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Human neutrophils ≠ murine neutrophils: does it matter?

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Summary

Human and murine neutrophils differ with respect to representation in blood, receptors, nuclear morphology, signaling pathways, granule proteins, NADPH oxidase regulation, magnitude of oxidant and hypochlorous acid production, and their repertoire of secreted molecules. These differences often matter and can undermine extrapolations from murine studies to clinical care, as illustrated by several failed therapeutic interventions based on mouse models. Likewise, coevolution of host and pathogen undercuts fidelity of murine models of neutrophil-predominant human infections.

However, murine systems that accurately model the human condition can yield insights into human biology difficult to obtain otherwise. The challenge for investigators who employ murine systems is to distinguish models from pretenders and to know when the mouse provides biologically accurate insights. Testing with human neutrophils observations made in murine systems would provide a safeguard but is not always possible. At a minimum, studies that use exclusively murine neutrophils should have accurate titles supported by data and restrict conclusions to *murine neutrophils* and not encompass all *neutrophils*.

For now, the integration of evidence from studies of neutrophil biology performed using valid murine models coupled with testing *in vitro* of human neutrophils combines the best of both approaches to elucidate the mysteries of human neutrophil biology.

Keywords

human neutrophiis; murine neutrophils; murine models; human infection

Perspective and purpose

Our understanding of many of the biological processes related to human health and disease derives in large part from two investigative approaches, namely clinical observations of patients afflicted with disease, either acquired or inherited, and animal experimental systems intended to model human disorders. Astute clinicians in search of alleviating the suffering of their patients drive basic and translational studies to identify underlying physiology, both normal and aberrant. Particularly powerful because of extensive background genetic

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information and well-characterized inbred strains, murine experimental systems have served to model a wide spectrum of human diseases, including infections, autoimmune disorders, inflammatory syndromes, and malignancies. Investigators often use murine experimental systems to assess the role of neutrophils in a specific disease, syndrome, or malignancy, either as their exclusive experimental tool or as an adjunct to their studies of human neutrophils *in vitro*. Enthusiasm for the use of murine systems as surrogates for human neutrophils is occasionally excessive and can distort perspective. For example, in a detailed review of the approaches available to study the contribution of neutrophils in murine model systems [1], the authors enthusiastically note in their concluding comments that "the use of mouse models for the study of neutrophils *in vivo* represents a milestone in the understanding of the biology of these cells and has enabled the deep interrogation of a number of pathways leading to diverse pathologies" [1]. As evidence of the success of this approach, the authors cite the use of DNase I as therapy for lung disease in cystic fibrosis "after the discovery that NETs [neutrophil extracellular traps] play a pivotal role in the disease". In fact, the Food and Drug Administration (FDA) approved the use of recombinant human DNase I for patients with cystic fibrosis in 1993, more than a decade *before* the publication of the first report of NETs [2]. Multiple studies of the role of the highly viscous, DNA-rich secretions created by the large number of spent and necrotic cells in the airways of patients with cystic fibrosis prompted the FDA's decision.

There may be many effective ways to manipulate neutrophils in murine experimental systems, but the intention in utilizing such systems is to have a tractable model of neutrophil-dependent biology that *both* mirrors the appearance of the original (*i.e.* the human) *and* recapitulates the same underlying mechanism(s). That is, to be an accurate and informative model, the mouse neutrophil must reproduce the behavior, biology, and biochemistry of human neutrophils in the process under study. In that way, investigators can probe the model to identify key regulatory molecules, critical pathways, and events that fuel pathophysiology caused by human neutrophils. A murine system that phenotypically mirrors the human situation but not the underlying mechanism is not a *bona fide* model and may prompt misleading conclusions.

One might ask what adverse consequences could arise from the use of experimental systems anchored only on the assumption that the same mechanisms underly a given phenotype for both mouse and man. At least two come to mind. From the perspective of the pursuit of knowledge, yet undiscovered aspects of human biology not represented in mice might be missed when the observations lack data on mechanism. Furthermore, extrapolation of observations from murine studies might fuel clinical trials in humans that prove unsuccessful because of the rationale for a particular intervention is flawed [3-7]. Hence, the incentive to know the fidelity of murine (or any animal) models should be great.

This review is neutrophil-centric, addressing only those settings where neutrophils represent critical contributors to the biological process under study. Consequently, my purposes are two-fold: first, to summarize the recognized differences in structure, composition, and biochemistry between murine and human neutrophils. The second goal is to present situations where such differences are such that the murine experimental system fails to

represent human biology faithfully and is unlikely to yield sound and clinically relevant insights.

The structure, composition, and biology of human neutrophils

Current understanding of the functional repertoire of human neutrophils, albeit incomplete, includes a wide variety of activities that contribute to host defense against threats, both infectious and inanimate, and, when excessive, can culminate in tissue damage [8-11]. Neutrophils are the predominant nucleated cell in human blood (50-70% of leukocytes) and serve as first responders to the invasion by microbes, the presence of foreign bodies, or the sequalae of tissue trauma.

In response to engagement of receptors on their surface, neutrophils adhere to activated endothelial cells, transmigrate into tissue, and crawl towards the origin of the stimulating agent. Neutrophils are phagocytic and respond to particulate stimuli, with or without opsonization with complement, antibody, or both, by ingesting the target or, when too large to eat, by adhering to its surface. Phagocytosis prompts a coordinated response whereby intracellular granules fuse with nascent phagosomes and cytosolic components of the NADPH oxidase assemble on the extraluminal face of the phagosomal membrane. With fusion, granules release their contents into the phagosome, delivering an array of enzymes, antimicrobial peptides, and other active biomolecules (Fig. 1). Activation of the NADPH oxidase promptly follows its assembly and transfers electrons from cytosolic NADPH to molecular oxygen in the phagosome, thereby generating first superoxide ion, which is rapidly converted to hydrogen peroxide that in turn interacts with the azurophilic granule protein myeloperoxidase (MPO) to produce the potently bactericidal agent, hypochlorous acid (HOCl) [12-16]. HOCl reacts with the luminal contents of the phagosome, including both the ingested target (*e.g.* a microbe) and the granule proteins, thereby generating a host of modified proteins that are themselves microbicidal (Fig. 2) [17-19]. The synergistic activities among the individual granule proteins and between granule proteins and oxidants create an environment highly toxic to most microbes [20-22].

The contribution of neutrophils to human innate immunity extends beyond the ingestion and killing of microbes [9-11]. Activated neutrophils release a host of chemokines and cytokines that recruit additional cells to the site and condition the milieu of the local inflammatory response [10]. Furthermore, under optimal conditions, activation prompts neutrophils to engage transcriptional pathways that trigger apoptosis and initiate events that culminate in termination of the inflammatory response [23-25]. Sometimes, as with interactions with particular bacteria, such as *Staphylococcus aureus*, or when stimulation is excessive, neutrophils undergo a necrotic cell death [24,26] and thereby release cytoplasmic contents that provide danger signals to drive additional inflammation. In some settings, neutrophils, as well as other cells, release extracellular traps (aka ETs), whose DNA may entrap microbes, contribute to autoimmune disease, or promote further inflammation [27,28].

In summary, human neutrophils play a prominent role in every stage of the inflammatory response, as first responders to many different types of threats, as sources of some of the

secreted immunomodulators released at the site, and as active participants in the termination of inflammation and the tissue repair needed to restore homeostasis [29].

Human vs Murine Neutrophils

Morphology and distribution

It may be news but not a surprise that differences in cellular organization, contents, and activities exist between species [30,31], largely reflecting customization to the lifestyles and needs of the individual species during evolution. Overall, however, human and murine neutrophils share many structural features

As noted earlier, neutrophils are the predominant nucleated cell in circulating human blood, at a concentration of 3-11 X 10^3/μL, and account for 57-75% of the leukocytes. The neutrophil predominance is typical for many, but not all, mammals [32]. Mice, like ruminants, have a lymphocyte-dominant hemogram, with lymphocytes at 75 to 90% of circulating leukocytes [33]. The maturation of neutrophils in humans and mice differs significantly, as CXCR4 binding retains human cells in bone marrow until they mature [9,34], whereas murine neutrophils appear to complete maturation outside of the bone marrow [35]. Whether these variables would differentially alter the kinetics of normal neutrophil turnover or influence the emergency granulopoiesis that operates during acute inflammation in humans [9] is unknown.

Mature human neutrophils possess lobed nuclei, typically with two to five lobes and 50% with three lobes. Certain drugs and some vitamin deficiencies (*e.g.* folic acid, vitamin B_{12}) can cause hypersegmentation of the nuclei of human neutrophils. The presence of multi-lobed nuclei is considered by some as an attribute that allows neutrophils to traverse tight spaces, as occurs during transmigration through vascular endothelium, because of the flexibility of the nucleus [36,37]. Apparently, high deformability allows human neutrophils to migrate rapidly and to accommodate the variety of vessel sizes encountered. Hypolobulated neutrophils exhibit reduced migratory behavior [38]. The multiple indentations in the nuclei of murine neutrophils can give an appearance of lobulation, although many cells possess nuclei with a ring form. The half-life of circulating neutrophils differs between humans and mice as well, with that for human neutrophils ~ 6.5 hours [39] and for murine cells 11.4 hours [40].

Receptors

In general, receptors on the cell surface engage ligands, soluble and particle-bound, to transmit signals intracellularly and initiate specific cellular responses. Consequently, the repertoire of receptors on a cell dictates the agonists that will be detected and the responsive pathways that will be activated. The interactions and crosstalk among signaling pathways sculpt the phenotype observed.

Although murine and human neutrophils share many receptors, significant differences have been identified in specific settings. In the case of the colony-stimulating factor receptor family (CSF1R/IL34/CSF1), studies of specific receptor knock-out mice demonstrate that CSF1R/CSF1 and CSF3R/CSF3 are required for normal development of monocytes and neutrophils, but their loss does not result in absolute neutropenia [reviewed in [41]]. In

fact, loss of CSF1 results in *increased* numbers of circulating murine neutrophils [42-44]. In contrast, mutations in the human gene encoding CSF3 and CSF3R result in severe congenital neutropenia, with affected children experiencing severe and life-threatening infections due to the absence of circulating neutrophils [45-47].

The balance between pro- and anti-inflammatory activities in neutrophils is maintained, in part, by the interplay between different types of immunoreceptors, which are defined as "transmembrane structure(s) containing an extracellular immunoglobulin-like domains and intracellular signaling via conserved immunoreceptor tyrosine-based activations (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMS)"[reviewed in [48]. The interplay between these receptors provides neutrophils with the functional plasticity necessary to adapt to the wide variety of situations they encounter. Notable differences in the array of immunoreceptors in human and murine neutrophils have been identified and were comprehensively reviewed by van Rees et al. [48] (Figure 3), although a few merit brief mention here.

Neutrophils in both species express receptors for Fc domains of aggregated IgG (FcγR) and participate in the ingestion of IgG-coated particles and microbes. Human neutrophils have three distinct Fcγ receptors, FcγRI (aka CD64), FcγRIIa (CD32a), and FcγRIIIb (CD16b), each with different affinities and behavior, and a receptor for IgA (FcαR). Both FcγRI and FcγRIIa have activating cytoplasmic motifs and signal via Syk, whereas FcγRIIIb is glycophosphatidylinositol-linked and lacks signaling motifs. Murine neutrophils lack FcRα, despite possessing IgA, and express an inhibitory FcγRIIb and activating FcγRIII and FcγRIV, the first signaling through SHIP and the latter two via Syk. Such differences in ligand recognition and signaling attributes (activating vs inhibitory) extend to other immunoreceptor types, including CD300 receptors, Siglec receptors, SIRP receptors and CEACAM receptors [see [48] for detailed discussion].

The differential expression of CEACAM receptors has significant implications relevant to human disease. The CEACAM receptors have a critical role in host defense against the exclusively human pathogen, *Neisseria gonorrhea* (Ngc). Clinically, a robust neutrophilic inflammatory response characterizes infections with Ngc [49]. Human neutrophils express three CEACAMs (CEACAM 1, 3, and 6) that bind colony opacity-associated (opa) outer membrane proteins on Ngc and mediate direct binding and uptake of Ngc by neutrophils [50-52]. Murine neutrophils lack the opa-binding CEACAMs and do not bind Ngc. Genetic manipulation to express the necessary human proteins in murine neutrophils corrects this specific short-coming [53].

In addition to the immunoreceptors, others differ between man and mouse, most notably the formyl peptide receptors (FPRs). N-formylated proteins from bacteria and mitochondria are the proteins first recognized as agonists for FPRs, but the list of ligands has grown significantly over time [54]. FPRs are G protein-coupled transmembrane proteins whose engagement by suitable ligands stimulates adhesion, degranulation, NADPH oxidase activation, and chemotaxis in neutrophils The human genome includes three genes that encode FPRs (*FPR1, FPR2, FPR3*), and human neutrophils express FPR1 and FPR2 on their plasma membrane. There are eight members of the murine FPR gene family, with

the orthologue for human FPR1, mFPR1, sharing many of the structural and functional features of the human receptor. One receptor, mFpr-rs1, is expressed on murine neutrophils and initially thought to share functional properties with human FPR2. However, studies in transfected cell lines demonstrate that the response profile of mFPR-rs1 does not parallel that of human FPR2 [55]. Other studies have identified differences in the FPR-dependent, agonist-induced activation of the NADPH oxidase in human and murine neutrophils [*e.g.* [56]]. The variety of specific ligands for the individual FPRs and, in the case of mouse, tissue expression in non-leukocytes likely reflect differential expansion of the FPR gene cluster between mouse and man during evolution [55,57,58] and may undermine the fidelity of murine experimental systems that aim to mirror FPR-dependent human disease. Such differences have prompted an effort to identify FPR1- and FPR2-specific ligands that operate in both species [*e.g.* [59]] and thus provide informative experimental tools

Signaling

Downstream of receptors are the biochemical pathways that relay signals to the intracellular machinery that elicits cellular responses. The Rho family GTPase Rac2 is a component required for activation of the phagocyte NADPH oxidase [60,61] and represents the predominant isoform in human neutrophils, with 80-95% Rac2 and the remainder Rac1 [62,63]. Murine neutrophils, on the other hand, express Rac1 and Rac2 in equal amounts. Mice deficient in Rac2 exhibit markedly depressed formyl peptide-stimulated F-actin polymerization, chemotaxis, and NADPH oxidase activity despite the presence of Rac1, which shares ~92% amino acid identity with Rac2 [64]. The Bokoch lab exploited these differences in Rac isoform expression between murine and human neutrophils to identify differential regulation of Rac1 and Rac2 over the gradient of fMLF concentration encountered as neutrophils crawl toward the source of chemoattractant [65]. Such studies illustrate how an appreciation of the differences between murine and human neutrophils can enable the dissection of complex human physiology.

Exposure to specific cytokines, chemokines, and other proinflammatory molecules converts neutrophils from their resting state to one in which they have a heightened responsiveness to stimuli, a process referred to as *priming*. The primed phenotype can include enhanced adhesion, chemotaxis, phagocytosis, degranulation, apoptosis, and NADPH oxidase activity, depending on the priming agent and the context [reviewed in [66]]. Typically, the priming agent does not itself activate neutrophils but instead prompts neutrophils to have an augmented sensitivity or response to subsequent exposure to a known agonist. Approximately 10% of circulating human and murine neutrophils exhibit a phenotype with increased expression of adhesion and activation markers and are “primed for recruitment” to a nascent inflammatory site [67].

Priming of the NADPH oxidase in human neutrophils depends on selective phosphorylation of a subset of serine residues whose phosphorylation controls oxidase assembly and activation [reviewed in [68]]. Critical to priming by GM-CSF, TNFα, and LPS, for formyl peptide-activation of the NADPH oxidase is MAPK phosphorylation of SER345 in $p47^{phox}$ [69]. Following its phosphorylation, proline-isomerase Pin1 binds to PRO346 and promotes conformational changes in $p47^{phox}$ that render other serine residues accessible for PKC-

dependent phosphorylation [70-72], which then exposes motifs in p47*phox* that mediate assembly of a complete and functional oxidase. Thus, the phosphorylation of SER345 and binding of Pin1 serve as essential early steps in the priming activity of GM-CSF, TNFα, and LPS on formyl peptide activation of NADPH oxidase in human neutrophils [71-73].

Exposure of murine neutrophils to LPS, TNFα, or cytochalasin B primes NADPH oxidase stimulation by FPR agonists [56,74], although it is unknown if the underlying mechanism is the same as that for the human phagocyte NADPH oxidase. In general, the phosphorylation sites in murine p47*phox* differ from those in the human protein (although rat p47*phox* is identical to human), and SER345 is not conserved in murine p47*phox*. Although murine p47*phox* has SER346, the required adjacent residue is not proline, making it unlikely that its phosphorylation by MAPK would mediate priming in a Pin1-dependent fashion [71,75]. However, THR356 in murine p47*phox* is phosphorylated by p38MAPK and has an adjacent proline residue, making it possible that the same mechanism, albeit with different residues, supports murine and human neutrophil priming [personal communication, Jamel El Benna, Ph.D., Research Director, Centre de Recherche sur l'Inflammation (CRI), Laboratoire d'Excellence Inflamex, Unversité de Paris, INSERM-U11499, Paris, France].

As part of the signaling pathway in TNFα-primed formyl peptide activation of the NADPH oxidase, there is sequential activation of different classes of phosphoinositide-3-kinases (PI3Ks), with the first phase dependent on PI3Kγ and the second on PI3Kδ [76]. TNFα primes murine neutrophils as well but less robustly than human neutrophils and with dependence on only PI3Kγ [76]. Although the authors offered no explanation for the differences in the observed PI3K isoform-dependent TNFα priming between human and murine neutrophils, they recommended caution when using murine experimental systems to predict the efficacy of PI3K inhibitors on responses of human neutrophils.

Secreted proteins

Granule proteins—Proteomic analyses of human [77,78] and murine [79,80] neutrophlls reveal many similarities, as expected, but some important differences [reviewed in [10]]. Neutrophils possess intracellular compartments, granules, in which antimicrobial proteins, enzymes, and other bioactive molecules are stored and ready for release during degranulation into nascent phagosomes or the extracellular space [reviewed [81]]. The azurophilic granules of human neutrophils possess three proteins (at least) that are absent from the granules of murine neutrophils: α-defensins, azurocidin (aka CAP37, aka heparin-binding protein, HBP), and bactericidal permeability increasing protein (BPI aka CAP57). Prominent contributors to host defense against infection and inflammation, these cationic antimicrobial peptides both damage or kill microbes as well as neutralize pathogen-associated factors that promote or exacerbate sepsis [82].

The α-defensins comprise nearly 50% of the protein in human azurophilic granules [83], exert broad antimicrobial action against viruses, bacteria (Gram-positive and Gram-negative), and fungi [84-86], and are chemotactic for T cell subtypes and immature dendritic cells [87]. Concentrations of α-defensins in plasma increase from ~ 42 ng/ml at rest to 900-170,000 ng/ml in sepsis, and α-defensins appear in pulmonary secretions during inflammatory lung disease [88]. The cationic antimicrobial peptide azurocidin meets infectious threats by

directly damaging ingested microbes and by summoning additional host defenses, thereby serving both as a chemoattractant and as an activator of monocytes and macrophages [89]. Furthermore, azurocidin acts on endothelial cells and fibroblasts, thereby contributing to tissue damage in some settings [89]. BPI, the third of the azurophilic granule proteins present in human but absent from murine neutrophils, binds avidly to the surface of Gram-negative bacteria, thereby compromising microbial integrity [90], and to endotoxin, thus neutralizing its proinflammatory action [91,92] and its contribution to the pathophysiology of many infectious and inflammatory diseases [93]. Taken together, the absence of these three antimicrobial proteins from murine neutrophils represents a substantial difference in the relative abilities of human and murine neutrophils to damage microbes, recruit additional host cells to the site of infection, and neutralize proinflammatory microbial components such as LPS.

But in addition to the absence from murine neutrophils of agents of host defense, there are differences in the relative amounts of some antimicrobial granule proteins, as is the case for MPO. Depending on the strain of mouse, murine neutrophils express 20 to 50% of the MPO present in human neutrophils [85,94], and the NADPH oxidase activity of murine neutrophils is 2- to 4-fold less than that of human neutrophils in response to conventional stimuli such as opsonized zymosan or phorbol myristate acetate (personal communication, Dr. Mary Dinauer, Department of Pediatrics and Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA). Thus, the capacity of murine neutrophils to generate HOCl and its reaction products (*vide infra*) is much less than that of human neutrophils. Because the MPO-H_2O_2-chloride system provides a potent and perhaps the most efficient oxygen-dependent antimicrobial activity in human neutrophil phagosomes [13,18,19,21,95,96], its absence would compromise intraphagosomal killing.

A comparison of how human and murine neutrophils interact with *Candida albicans* illustrates the biological consequences of generating too little HOCl. Human neutrophils kill *C.albicans*, a common human commensal and one of the predominant fungal pathogens for humans [97], and prevent fungal filamentation in an MPO-dependent fashion [98]. Neutrophils from individuals with an inherited deficiency of MPO fail to kill *C.albicans in vitro* or to damage hyphal elements in *C.albicans* and *Aspergillus fumigatus* [99-104]. The defective candidacidal activity *in vitro* due to insufficient HOCl production has clinical manifestations, as four of the first six MPO-deficient humans had severe infection with disseminated or visceral candidiasis [101-103,105]. In many ways, the interaction of murine neutrophils with *C.albicans* resembles that of MPO-deficient human neutrophils. Murine neutrophils kill *C.albicans* less effectively than do human neutrophils and fail to prevent *Candida* germ tube formation [98]. In fact, hyphal growth of ingested *C.albicans* results in the death of the murine neutrophil. Ineffective fungal killing occurs in murine blood as well as in isolated neutrophils, with significantly greater fungal survival and filamentation in murine blood than in human blood [106]. Although the defective candidacidal action of murine neutrophils likely reflects both the absence of α-defensins and the lesser amounts of MPO, the depressed activity of whole blood suggests that that soluble factors that act against *C.albicans* in mice are relatively ineffective as well. Together these inadequacies in cellular and soluble factor defenses constitute a serious short-coming in the use of murine experimental systems to model human immune response to *C.albicans*.

Cytokines—Secreted biomolecules that are immunoregulatory and serve to tune the immune response, both at the site of acute inflammation and systemically, cytokines were once not considered part of the repertoire of human neutrophil activity. However, since the early 1990s when investigators demonstrated that human neutrophils produced IL-8 and TNFα in response to phagocytosis [107,108], subsequent studies have identified additional cytokines produced by human neutrophils in response to a variety of agonists and in diverse experimental settings [reviewed in[10,109,110] [111]. Neutrophil-derived cytokines include both those stored intracellularly [*e.g.* TNFα-related apoptosis-inducing ligand (TRAIL) [112,113]] as well as those synthesized *de novo* and secreted in response to *in vitro* stimulation. The list includes both pro- and anti-inflammatory cytokines, chemokines, colony stimulating factors, TNFα family members, and factors that promote angiogenesis and fibrogenesis [111]. Although neutrophils express only ~10 to 20% the RNA of other leukocytes [114] and therefore produce and secrete relatively less cytokine per cell, the enormous number of neutrophils at an inflammatory site enables neutrophil-derived agents to influence significantly local events. Secreting such bioactive agents, stimulated neutrophils communicate with other cells, both near and far, to modulate the inflammatory response [9].

Human and murine neutrophils differ in the cytokines expressed and secreted, summarized in [111]. (Figure 4). Human neutrophils do not produce IFNβ, IL-10, or IL-17 [115] [116], for example. Considering the far-reaching and multiple immunomodulatory properties of cytokines, one anticipates that the inflammatory milieu in many contexts would differ in mice and humans and, more importantly, that it would be difficult to predict the ways in which events in the two species would differ. It is possible that the cytokines and granule proteins act in concert to recruit and activate immune cells in an inflammatory process [10], thus adding to both the complexity of the biology and to the uncertainty in predicting species' differences and their consequences.

Experimental studies using mice

The significant differences in the genetic composition of mice and humans comes as no surprise, given the divergence of the two species during evolution 65 to 75 million years ago. Although the mouse genome contains more genes than does that of humans (20,210 vs 19,042 [6]), a large majority of genes (75% of murine, 80% of human) are closely related functionally [reviewed in [6]]. However, mice and humans differ markedly in their genomic responses to proinflammatory stimuli such as trauma, burns, and endotoxemia [117]. In part, these differences may reflect the contrasting strategies that mice and humans have developed to optimize their odds of survival in an environment replete with infectious threats.

In general, multicellular organisms can adopt one of two distinct strategies to encounters with infectious threats: resistance or tolerance [6,118-120]. Resistance mechanisms include those targeted to kill (or at least damage) and eliminate invading microbes. Alternatively, tolerance allows the host to maintain function despite the presence of large numbers of potential pathogens. Although neither mice nor man utilizes one strategy at the expense of the other, resistance mechanisms predominate in human response to infection, particularly with respect to the innate immune system, whereas mice rely more on tolerance to endure

infectious threats. Detailed discussions of these alternative approaches to infection have been presented more extensively elsewhere [119,120], but the contrasting sensitivities of murine and human innate immune responses to lipopolysaccharide (LPS) illustrate the differences between resistance and tolerance. Simply put, mice tolerate endotoxemia and are highly resistant to LPS, with a lethal dose ~ 10 mg/kg. In contrast, humans mount a robust immune response to LPS and are exquisitely sensitive, with ~15 μg/kg triggering shock [6,7]. These diametrically opposite responses to endotoxin reflect differences in the determinants of sentinel and reactive immune cells and in the soluble factors that contribute critically to detection of LPS [121]. Such genetic and phenotypic differences in the innate immune responses to a prototypical microbial proinflammatory agonist such as LPS may explain, at least in part, the legacy of failed therapies to target endotoxemia and sepsis: the murine experimental systems used to mimic the human response to endotoxin were imposters, not models. Consequently, the extrapolations from mouse to man fell short of expectations [5-7].

The differences in genomic responses of mice and humans include proinflammatory stimuli in addition to those to endotoxin [117]. Many investigators working with murine experimental systems to explore physiologic or pathophysiologic mechanisms that relate to human neutrophil biology or innate immune responses have identified some of the limitations of such systems that approximate phenomenology but do not mirror the human condition with respect to underlying mechanism. Several examples illustrate this important point.

- Neutrophils are critical contributors to the pathophysiology of many autoimmune diseases and targets for therapy with intravenous immunoglobulin G (IVIG), which is frequently used clinically in many inflammatory and autoimmune diseases [122]. IVIG contains antibodies to the death receptor Fas (aka CD95) and to Siglec-9, thereby prompting cytokine-dependent neutrophil death when infused into humans and mediating a beneficial clinical response [123-126]. In contrast to the effects on human neutrophils, IVIG, $F(ab')_2$ or Fc fragments of IgG, pooled murine IgG, or agonistic monoclonal antibodies to Fas or Siglec-9 have no effect on murine neutrophils. These contrasting results prompted the authors to underscore “the need to establish experimental systems that take into account the divergent species-related effects of IVIG on neutrophils” [127].
- The α-defensins released from activated human neutrophils promotes damage in acute lung injury by disrupting capillary-epithelial cell barriers, and the absence of α-defensins from murine neutrophils disqualifies unmodified murine systems as potential models of acute human lung injury. The creation of transgenic mice expressing α-defensins in their neutrophils was required to provide a better approximation of acute lung injury in humans for study [88].
- Although both human and murine neutrophils generate extracellular traps in response to larval *Strongyloides stercoralis*, the extracellular traps from human, but not murine, neutrophils are required for killing of larvae [128].
- Whereas binding of Shiga toxin to murine neutrophils depends on the presence of globotriaosylceramide 3 (Gb_3) on the plasma membrane, its association with human neutrophils does not. Consequently, human serum amyloid P protects mice but not humans from the deleterious effects of Shiga toxin 2, an important

virulence factor in hemolytic uremic syndrome caused by enteropathogenic *E.coli* O157:H7 [129].

- As discussed earlier, the reduced capacity of murine neutrophils to generate HOCl undermines the accuracy, at the mechanistic level, of studies to elucidate the role of neutrophils in human infections with *C.albicans*. Similarly, murine neutrophils do not respond to *Mycobacterium tuberculosis* in the same MPO-dependent fashion as do human neutrophils [130]. Furthermore, MPO activity figures prominently in sterile settings of chronic inflammation, including atherosclerosis [131]. Whereas MPO-dependent biochemistry has been implicated in the initiation and progression of human atherosclerosis, some murine systems used to study atherosclerosis lack evidence for a contribution from MPO [132,133].
- In many settings, the behavior of tumors in mice fails to mirror critical aspects of human disease [134]. In part, the discrepancies likely reflect the absence of immunoediting, a fundamental determinant in the selection and development of human malignancies [135]. Many human tumors are extensively infiltrated with neutrophils[136], and the presence of tumor-associated neutrophils has been implicated in tumor angiogenesis [137]. Neutrophil infiltration of human tumors has been assigned prognostic significance, and some investigators in the field have advocated for neutrophil-targeted therapy as part of patient management [135,137-139], which together underscore the clinical importance of having valid animal models.

Modeling human infections in which neutrophils play a central role

The examples mentioned above demonstrate that in some biological settings where neutrophils serve a defining role, the murine experimental system does not mirror the human condition with sufficient accuracy to provide informative insights into the mechanism(s) underlying human disease. However, many investigators have utilized murine models successfully to explore the pathogenesis of human clinical disorders, thus providing evidence that in the *right* setting, a murine model can serve as a powerful and informative experimental tool. Unfortunately, the identification of the *right* situation is a challenge often unrecognized by reviewers of grants or manuscripts who greet data derived from *in vitro* studies of human neutrophils with a request for the applicant or authors to test the hypothesis in question in a mouse model or knock-out. Unfortunately, such requests often prompt investigators to comply and thereby generate data that may be compelling but too often lack a connection with the human biology intended to be explicated.

To suggest using a mouse system to model human infections in which neutrophils figure prominently fails to consider the coevolution of humans and human-adapted microbes. Humans and their pathogens have coevolved to the extent that reciprocal adaptive changes have occurred in each and have thereby exerted selective pressures on each. This coevolution of host and pathogen serves as a potent agent in the biology of human infection [140]. Accordingly, the human immune system has specific strategies to target certain microbes, and, on the other hand, microbes that are successful pathogens employ adaptive responses to resist, evade, or counteract host responses. The coadaptation of microbes to humans and of

human innate immunity to microbes has fostered the development of reciprocal constructs and responses that, in some cases, are so finely tuned that the introduction of a given microbe into a non-human species yields no valuable or valid insights, regardless of how similar the phenotype or entertaining the result of the murine infection. Some examples of the disconnect between mouse and man are obvious. Exclusively human pathogens such as *Neisseria gonorrhoeae* cannot be studied in a mouse model without significant genetic retooling of the mouse, as the organism is exquisitely and exclusively adapted to infect humans. For other microbes, it may be less readily apparent why a mouse model is in many ways inappropriate, as is the case for *Staphylococcus aureus*.

Staphylococcal infections in murine experimental systems

S.aureus is a human commensal that causes frequent and serious infections in humans, often complicated by local tissue necrosis, distant metastatic spread, and commonly death [141]. Early host defense against *S.aureus* is neutrophil-mediated and oxidant-dependent [142], convincingly demonstrated by the morbidity and mortality of staphylococcal disease in individuals with chronic granulomatous disease, who lack a functional NADPH oxidase [143] and are particularly susceptible to infection with *S.aureus* [144]. *S.aureus* has adapted successfully to man and become a prominent cause of infection in even the immunocompetent host.

Evidence from *in vitro* studies of normal human and of MPO-deficient neutrophils demonstrates that optimal killing of ingested *S.aureus* by human neutrophils is MPO-dependent [95,145]. However, neutrophils fail to kill all ingested staphylococci, and surviving staphylococci within neutrophils often trigger necrotic cell death of the host cell [146], thereby releasing proinflammatory agonists that promote more inflammation and tissue destruction locally and metastatic infection to bone, heart, lungs and other vital organs. Adaptations during co-evolution with humans have brought *S.aureus* success as a human pathogen. In contrast to its relationship with humans, *S.aureus* is not an organism that naturally infects mice, although strains may be adapted to cause transmissible disease among mice [147].

Strategies that *S.aureus* has employed to gain foothold in humans include those that block chemotaxis, ingestion, and killing by neutrophils [reviewed in [148]]. For example, the chemotaxis inhibitory protein of *S.aureus* (CHIPS) binds the C5a and formyl peptide receptors of human neutrophils better than murine receptors to inhibit human neutrophil chemotaxis [149]. Secreted staphylococcal proteins such as staphylococcal complement inhibitor (SCIN) and its homologues block steps in complement activation in the cascade that culminates in opsonization of the organism and phagocytosis by neutrophils [150,151]. *S.aureus* secretes a variety of leukotoxins, including Panton Valentine Leukocidin (PVL), which target specifically human cells to promote their lytic cell death [152-155]. Some have little or /no binding to murine leukocytes [reviewed [153]] (Figure 5). Even within the phagosome, *S.aureus* targets specifically human neutrophil attack by secreting SPIN (staphylococcal peroxidase inhibitor), a protein that binds to and inhibits the activity of human MPO [reviewed [156]]. *S.aureus* increase expression of SPIN within the phagosomes of human neutrophils, and SPIN inhibits MPO activity and the generation of HOCl [157,158].

SPIN does not bind or inhibit the MPO from rabbit, mouse, horse, or cow neutrophils, providing evidence of the remarkable human adaptation achieved by *S.aureus* [156,157].

Thus, during its coevolution with man, human-adapted strains of *S.aureus* have acquired attributes and behaviors finely tuned to elements of the human innate immune system in ways that cannot be precisely mimicked in unmodified murine experimental systems.

Summary

I chose this topic with great trepidation, since I have previously written about the functionally relevant differences between human and murine neutrophils and their potential consequences with respect to the use of murine experimental systems to model human neutrophil-dependent biology [133]. Furthermore, others have contrasted human and murine immune systems in great detail [6,31,106,159,160], including a recently published thought-provoking perspective [161]. However, I decided that the exercise has merit, but neither as an indictment of murine models, since that would be unjustified and not my intention, nor as a tribute to *in vitro* studies of human neutrophils.

Of course, the *in vitro* study of isolated human neutrophils, the experimental approach that has anchored my scientific career, has many shortcomings. The process of isolating neutrophils from venous blood itself imposes stresses on cells that are non-physiologic (exposure to plastics, dilute buffers, anticoagulants, centrifugal forces, density gradient media *etc*) and likely perturb neutrophils. Are isolated neutrophils ever “resting”? Some investigators keep isolated neutrophils at 4°C (or colder in an ice bath) before starting experiments in order to avoid inadvertent stimulation. What changes in membrane behavior or neutrophil metabolism accompany such cold exposure? Most consequential to the behavior of isolated neutrophils however is the absence from such studies of soluble factors and other cells, circulating as well as tissue-bound, with which neutrophils communicate. In the absence of such trophic factors, neutrophils isolated from venous blood only approximate neutrophils *in vivo*. Animal models avoid all these shortcomings (and likely more).

How best to proceed? Without question, human and murine neutrophils differ in many ways: differences in relative abundance in circulation blood, in receptors, signaling pathways, granule proteins, NADPH oxidase regulation, magnitude of oxidant and HOCl production, and repertoire of cytokines and other secreted molecules they produce. Consequently, authors should use and editors should demand accurate titles for manuscripts. If all the data in a report derive from the study of murine neutrophils or a murine model exclusively, then the correct and scientifically accurate title of the manuscript should include *mouse/murine* to modify the word neutrophils. Sometimes, but not always, the differences matter greatly and can derail extrapolations from murine studies to clinical care, as illustrated by several failed clinical interventions based on presumed murine models, such as therapy for sepsis.

However, when *bona fide* models, murine experimental systems can provide insights into human biology difficult to obtain in any other way. Furthermore, when murine models may fall short of explicating mechanisms underlying certain human diseases, the

novel insights into general immune mechanisms have been remarkable. The challenge for investigators who employ murine experimental systems, however, is to distinguish models from pretenders and to know when the mouse provides accurate insights into the human biology. Testing in humans those observations made in the murine systems would provide a safeguard, but such experiments are not always possible. The expense and the housing requirements can preclude the use of animal models that better mimic human neutrophils, such as rabbit or pig. For now, the integration of evidence from studies of neutrophil biology performed using sound murine models coupled with testing *in vitro* of human neutrophils will combine the best of both approaches to elucidate the mysteries of human neutrophil biology.

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Abbreviations:

fMLF	formyl-methionyl-leucyl-phenylalanine
FPR	formyl peptide receptor
HOCl	hypochlorous acid
LPS	lipopolysaccharide
MPO	myeloperoxidase
NETs	neutrophil extracellular traps
TNFα	tumor necrosis factor α

REFERENCES

1. Stackowicz J, Jönsson F, Reber LL. Mouse Models and Tools for the in vivo Study of Neutrophils. Front Immunol. 2019;10:3130. [PubMed: 32038641]
2. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–1535. [PubMed: 15001782]
3. Panitch HS, Hirsch RL, Haley AS, Johnson KP. Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet. 1987;1(8538):893–895. [PubMed: 2882294]
4. Monaco AP. Chimerism in organ transplantation: conflicting experiments and clinical observations. Transplantation. 2003;75(9 Suppl):13s–16s. [PubMed: 12819484]
5. Munford RS. Murine responses to endotoxin: another dirty little secret? JInfectDis. 2010;201:175–177.
6. Zschaler J, Schlorke D, Arnhold J. Differences in innate immune response between man and mouse. Crit Rev Immunol. 2014;34(5):433–454. [PubMed: 25404048]
7. Cavaillon JM, Singer M, Skirecki T. Sepsis therapies: learning from 30 years of failure of translational research to propose new leads. EMBO Mol Med. 2020;12(4):e10128. [PubMed: 32176432]

8. DeLeo FR, Nauseef WM. Granulocytic Phagocytes. In: Bennett JE, Dolin R, Blaser MJ, eds. Principles and Practice of Infectious Diseases. 9 ed. Philadelphia, PA: Elsevier; 2020:83–98.
9. Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol. 2014;15(7):602–611. [PubMed: 24940954]
10. Cassatella MA, Ostberg NK, Tamassia N, Soehnlein O. Biological Roles of Neutrophil-Derived Granule Proteins and Cytokines. Trends Immunol. 2019;40(7):648–664. [PubMed: 31155315]
11. Ley K, Hoffman HM, Kubes P, et al. Neutrophils: New insights and open questions. Science immunology. 2018;3(30).
12. Nauseef WM. The Neutrophil NADPH Oxidase. In: Vissers M, Hampton MB, Kettle AJ, ed. Hydrogen Peroxide Metabolism in Health and Disease. Boca Raton, FL: CRC Press; 2018:237–277.
13. Forbes LV, Kettle AJ. Myeloperoxidase: Unleashing the Power of Hydrogen Peroxide. In: Vissers MCM, Hampton MB, Kettle AJ, eds. Hydrogen Peroxide Metabolism in Health and Disease. Boca Rotan: CRC Press; 2018:281–304.
14. Albrett AM, Ashby LV, Dickerhof N, Kettle AJ, Winterbourn CC. Heterogeneity of hypochlorous acid production in individual neutrophil phagosomes revealed by a rhodamine-based probe. J Biol Chem. 2018;293:15715–15724. [PubMed: 30135208]
15. Kettle AJ, Anderson RF, Hampton MB, Winterbourn CC. Reactions of superoxide with myeloperoxidase. Biochemistry. 2007;46:4888–4897. [PubMed: 17381162]
16. Kettle AJ, Winterbourn CC. Superoxide modulates the activity of myeloperoxidase and optimizes the production of hypochlorous acid. Biochem J. 1988;252:529–536. [PubMed: 2843172]
17. Green JN, Chapman ALP, Bishop CJ, Winterbourn CC, Kettle AJ. Neutrophil granule proteins generate bactericidal ammonia chloramine on reaction with hydrogen peroxide. Free Radic Biol Med. 2017;113:363–371. [PubMed: 29055823]
18. Winterbourn CC, Kettle AJ, Hampton MB. Reactive Oxygen Species and Neutrophil Function. Annu Rev Biochem. 2016;85:765–792. [PubMed: 27050287]
19. Kettle AJ, Winterbourn CC. Myeloperoxidase: Structure and Function of the Green Heme Peroxidase of Neutrophils. In: Heme Peroxidases. 4 ed.: the Royal Society of Chemistry; 2016:272–308.
20. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. Immunol Rev. 2007;219:88–102. [PubMed: 17850484]
21. Nauseef WM. The phagocyte NOX2 NADPH oxidase in microbial killing and cell signaling. Curr Opin Immunol. 2019;60:130–140. [PubMed: 31302569]
22. Odeberg H, Olsson I. Microbicidal mechanisms of human granulocytes: synergistic effects of granulocyte elastase and myeloperoxidase or chymotrypsin-like cationic protein. Infect Immun. 1976;14:1276–1283. [PubMed: 12111]
23. Kobayashi SD, DeLeo FR. Role of neutrophils in innate immunity: a systems biology-level approach. Wiley Interdiscip Rev Syst Biol Med. 2009;1(3):309–333. [PubMed: 20836000]
24. Kobayashi SD, Malachowa N, DeLeo FR. Influence of Microbes on Neutrophil Life and Death. Frontiers in cellular and infection microbiology. 2017;7:159. [PubMed: 28507953]
25. Kennedy AD, DeLeo FR. Neutrophil apoptosis and the resolution of infection. Immunol Res. 2009;43:25–61. [PubMed: 19066741]
26. Rungelrath V, Porter AR, Malachowa N, et al. Further Insight into the Mechanism of Human PMN Lysis following Phagocytosis of Staphylococcus aureus. Microbiology spectrum. 2021;9(2):e0088821. [PubMed: 34704790]
27. Wang M, Ishikawa T, Lai Y, Nallapothula D, Singh RR. Diverse Roles of NETosis in the Pathogenesis of Lupus. Front Immunol. 2022;13:895216. [PubMed: 35686129]
28. Hidalgo A, Libby P, Soehnlein O, Aramburu IV, Papayannopoulos V, Silvestre-Roig C. Neutrophil extracellular traps: from physiology to pathology. Cardiovasc Res. 2021.
29. Shim HB, Deniset JF, Kubes P. Neutrophils in homeostasis and tissue repair. Int Immunol. 2022;34(8):399–407. [PubMed: 35752158]
30. Styrt B Species variation in neutrophil biochemistry and function. J Leukoc Biol. 1989;46(1):63–74. [PubMed: 2543725]

31. Mestas J, Hughes CCW. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172:2731–2738. [PubMed: 14978070]
32. Fingerhut L, Dolz G, de Buhr N. What Is the Evolutionary Fingerprint in Neutrophil Granulocytes? International journal of molecular sciences. 2020;21(12).
33. Doeing DC, Borowicz JL, Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. BMC Clin Pathol. 2003;3(1):3. [PubMed: 12971830]
34. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. Trends Immunol. 2010;31:318–324. [PubMed: 20620114]
35. Deniset JF, Surewaard BG, Lee WY, Kubes P. Splenic Ly6G(high) mature and Ly6G(int) immature neutrophils contribute to eradication of S. pneumoniae. J Exp Med. 2017;214(5):1333–1350. [PubMed: 28424248]
36. Hoffmann K, Sperling K, Olins AL, Olins DE. The granulocyte nucleus and lamin B receptor: avoiding the ovoid. Chromosoma. 2007;116(3):227–235. [PubMed: 17245605]
37. Li Y, Chen M, Chang W. Roles of the nucleus in leukocyte migration. J Leukoc Biol. 2022.
38. Gaines P, Tien CW, Olins AL, et al. Mouse neutrophils lacking lamin B-receptor expression exhibit aberrant development and lack critical functional responses. Exp Hematol. 2008;36(8):965–976. [PubMed: 18550262]
39. Mauer AM, Athens JW, Ashenbrucker H, Cartwright GE, Wintrobe MM. LEUKOKINETIC STUDIES. II. A METHOD FOR LABELING GRANULOCYTES IN VITRO WITH RADIOACTIVE DIISOPROPYLFLUOROPHOSPHATE (DFP). J Clin Invest. 1960;39(9):1481–1486. [PubMed: 16695832]
40. Basu S, Hodgson G, Katz M, Dunn AR. Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. Blood. 2002;100(3):854–861. [PubMed: 12130495]
41. Pinheiro D, Mawhin MA, Prendecki M, Woollard KJ. In-silico analysis of myeloid cells across the animal kingdom reveals neutrophil evolution by colony-stimulating factors. Elife. 2020;9.
42. Dai XM, Ryan GR, Hapel AJ, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood. 2002;99(1):111–120. [PubMed: 11756160]
43. Yoshida H, Hayashi S, Kunisada T, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature. 1990;345(6274):442–444. [PubMed: 2188141]
44. Hibbs ML, Quilici C, Kountouri N, et al. Mice lacking three myeloid colony-stimulating factors (G-CSF, GM-CSF, and M-CSF) still produce macrophages and granulocytes and mount an inflammatory response in a sterile model of peritonitis. J Immunol. 2007;178(10):6435–6443. [PubMed: 17475873]
45. Lakshman R, Finn A. Neutrophil disorders and their management. J Clin Pathol. 2001;54(1):7–19. [PubMed: 11271792]
46. Xia J, Miller CA, Baty J, et al. Somatic mutations and clonal hematopoiesis in congenital neutropenia. Blood. 2018;131(4):408–416. [PubMed: 29092827]
47. Walkovich K, Connelly JA. Congenital Neutropenia and Rare Functional Phagocyte Disorders in Children. Hematol Oncol Clin North Am. 2019;33(3):533–551. [PubMed: 31030818]
48. van Rees DJ, Szilagyi K, Kuijpers TW, Matlung HL, van den Berg TK. Immunoreceptors on neutrophils. Semin Immunol. 2016;28(2):94–108. [PubMed: 26976825]
49. Unemo M, Seifert HS, Hook EW 3rd, Hawkes S, Ndowa F, Dillon JR. Gonorrhoea. Nat Rev Dis Primers. 2019;5(1):79. [PubMed: 31754194]
50. Swanson J, Sparks E, Zeligs B, Siam MA, Parrott C. Studies on gonococcus infection. V. Observations on in vitro interactions of gonococci and human neutrophils. Infect Immun. 1974;10(3):633–644. [PubMed: 4214776]
51. Virji M, Watt SM, Barker S, Makepeace K, Doyonnas R. The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of Neisseria meningitidis and Neisseria gonorrhoeae. Mol Microbiol. 1996;22(5):929–939. [PubMed: 8971714]

52. Gray-Owen SD, Dehio C, Haude A, Grunert F, Meyer TF. CD66 carcinoembryonic antigens mediate interactions between Opa-expressing Neisseria gonorrhoeae and human polymorphonuclear phagocytes. EMBO J. 1997;16(12):3435–3445. [PubMed: 9218786]
53. Sarantis H, Gray-Owen SD. Defining the roles of human carcinoembryonic antigen-related cellular adhesion molecules during neutrophil responses to Neisseria gonorrhoeae. Infect Immun. 2012;80(1):345–358. [PubMed: 22064717]
54. He HQ, Ye RD. The Formyl Peptide Receptors: Diversity of Ligands and Mechanism for Recognition. Molecules. 2017;22(3).
55. Wang ZG, Ye RD. Characterization of two new members of the formyl peptide receptor gene family from 129S6 mice. Gene. 2002;299(1-2):57–63. [PubMed: 12459252]
56. Bylund J, Samuelsson M, Collins LV, Karlsson A. NADPH-oxidase activation in murine neutrophils via formyl peptide receptors. Exp Cell Res. 2003;282(2):70–77. [PubMed: 12531693]
57. Li L, Chen K, Xiang Y, et al. New development in studies of formyl-peptide receptors: critical roles in host defense. J Leukoc Biol. 2016;99(3):425–435. [PubMed: 26701131]
58. Gao JL, Chen H, Filie JD, Kozak CA, Murphy PM. Differential expansion of the N-formylpeptide receptor gene cluster in human and mouse. Genomics. 1998;51(2):270–276. [PubMed: 9722950]
59. Skovbakke SL, Winther M, Gabl M, et al. The peptidomimetic Lau-(Lys-βNSpe)(6)-NH(2) antagonizes formyl peptide receptor 2 expressed in mouse neutrophils. Biochem Pharmacol. 2016;119:56–65. [PubMed: 27614010]
60. Abo A, Pick E, Hall A, Totty N, Teahan CG, Segal AW. Activation of the NADPH oxidase involves the small GTP-binding protein p21 rac1 Nature. 1991;353:668–670. [PubMed: 1922386]
61. Knaus UG, Heyworth PG, Evans T, Curnutte JT, Bokoch GM. Regulation of phagocyte oxygen radical production by the GTP-binding protein Rac 2. Science. 1991;254:1512–1515. [PubMed: 1660188]
62. Heyworth PG, Bohl BP, Bokoch GM, Curnutte JT. Rac translocates independently of the neutrophil NADPH oxidase components p47 phox and p67 phox . Evidence for its interaction with flavocytochrome b 558 J Biol Chem. 1994;269:30749–30752. [PubMed: 7982999]
63. Quinn MT, Evans T, Loetterle LR, Jesaitis AJ, Bokoch GM. Translocation of Rac correlates with NADPH oxidase activation. Evidence for equimolar translocation of oxidase components. J Biol Chem. 1993;268:20983–20987. [PubMed: 8407934]
64. Li S, Yamauchi A, Marchal CC, Molitoris JK, Quilliam LA, Dinauer MC. Chemoattractant-stimulated Rac activation in wild-type and Rac2-deficient murine neutrophils: preferential activation of Rac2 and Rac2 gene dosage effect on neutrophil functions. J Immunol. 2002;169(9):5043–5051. [PubMed: 12391220]
65. Zhang H, Sun C, Glogauer M, Bokoch GM. Human neutrophils coordinate chemotaxis by differential activation of Rac1 and Rac2. J Immunol. 2009;183(4):2718–2728. [PubMed: 19625648]
66. Miralda I, Uriarte SM, McLeish KR. Multiple Phenotypic Changes Define Neutrophil Priming. Frontiers in cellular and infection microbiology. 2017;7:217. [PubMed: 28611952]
67. Fine N, Barzilay O, Sun C, et al. Primed PMNs in healthy mouse and human circulation are first responders during acute inflammation. Blood advances. 2019;3(10):1622–1637. [PubMed: 31138591]
68. El Benna J, Dang PM, Gougerot-Pocidalo MA, Marie JC, Braut-Boucher F. p47phox, the phagocyte NADPH oxidase/NOX2 organizer: structure, phosphorylation and implication in diseases. Exp Mol Med. 2009;41(4):217–225. [PubMed: 19372727]
69. Dang PMC, Stensballe A, Boussetta T, et al. A specific p47 phox -serine phosphorylated by convergent MAPKs mediates neutrophil NADPH oxidase priming at inflammatory sites. JClinInvest. 2006;116 (7):2033–2043
70. Makni-Maalej K, Boussetta T, Hurtado-Nedelec M, Belambri SA, Gougerot-Pocidalo MA, El-Benna J. The TLR7/8 agonist CL097 primes N-formyl-methionyl-leucyl-phenylalanine-stimulated NADPH oxidase activation in human neutrophils: critical role of p47phox phosphorylation and the proline isomerase Pin1. J Immunol. 2012;189(9):4657–4665. [PubMed: 23002436]

71. Boussetta T, Gougerot-Pocidalo MA, Hayem G, et al. The prolyl isomerase Pin1 acts as a novel molecular switch for TNF-α-induced priming of the NADPH oxidase in human neutrophils. Blood. 2010;116(26):5795–5802. [PubMed: 20956805]
72. El Benna J, Hurtado-Nedelec M, Marzaioli V, Marie JC, Gougerot-Pocidalo MA, Dang PM. Priming of the neutrophil respiratory burst: role in host defense and inflammation. Immunol Rev. 2016;273(1):180–193. [PubMed: 27558335]
73. Liu M, Bedouhene S, Hurtado-Nedelec M, et al. The Prolyl Isomerase Pin1 Controls Lipopolysaccharide-Induced Priming of NADPH Oxidase in Human Neutrophils. Front Immunol. 2019;10:2567. [PubMed: 31736979]
74. Onnheim K, Bylund J, Boulay F, Dahlgren C, Forsman H. Tumour necrosis factor (TNF)-alpha primes murine neutrophils when triggered via formyl peptide receptor-related sequence 2, the murine orthologue of human formyl peptide receptor-like 1, through a process involving the type I TNF receptor and subcellular granule mobilization. Immunology. 2008;125(4):591–600. [PubMed: 18710405]
75. Liou YC, Zhou XZ, Lu KP. Prolyl isomerase Pin1 as a molecular switch to determine the fate of phosphoproteins. Trends Biochem Sci. 2011;36(10):501–514. [PubMed: 21852138]
76. Condliffe AM, Davidson K, Anderson KE, et al. Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils. Blood. 2005;106(4):1432–1440. [PubMed: 15878979]
77. Rieckmann JC, Geiger R, Hornburg D, et al. Social network architecture of human immune cells unveiled by quantitative proteomics. Nat Immunol. 2017;18(5):583–593. [PubMed: 28263321]
78. Grabowski P, Hesse S, Hollizeck S, et al. Proteome Analysis of Human Neutrophil Granulocytes From Patients With Monogenic Disease Using Data-independent Acquisition. Mol Cell Proteomics. 2019;18(4):760–772. [PubMed: 30630937]
79. Wang X, Zhao J, Cai C, et al. A Label-Free Quantitative Proteomic Analysis of Mouse Neutrophil Extracellular Trap Formation Induced by Streptococcus suis or Phorbol Myristate Acetate (PMA). Front Immunol. 2018;9:2615. [PubMed: 30555459]
80. Abdel Malik R, Zippel N, Frömel T, et al. AMP-Activated Protein Kinase α2 in Neutrophils Regulates Vascular Repair via Hypoxia-Inducible Factor-1α and a Network of Proteins Affecting Metabolism and Apoptosis. Circ Res. 2017;120(1):99–109. [PubMed: 27777247]
81. Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. Immunol Rev. 2016;273(1):11–28. [PubMed: 27558325]
82. Winstel V, Schneewind O, Missiakas D. Staphylococcus aureus Exploits the Host Apoptotic Pathway To Persist during Infection. mBio. 2019;10(6).
83. Gabay JE, Almeida RP. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. Curr Opin Immunol. 1993;5(1):97–102. [PubMed: 8452681]
84. Ganz T, Lehrer RI. Antimicrobial peptides of leukocytes. Curr Opin Hematol. 1997;4:53–58. [PubMed: 9050380]
85. Lehrer RI, Ganz T. Defensins of vertebrate animals. Curr Opin Immunol. 2002;14(1):96–102. [PubMed: 11790538]
86. Xu D, Lu W. Defensins: A Double-Edged Sword in Host Immunity. Front Immunol. 2020;11:764. [PubMed: 32457744]
87. Yang D, Chertov O, Oppenheim JJ. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). J Leukoc Biol. 2001;69(5):691–697. [PubMed: 11358975]
88. Bdeir K, Higazi AA, Kulikovskaya I, et al. Neutrophil alpha-defensins cause lung injury by disrupting the capillary-epithelial barrier. Am J Respir Crit Care Med. 2010;181(9):935–946. [PubMed: 20093642]
89. Soehnlein O, Lindbom L. Neutrophil-derived azurocidin alarms the immune system. J Leukoc Biol. 2009;85(3):344–351. [PubMed: 18955543]
90. Weiss J Bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP): structure, function and regulation in host defense against gram-negative bacteria. Biochem Soc. 2003;31:785–790.

91. Gioannini TL, Teghanemt A, Zarember K, Weiss JP. Regulation of endotoxin with host cells. JEndotoxin Research. 2003;9:401–408. [PubMed: 14733729]
92. Levy O Antimicrobial proteins and peptides: anti-infective molecules of mammalian leukocytes. JLeukocBiol. 2004;76:909–925.
93. Schultz H, Weiss J. The bactericidal / permeability-increasing protein (BPI) in infection and inflammatory disease. Clin Chim Acta. 2007;384:12–23. [PubMed: 17678885]
94. Rausch PG, Moore TG. Granule enzymes of polymorphonuclear neutrophils: a phylogenetic comparison. Blood. 1975;46:913–919. [PubMed: 173439]
95. Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM. Myeloperoxidase: a front-line defender against phagocytosed microorganisms. JLeukBiol. 2013;93:185–198.
96. Nauseef WM. Myeloperoxidase in human neutrophil host defence. Cell Microbiol. 2014;16(8):1146–1155. [PubMed: 24844117]
97. Netea MG, Joosten LA, van der Meer JW, Kullberg BJ, van de Veerdonk FL. Immune defence against Candida fungal infections. Nat Rev Immunol. 2015;15(10):630–642. [PubMed: 26388329]
98. Ermert D, Niemiec MJ, Rohm M, Glenthoj A, Borregaard N, Urban CF. Candida albicans escapes from mouse neutrophils. J Leukoc Biol. 2013;94(2):223–236. [PubMed: 23650619]
99. Cech P, Papathanassiou A, Boreux G, Roth P, Miescher PA. Hereditary myeloperoxidase deficiency. Blood. 1979;53:403–411. [PubMed: 216438]
100. Larrocha C, de Castro MF, Fontan G, Vitoria A, Fernandez-Chacon JL, Jimenez C. Hereditary myeloperoxidase deficiency: study of 12 cases. Scand J Haematol. 1982;29:389–397. [PubMed: 6296995]
101. Lehrer RI, Cline MJ. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infection. JClinInvest. 1969;48:1478–1488.
102. Moosmann K, Bojanowsky A. Rezidivierende candidiosis bei myeloperoxydasemangel. MonatsschrKinderheilkd. 1975;123:407–409.
103. Parry MF, Root RK, Metcalf JA, Delaney KK, Kaplow LS, Richar NJ. Myeloperoxidase deficiency: prevalence and clinical significance. AnnInternMed. 1981;95:293–301.
104. Diamond RD, Clark RA, Haudenschild CC. Damage to Candida albicans hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism in vitro. JClinInvest. 1980;66:908–917.
105. Cech P, Stalder H, Widmann JJ, Rohner A, Miescher PA. Leukocyte myeloperoxidase deficiency and diabetes mellitus associated with Candida albicans liver abscess. Am J Med. 1979;66(1):149–153. [PubMed: 217268]
106. Machata S, Sreekantapuram S, Hünniger K, et al. Significant Differences in Host-Pathogen Interactions Between Murine and Human Whole Blood. Front Immunol. 2020;11:565869. [PubMed: 33519798]
107. Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. J Exp Med. 1991;173:771–774. [PubMed: 1997655]
108. Bazzoni F, Cassatella MA, Laudanna C, Rossi F. Phagocytosis of opsonized yeast induces tumor necrosis factor-α mRNA accumulation and protein release by human polymorphonuclear leukocytes. J Leukoc Biol. 1991;50:223–228. [PubMed: 1856593]
109. Conlon BP, Rowe SE, Gandt AB, et al. Persister formation in Staphylococcus aureus is associated with ATP depletion. Nature microbiology. 2016;1.
110. Tamassia N, Cassatella MA, Bazzoni F. Fast and Accurate Quantitative Analysis of Cytokine Gene Expression in Human Neutrophils by Reverse Transcription Real-Time PCR. Methods Mol Biol. 2020;2087:243–260. [PubMed: 31728997]
111. Tecchio C, Micheletti A, Cassatella MA. Neutrophil-derived cytokines: facts beyond expression. Front Immunol. 2014;5:508. [PubMed: 25374568]
112. Cassatella MA. On the production of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) by human neutrophils. JLeukBiol. 2006;79:1140–1149.

113. Simons MP, Leidal KG, Nauseef WM, Griffith TS. TNF-related apoptosis-inducing ligand (TRAIL) is expressed throughout myeloid development, resulting in a broad distribution among neutrophil granules. JLeukocBiol. 2008;83(3):621–629.
114. 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. [PubMed: 10618514]
115. Tamassia N, Cassatella MA, Bazzoni F. Fast and Accurate Quantitative Analysis of Cytokine Gene Expression in Human Neutrophils by Reverse Transcription Real-Time PCR. In: Quinn MT, DeLeo FR, eds. Neutrophil Methods and Protocols. Vol 2087. Third ed. New York, NY: Humana Press; 2020:243–261.
116. Tamassia N, Bianchetto-Aguilera F, Arruda-Silva F, et al. Cytokine production by human neutrophils: Revisiting the "dark side of the moon". Eur J Clin Invest. 2018;48 Suppl2:e12952. [PubMed: 29772063]
117. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2013;110(9):3507–3512. [PubMed: 23401516]
118. Ayres JS, Schneider DS. Tolerance of infections. Annu Rev Immunol. 2012;30:271–294. [PubMed: 22224770]
119. Schneider DS, Ayres JS. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat Rev Immunol. 2008;8(11):889–895. [PubMed: 18927577]
120. Råberg L, Sim D, Read AF. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science. 2007;318(5851):812–814. [PubMed: 17975068]
121. Warren HS, Fitting C, Hoff E, et al. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis. 2010;201(2):223–232. [PubMed: 20001600]
122. Norris PAA, Kaur G, Lazarus AH. New insights into IVIg mechanisms and alternatives in autoimmune and inflammatory diseases. Curr Opin Hematol. 2020;27(6):392–398. [PubMed: 32868670]
123. Schaub A, von Gunten S, Vogel M, et al. Dimeric IVIG contains natural anti-Siglec-9 autoantibodies and their anti-idiotypes. Allergy. 2011;66(8):1030–1037. [PubMed: 21385183]
124. von Gunten S, Schaub A, Vogel M, Stadler BM, Miescher S, Simon HU. Immunologic and functional evidence for anti-Siglec-9 autoantibodies in intravenous immunoglobulin preparations. Blood. 2006;108(13):4255–4259. [PubMed: 16902148]
125. Casulli S, Topçu S, Fattoum L, et al. A differential concentration-dependent effect of IVIg on neutrophil functions: relevance for anti-microbial and anti-inflammatory mechanisms. PLoS One. 2011;6(10):e26469. [PubMed: 22065996]
126. Altznauer F, von Gunten S, Späth P, Simon HU. Concurrent presence of agonistic and antagonistic anti-CD95 autoantibodies in intravenous Ig preparations. J Allergy Clin Immunol. 2003;112(6):1185–1190. [PubMed: 14657880]
127. Schneider C, Wicki S, Graeter S, et al. IVIG regulates the survival of human but not mouse neutrophils. Sci Rep. 2017;7(1):1296. [PubMed: 28465620]
128. Bonne-Année S, Kerepesi LA, Hess JA, et al. Extracellular traps are associated with human and mouse neutrophil and macrophage mediated killing of larval Strongyloides stercoralis. Microbes and infection / Institut Pasteur. 2014;16(6):502–511.
129. Griener TP, Mulvey GL, Marcato P, Armstrong GD. Differential binding of Shiga toxin 2 to human and murine neutrophils. J Med Microbiol. 2007;56(Pt 11):1423–1430. [PubMed: 17965340]
130. Linnemann LC, Schaible UE, Dallenga TK. Evaluation of Myeloperoxidase as Target for Host-Directed Therapy in Tuberculosis In Vivo. International journal of molecular sciences. 2022;23(5).
131. Davies MJ, Hawkins CL. The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. Antioxid Redox Signal. 2020;32(13):957–981. [PubMed: 31989833]
132. Brennan ML, Anderson MM, Shih DM, et al. Increased atherosclerosis in myeloperoxidase-deficient mice. JClinInvest. 2001;107(4):419–430.
133. Nauseef WM. The proper study of mankind. JClinInvest. 2001;107(4):401–403.

134. Eruslanov EB, Singhal S, Albelda SM. Mouse versus Human Neutrophils in Cancer: A Major Knowledge Gap. Trends in cancer. 2017;3(2):149–160. [PubMed: 28718445]

135. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565–1570. [PubMed: 21436444]

136. Quail DF, Amulic B, Aziz M, et al. Neutrophil phenotypes and functions in cancer: A consensus statement. J Exp Med. 2022;219(6).

137. Ozel I, Duerig I, Domnich M, Lang S, Pylaeva E, Jablonska J. The Good, the Bad, and the Ugly: Neutrophils, Angiogenesis, and Cancer. Cancers (Basel). 2022;14(3).

138. Bui TM, Yalom LK, Sumagin R. Tumor-associated neutrophils: orchestrating cancer pathobiology and therapeutic resistance. Expert Opin Ther Targets. 2021;25(7):573–583. [PubMed: 34236924]

139. Que H, Fu Q, Lan T, Tian X, Wei X. Tumor-associated neutrophils and neutrophil-targeted cancer therapies. Biochim Biophys Acta Rev Cancer. 2022;1877(5):188762. [PubMed: 35853517]

140. Woolhouse ME, Webster JP, Domingo E, Charlesworth B, Levin BR. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nat Genet. 2002;32(4):569–577. [PubMed: 12457190]

141. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr.Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–661. [PubMed: 26016486]

142. Lu T, Porter AR, Kennedy AD, Kobayashi SD, Deleo FR. Phagocytosis and Killing of Staphylococcus aureus by Human Neutrophils. J Innate Immun. 2014;6(5):639–649. [PubMed: 24713863]

143. Dinauer MC. Neutrophil Defects and Diagnosis Disorders of Neutrophil Function: An Overview. Methods Mol Biol. 2020;2087:11–29. [PubMed: 31728980]

144. Marciano BE, Spalding C, Fitzgerald A, et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis. 2015;60(8):1176–1183. [PubMed: 25537876]

145. Albrich JM, Hurst JK. Oxidative inactivation of Escherichia coli by hypochlorous acid. Rates and differentiation of respiratory from other reaction sites. FEBS Lett. 1982;144:157–161. [PubMed: 6286355]

146. Greenlee-Wacker M, DeLeo FR, Nauseef WM. How methicillin-resistant Staphylococcus aureus evade neutrophil killing. Curr Opin Hematol. 2014.

147. Holtfreter S, Radcliff FJ, Grumann D, et al. Characterization of a mouse-adapted Staphylococcus aureus strain. PLoS One. 2013;8(9):e71142. [PubMed: 24023720]

148. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. Nature reviews Microbiology. 2015;13(9):529–543. [PubMed: 26272408]

149. de Haas CJC, Veldkamp KE, Peschel A, et al. Chemotaxis inhibitory protein of Staphylococcus aureus , a bacterial antiinflammatory agent. J Exp Med. 2005;199:687–695.

150. Rooijakkers SHM, Ruyken M, Roos A, et al. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol. 2005;6(9):920–927. [PubMed: 16086019]

151. Jongerius I, Köhl J, Pandey MK, et al. Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med. 2007;204(10):2461–2471. [PubMed: 17893203]

152. Spaan AN, Henry T, van Rooijen WJ, et al. The staphylococcal toxin panton-valentine leukocidin targets human c5a receptors. Cell Host Microbe. 2013;13(5):584–594. [PubMed: 23684309]

153. Spaan AN, van Strijp JAG, Torres VJ. Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nature reviews Microbiology. 2017;15(7):435–447. [PubMed: 28420883]

154. Fromageau A, Gilbert FB, Prévost G, Rainard P. Binding of the Staphylococcus aureus leucotoxin LukM to its leucocyte targets. Microb Pathog. 2010;49(6):354–362. [PubMed: 20624452]

155. Spaan AN, Schiepers A, de Haas CJ, et al. Differential Interaction of the Staphylococcal Toxins Panton-Valentine Leukocidin and γ-Hemolysin CB with Human C5a Receptors. J Immunol. 2015;195(3):1034–1043. [PubMed: 26091719]

156. Allison M, Mishra N, Geisbrecht BV. Structure, function, and mechanistic insights into a novel family of myeloperoxidase inhibitory proteins expressed by staphylococci. In: Hawkins CL, Nauseef WM, eds. Mammalian Heme Peroxidases. Boca Raton, FL: CRC Press; 2022:307–321.

157. de Jong NWM, Ramyar KX, Guerra FE, et al. Immune evasion by a staphylococcal inhibitor of myeloperoxidase. Proc Natl Acad Sci U S A. 2017;114(35):9439–9444. [PubMed: 28808028]

158. de Jong NWM, Ploscariu NT, Ramyar KX, et al. A structurally dynamic N-terminal region drives function of the staphylococcal peroxidase inhibitor (SPIN). J Biol Chem. 2018;293(7):2260–2271. [PubMed: 29306874]

159. Rosen H Editorial: of mice and men--yet again. J Leukoc Biol. 2013;94(2):210–212. [PubMed: 23906966]

160. Davis MM. A prescription for human immunology. Immunity. 2008;29:1–4. [PubMed: 18631448]

161. Medetgul-Ernar K, Davis MM. Standing on the shoulders of mice. Immunity. 2022;55(8):1343–1353. [PubMed: 35947979]

162. Davies MJ. Reactivity of peroxidase-derived oxidants with proteins, glycoproteins, and proteoglycans. In: Hawkins CL, Nauseef WM, eds. Mammalian Heme Peroxidases. Boca Raton, FL: CRC Press; 2022:53–78.

163. Hawkins CL. Reactivity of hypochlorous acid (HOCl) with nucleic acids, RNA, and DNA. In: Hawkins CL, Nauseef WM, eds. Mammalian Heme Peroxidases. Boca Raton, FL: CRC Press; 2022:79–94.

164. Pike DP, Ford DA. Reactivity of peroxidase oxidants with lipids: the generation of biologically important modified lipids. In: Hawkins CL, Nauseef WM, eds. Mammalian Heme Peroxidases. Boca Raton: CRC Press; 2022:95–114.

165. Hallberg LAE, Thorsen NW, Hartsema EA, Hägglund PM, Hawkins CL. Mapping the modification of histones by the myeloperoxidase-derived oxidant hypochlorous acid (HOCl). Free Radic Biol Med. 2022.

166. Shearer HL, Hampton MB, Dickerhof N. Bactericidal Activity of the Oxidants Derived from Mammalian Heme Peroxidases. In: Hawkins CL, Nauseef WM, eds. Mammalian Heme Peroxidases. Boca Raton, London, New York: CRC Press; 2022:171–188.

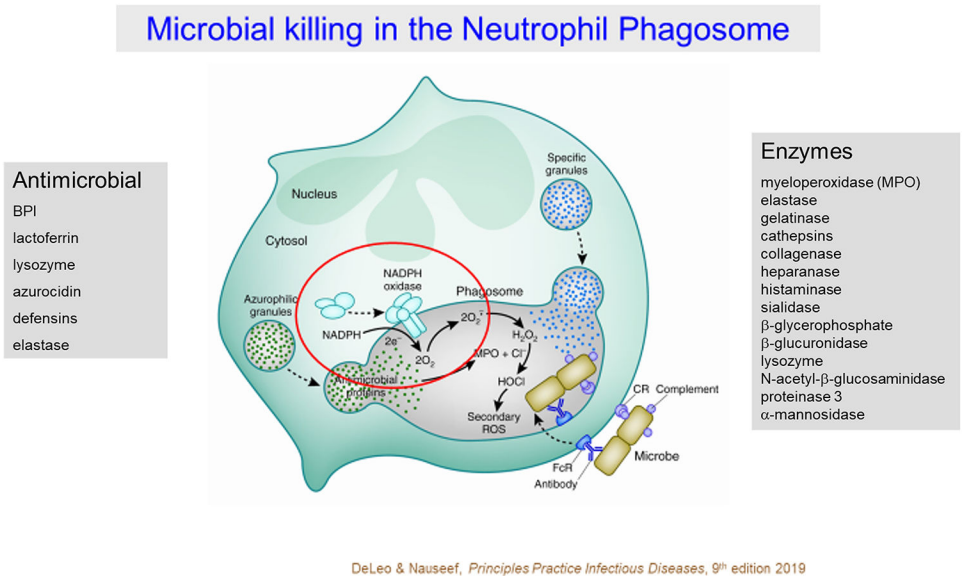

Figure 1. Microbicidal action in human neutrophil phagosomes

Antimicrobial events in the phagosomes of human neutrophils reflect the synergistic actions of granule proteins and oxidants. Granule proteins synthesized during granulocyte development in the bone marrow are packaged into one of several granule compartments (*e.g.* azurophilic and specific granules) and released into phagosomes by fusion with the nascent phagosome. Granule proteins include enzymes and proteins with direct bactericidal activity. Coincident with phagocytosis, the multicomponent NADPH oxidase assembles on the nascent phagosome and shuttles electrons from cytoplasmic NADPH to molecular O_2 in the lumen of the phagosome, thereby generating superoxide anion (O_2^-). Dismutation ofO_2^- produces H_2O_2 that in turn reacts with MPO to form HOCl. Most of the oxygen consumed by stimulated human neutrophils can be recovered as HOCl. Interactions among the granule proteins and between granule proteins and oxidants creates an environment toxic, and often lethal, for a wide variety of microbes [Figure from [8]].

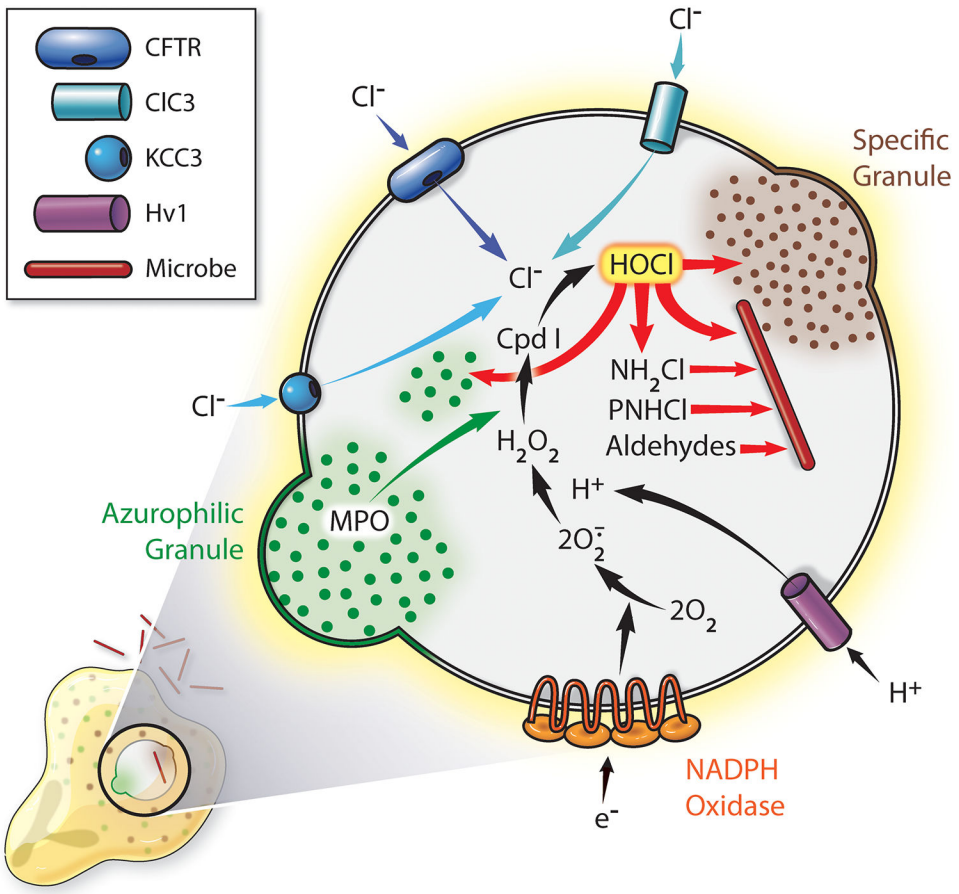

Figure 2. MPO-related events in phagosomes of human neutrophils

At the onset of phagocytosis, granules and secretory vesicles fuse with nascent phagosomes, delivery granule proteins into the phagosomal lumen and inserting functionally important membrane proteins, including the voltage-gated proton channel Hv1 and chloride channels ClC3, KCC3, and cystic fibrosis transmembrane conductance regular (CFTR), into the membrane of the phagosomes. Granule fusion delivers MPO, and CFTR supports transport of Cl^- from cytoplasm into the phagosome. H_2O_2 generated by the NADPH oxidase, reacts with MPO to form Compound I (Cpd I), which mediates the two-electron oxidation of Cl^- to Cl^+ and the production of HOCl. HOCl reacts with a wide variety of biomolecules, including proteins, lipids, carbohydrates, histones, and nucleic acids [162-165]. With respect to proteins, HOCl reacts most readily with sulfur-containing residues, notably methionine and cysteine, and generates monochloramines (NH_4Cl), protein chloramines (PNHCl), aldehydes, and other products, some of which exert sustained antimicrobial action [reviewed in [166]]. It is important to recognize that HOCl does not react preferentially with microbial proteins rather than host targets; all susceptible substrates are attacked regardless of their origin. Given that host proteins predominate in the phagosomes, most of the chlorinated and oxidized proteins are of host origin. [Figure from [96]]

Human					Mouse						
Receptor	Alternative names	Gene	Cytoplasmic motif or adaptor	Signaling	Receptor	Alternative names	Gene	Cytoplasmic motif or adaptor	Signaling	Additional information	References
Fcγ receptors (ligand: Fc domain of Immunoglobulin G)											
FcγRI	CD64	FCGR1A	FcRγ	Syk	FcγRIIb	CD32, Ly-17, Fcgr2b	Fcgr2	ITIM	SHIP	hFcγRI: inducible upon activation.	80, 83, 84, 86
FcγRIIa/c	CD32a/c	FCGR2A/C	ITAM	Syk	FcγRIII	CD16, FcRIII	Fcgr3	ITAM	Syk		
FcγRIIIb	CD16b	FCGR3B	GPI-linked		FcγRIV	Fcgr4, Fcgr3a	Fcgr4	ITAM	Syk		
Fcα receptors (ligand: Fc domain of Immunoglobulin A)											
FcαR	CD89	FCAR	FcRγ	Syk, SHP-1	-					Can mediate inhibitory signaling via SHP-1.	70, 71, 87
CD300 (ligand: lipids)											
CD300a	CMRF-35H, IRC1, IRC2, IRp60	CD300A	3 ITIMs	SHP-1	CLM-8	CLM-8, LMIR-1, MAIR-I	Cd300a	2 ITIMs	SHP-1	May associate with SHP-2 and SHIP as well. Contains additional Grb2 and PI3K binding sites. hCD300f is expressed on low levels on resting neutrophils.	88, 89, 91-94, 97, 98
CD300f	CD300lf, IgSF13, IREM-1	CD300LF	2 ITIMs	SHP-1, SHP-2	CLM-7	LMIR-5, CD300b, mIREM3	Cd300lb	DAP12	Syk		
CD300b	CD300lb, IREM-3	CD300LB	DAP12	Grb2, Syk	CLM-1	DlgR2, LMIR-3, MAIR-V	Cd300lf	2 ITIMs	SHP-1, SHP-2, PI3K, Grb2		
CD300c	CMRF-35A	CD300C	DAP12, FcRγ	Syk	CLM-5	MAIR-IV, LMIR-4	Cd300ld	FcRγ	Syk		
Siglecs (ligand: sialic acids in various linkages)											
Siglec-3	CD33, gp67	CD33	2 ITIMs	SHP-1, SHP-2	Siglec-3	-	Cd33	ITIM-like	SHP-1, SHP-2		99, 100, 102, 104
Siglec-5	CD170, CD33L2, OBBP2	SIGLEC5	2 ITIMs	SHP-1, SHP-2	Siglec-E	Siglec12, Siglec5, Siglec1	Siglec12	ITIM	SHP-1, SHP-2		
Siglec-9	CD329	SIGLEC9	2 ITIMs	SHP-1, SHP-2							
Siglec-14	-	SIGLEC14	DAP12	Syk							
CEACAM (ligand: CEACAM molecules, Opa)											
CEACAM-1	CD66a, BGP	CEACAM1	2 ITIMs	SHP-1	CEACAM-1	Bgp-1, CD66a, MHV-R	Ceacam-1	2 ITIMs	SHP-1		105, 109, 115, 117, 175
CEACAM-3	CD66d, CGM1	CEACAM3	ITAM-like	Syk							
CEACAM-4	CGM7	CEACAM4	ITAM-like	PI3K, Nck							
CEACAM-6	CD66c, NCA	CEACAM6	GPI-linked								
CEACAM-8	CD66b, CD67, CGM6	CEACAM8	GPI-linked								
SIRP (ligand: SIRPα: CD47; SIRPβ: unknown)											
SIRPα	BIT, CD172A, PTPNS1, SHPS1	SIRPA	2 ITIMs	SHP-1, SHP-2	SIRPα	Bit, Myd1, Ptpns1, Shps1	Sirpa	ITIM	SHP-1, SHP-2		120, 121
SIRPβ1	CD172B	SIRPB1	DAP12	Syk							
SIRPβ2	PTPN1L, PTPNS1L3	SIRPB2	DAP12	Syk							
ILT & PIR (ligand: HLA class I molecules)											
ILT-1	LIR-7, CD85H	LILRA2	ITAM-FcRγ	Syk	PIR-A	-	Pira	FcRγ	Syk		78, 132-134, 136, 137
ILT-4	LIR-2, CD85D, MIR-10	LILRB2	4 ITIMs	SHP-1, SHP-2, SHIP	PIR-B	LIR-3	Lilrb3	3 ITIMs	SHP-1, SHP-2		
ILT-5	LIR-3, CD85A	LILRB3	4 ITIMs	SHP-1, SHP-2, SHIP							
ILT-3	LIR-5, CD85K	LILRB4	2 ITIMs	SHP-1, SHP-2, SHIP							
PILR (sialylated proteins, CD99)											
PILRα	FDF03	PILRA	2 ITIMs	SHP-1, SHP-2	PILRα	FDF03	Pilra	2 ITIMs	SHP-1, SHP-2	Binds SHP-2 with greater affinity.	139, 140, 143
PILRβ	FDFACT	PILRB	DAP12	Syk	PILRβ	Fdfact	Pilrb	DAP12	Syk		
TREM (ligand: unknown)											
TREM-1	CD354	TREM1	DAP12	Syk	TREM-1	CD354	Trem1	DAP12	Syk		145, 150, 151, 154
TLT-2	-	TREML2	?		TLT-2	-	Treml2	?			
CD200R (human ligand: CD200 (OX2); mouse ligand: unknown)											
CD200R	CRTR2, MOX2R, OX2R	CD200R1	NPXY motif	Dok-1, Dok-2, SHIP	CD200R	Mox2	Cd200	NPXY motif	Dok-1, Dok-2, SHIP	Contains no ITIM; but is still inhibitory. Recruits Dok-1 and -2 that in turn recruit SHIP.	31, 156, 158, 59
SIRL (ligand: unknown)											
SIRL-1	-	VSTM1	2 ITIMs	SHP-1, SHP-2	?						162, 163
LAIR-1 (ligand: collagens)											
LAIR-1	CD305	LAIR1	2 ITIMs	SHP-1, SHP-2, Csk	mLAIR-1	CD305	Lair1	2 ITIMs	SHP-2	Inducible upon activation. hLAIR-1: SHP-1 predominantly mediates inhibitory signaling.	165, 168-170

Figure 3. Immunoreceptors on human and murine neutrophils

Expression of members of the immunoreceptor family on human and murine neutrophils, including those with ITAM (green), ITIM (red), or other (gray) signaling pathways. Table originally published in [48]

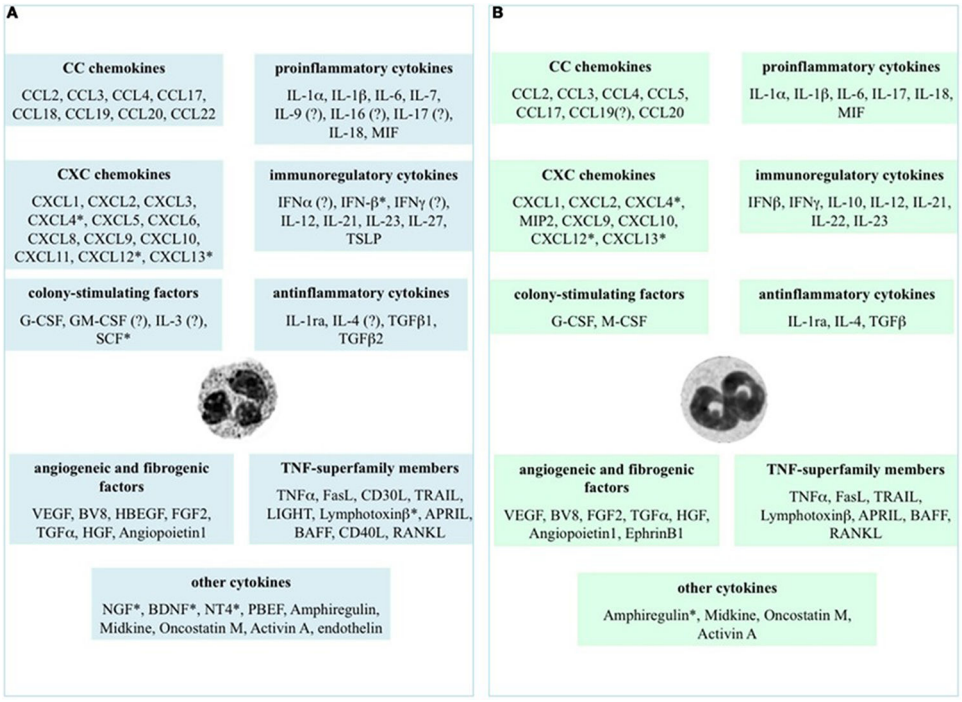

Figure 4. Chemokines and cytokines

Chemokines and cytokines potentially expressed or produced by human (panel A) and murine (panel B) neutrophils. The evidence for expression came from assessing gene expression, immunochemical staining, protein quantitation by enzyme-linked immunoabsorbant assays as well as some biological assays. The * indicates that the evidence was limited to mRNA detection and ? indicates data that are controversial at the time of this publication (2014) Table originally published in [111]

Leukocidin			Receptors		Specificity*	Species (activity)‡
Name	Cognate pairs	Non-cognate pairing§	Myeloid receptors	Erythroid receptors	Target cells	Human, rabbit, mouse
PVL	LukS–PV (S type) and LukF–PV (F type)	• LukD • HlgB	• C5aR1 • C5aR2		• Neutrophils • Monocytes • Macrophages	• Human (high) • Rabbit (medium) • Mouse (none)
LukED	LukE (S type) and LukD (F type)	• LukF–PV • HlgB	• CCR5 • CXCR1 • CXCR2	DARC‖	• Neutrophils • Monocytes • Macrophages • Dendritic cells • T cells • Erythrocytes • NK cells	• Human (high) • Mouse (high)
HlgAB¶	HlgA (S type) and HlgB (F type)	• LukF–PV • LukD	• CCR2 • CXCR1 • CXCR2	DARC‖	• Neutrophils • Monocytes • Macrophages • Erythrocytes	• Human (high) • Mouse (medium)
HlgCB	HlgC (S type) and HlgB (F type)	• LukF–PV • LukD	• C5aR1 • C5aR2		• Neutrophils • Monocytes • Macrophages	• Human (high) • Rabbit (medium) • Mouse (low)
LukAB (also known as LukGH)	LukA (LukH; S type) and LukB (LukG; F type)	None	CD11b		• Neutrophils • Monocytes • Macrophages • Dendritic cells	• Human (high) • Rabbit (medium) • Mouse (low)

Figure 5. Staphylococcal bicomponent leukotoxins

Human-adapted *S.aureus* produce distinct leukocidins that target myeloid and erythroid receptors with species-specific differences in susceptibility as demonstrated by experimental data (*). § designates potential pairs of the S component with an F component from another leukocidin; II indicates that DARC makes erythrocytes susceptible to the actions of LukED and HlgAB; ¶ HlgAB engages CCR2 and DARC in both human and murine cells, but only human CXCR1 and CXCR2. C5aR1, C5a anaphylatoxin chemotactic receptor 1; CCR2, CC-chemokine receptor 2; CXCR1, CXC chemokine receptor 1; DARC, Duffy antigen receptor for chemokines; HlgA, γ-haemolysin A; LukA, leukocidin A; NK cells, natural killer cells; PVL, Panton–Valentine leukocidin. Table originally published in [153]

*Shown are the leukocytes for which there are experimental data in the literature.

‡On the basis of published susceptibility of tested primary cells. §Potential non-cognate pairing of the S component with an F component of another leukocidin. which results in functional mixed pores or inactive hybrid complexes, depending on the pair. ‖DARC renders erythrocytes susceptible to the haemolytic activity of LukED and HlgAB. ¶HlgAB targets both human and murine CCR2 and DARC, but only human CXCRl and CXCR2. CSaRl. C5a anaphylatoxin chemotactic receptor 1; CCR2, CC-chemokine receptor 2; CXCRl. CXC chemokine receptor 1; DARC. Duffy antigen receptor for chemokines: HlgA, y-haemolysin A: LukA. leukocidin A; NK cells, natural killer cells: PVL. Panton-Valentine leukocidin.