. The granulocytes include variable percentages of eosinophils and a homogeneous population of NDNs that, in healthy donors, consist of resting mature neutrophils (left). By contrast, density gradient centrifugation of blood from patients with disease reveals the presence of activated neutrophils within the NDNs, as well as of heterogeneous populations of LDNs within the PBMCs, which may include both immature neutrophils and activated mature neutrophils in different ratios (top right). Depending on the disease, LDNs may manifest either immunosuppressive or proinflammatory properties. Immunosuppressive LDNs are also known as G-MDSCs, and proinflammatory LDNs are known as LDGs. TGFβ, transforming growth factor–β; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; HB-EGF, heparin-binding epidermal growth factor–like growth factor; T_H1, T helper 1 cell.

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Neutrophil subsets

Studies aimed at examining blood neutrophils from patients have identified discrete populations of $CD66b^+$ neutrophils (or neutrophil-like cells) that, depending on the disease, exert either immunosuppressive or proinflammatory properties (Fig. 3C) (*81*). Some of these neutrophil populations, known as "low-density neutrophils" (LDNs), settle within the peripheral blood mononuclear cell (PBMC) fraction after density gradient centrifugation of the blood. This fraction includes immature neutrophils and activated mature neutrophils at different ratios (*81*). In patients with tumors, LDNs inhibit T cell proliferation and functions (mainly through arginase-1 and ROS release) and are more commonly known as granulocytic myeloid–derived suppressor cells (G-MDSCs) (*85*). By contrast, in patients with SLE or psoriasis, LDNs exert proinflammatory activities [for example, they are more prone to release proinflammatory cytokines and NETs, similar to normal-density neutrophils (NDNs) as outlined in the previous section], and are called low-density granulocytes (LDGs) (*86*). Because of the lack of specific markers that could allow their selective identification and isolation, the precise phenotypic and functional properties of these LDN subsets remain poorly understood (*81*, *82*). Future studies are necessary to understand whether these various neutrophil populations represent bona fide subsets—for example, fully differentiated and committed to specialized functions—or instead are "modified phenotypes" contextual to the presence of trophic factors that they are exposed to. We currently do not understand the relationship either between circulating NDNs and immunosuppressive LDNs/NDNs or between the latter cell populations and tumor-associated neutrophils (TANs). In this context, mouse TANs are known to polarize into either an antitumorigenic (TAN_1) or a protumorigenic (TAN_2) phenotype (*87*), whereas the immunoregulatory properties of human TANs are currently less well defined (*81*, *85*).

MONOGENIC HUMAN NEUTROPHIL DISORDERS

Neutropenia syndromes

Identification of disease genes and recent research concerning disease pathogenesis have provided insights into normal neutrophil homeostasis, or the balance between differentiation, migration, and apoptosis. This is a highly regulated process because the failure of multiple and diverse pathways of differentiation or migration results in low circulating neutrophil

numbers. Neutrophil maturation arrest is the final common pathologic mechanism of a group of inherited neutropenic disorders associated with mutations in three different genes (*HAX1*, *AK2*, and *GFI1*). Loss-of-function mutations in hematopoietic lineage cell-specific protein-1–associated protein X-1 (*HAX1*), which is responsible for the classic autosomal recessive neutropenia known as Kostmann disease, demonstrate a defect in G-CSF signaling. G-CSF is a key player in bone marrow production and release of neutrophils into the blood. It is therefore not surprising that the standard therapy of most neutropenia disorders is recombinant G-CSF. Mutations in adenylate kinase 2 (*AK2*), which is associated with cartilage hair hypoplasia, result in mitochondrial dysfunction, thus illustrating an important role for this mitochondrial metabolism pathway in neutrophil differentiation (*88*). Mutations in the transcription repressor GFI1, which has been observed in some patients with severe congenital neutropenia, affect the complex epigenetic regulation of transcription factors crucial to myeloid differentiation (*89*). Neutropenia is also observed when the neutrophils are unable to leave the bone marrow as observed in WHIM (warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis) syndrome because of mutations in *CXCR4*, a chemokine receptor that plays a crucial role not only in homing of circulating neutrophils from bone marrow to blood and back but also between blood and other tissues. Disease-associated gain-of-function mutations impair this protein's intracellular trafficking, resulting in increased responsiveness to various chemokines and retention of neutrophils in the bone marrow (*90*).

Neutropenia is also observed when neutrophils are driven to apoptosis through a variety of upstream pathways. The most common cause of severe chronic neutropenia is gain-of-function mutations in NE (*ELANE*). Recent studies with *ELANE* mutants suggest that mutations result in protein misfolding and demonstrate a complex dysregulation of the unfolded protein response resulting in endothelial reticulum (ER) stress and hence neutrophil death. The multiple steps of this complex process may explain why patients with the same mutations may present with cyclic neutropenia with low neutrophil numbers only intermittently (*91*). ER stress leading to apoptosis appears to be the end result of other upstream molecular mechanisms in patients with glucose-6-phosphatase catalytic subunit 3 (*G6PC3*) mutations who have an inactive ER enzyme or jagunal homolog 1 (*JAGN1*) mutations who have defective ER secretory pathways (*92*). This common pathway demonstrates that neutrophils appear to be particularly sensitive to ER stress.

Neutropenia is a feature of several immunodeficiency disorders that primarily involve adaptive immunity. Neutropenia may be the initial presentation of patients with X-linked agammaglobulinemia. Although mutations in Bruton's tyrosine kinase (*BTK*) have been shown to affect several neutrophil functions (*93*), the underlying cause of neutropenia has not been elucidated. Specific gain-of-function mutations in the Wiskott-Aldrich syndrome gene (WAS) are associated with X-linked neutropenia that are distinct from the mutations associated with classic Wiskott-Aldrich syndrome, which is characterized classically by thrombocytopenia, immunodeficiency, and eczema. These WAS mutations result in defects in actin polymerization, mitosis, and cytokinesis, resulting in neutrophils that are susceptible to cell-cycle arrest and apoptosis (*94*). Neutropenia is also a common clinical feature of X-linked hyper–immunoglobulin M (IgM) syndrome due to mutations in *CD40L*. Recent studies illustrate the role of CD40L in a variety of neutrophil functions that may explain this phenotype (*95*). Patients with STK4 deficiency not only have lymphopenia but also have neutrophils with increased susceptibility to apoptosis, likely due to mitochondrial dysfunction (*96*).

Neutrophil dysfunction diseases

Monogenic diseases have been identified that affect all aspects of neutrophil function discussed in this Review, including neutrophil recruitment, vesicular trafficking, NET formation, and immune regulation. The traditional disorders of neutrophil dysfunction include leukocyte adhesion or neutrophil granule defects and CGD (*36*). Severe glucose-6-phosphate dehydrogenase (G6PD) deficiency is known to be associated with reduced NADPH oxidase function similar to CGD. However, recent studies have also shown additional defects in NETosis (*97*). Patients with MyD88 deficiency are known to have impaired CD62L shedding on neutrophils and absent cytokine responses to TLR agonists and IL-1β (*98*). MyD88 has recently been shown to play a critical role in microbiome-directed neutrophil aging and numerous other neutrophil functions (*8*). Homozygous mutations in vacuolar protein sorting 45 homolog (*VPS45*) result in defective membrane trafficking and neutrophil migration defects that may have disease mechanisms similar to Cohen syndrome due to mutations in VPS13b, which is another VPS protein family member (*99*). Two previously unidentified disorders described in the last few years are associated with defective neutrophil function owing to defects in actin, including WD repeat domain 1 (WDR1) and myosin light chain kinase (MKL1). Homozygous mutations WDR1 lead to impaired chemotaxis and chemokinesis. WDR1 encodes for an actin-interacting protein that regulates actin disassembly that is important for the rapid remodeling of the cytoskeleton in neutrophils (*53*). Homozygous mutations in MKL1 lead to defective migration and impaired phagocytosis due to loss of protein expression of this transcriptional regulator of actin and actin cytoskeleton genes, resulting in abnormal actin assembly (*54*).

Autoinflammatory disorders

Although neutrophil dysfunction may lead to immunodeficiency, it can also result in uncontrolled neutrophil-mediated inflammation. This is not only exemplified in some of the clinical features of CGD but is also observed in a group of monogenic diseases known as the autoinflammatory disorders, including familial Mediterranean fever and cryopyrin-associated periodic syndrome. These conditions are associated with primarily innate immune activation that results from mutations in genes that normally keep inflammation in check. Gain-of-function mutations in the Mediterranean fever gene (*MEFV*) and *NLRP3* result in uncontrolled oligomerization of intracellular protein complexes known as inflammasomes that are expressed in neutrophils and other myeloid cells and are associated with actin (*100*). Activation of these complexes leads to cleavage and activation of caspase-1, release of the proinflammatory mediators IL-1β and IL-18, and various forms of cell death, ultimately resulting in neutrophil influx into the blood and tissues (*101*). Although the research focus of inflammasomopathies has been on monocytes and macrophages, it is becoming increasingly clear that these intracellular inflammatory regulatory complexes are also expressed in neutrophils, so these cells are playing more than just a downstream effector role in pathogenic disease mechanisms.

CONCLUDING REMARKS

Neutrophils have a distinct ability to get into any tissue, through any blood vessel wall and any epithelium. Although enormous

progress has been made in understanding neutrophil transmigration through the venular endothelium and basement membrane, other endothelia and epithelia are much less studied. Why have neutrophils evolved a multilobated nucleus? How can they be so enormously deformable? What are the molecular mechanisms by which tethers and slings are formed? How do tethers retract? How is granule trafficking differentially regulated, and what are the consequences to inflammation? How do neutrophils decide between phagocytosis and NETosis? How do neutrophil subsets affect immunity? Are there additional neutrophil progenitors? How do these newly identified progenitors contribute to neutrophil heterogeneity in disease? Many aspects of neutrophil development and function in vivo remain enigmatic. The role of neutrophils in infections is clear, but their role in other innate immune responses, adaptive immunity, and daily homeostatic functions require further studies. Undoubtedly, answers to these questions have the potential to lead to better therapeutics for inflammatory diseases.

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