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Towards a comprehensive understanding of the role of neutrophils in innate immunity: a systems biology-level approach

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Abstract

The innate immune system is the first line of host defense against invading microorganisms. Polymorphonuclear leukocytes (PMNs or neutrophils) are the most abundant leukocyte in humans and essential to the innate immune response against invading pathogens. Compared to the acquired immune response, which requires time to develop and is dependent on previous interaction with specific microbes, the ability of neutrophils to kill microorganisms is immediate, non-specific, and not dependent on previous exposure to microorganisms. Historically, studies of PMN-pathogen interaction focused on the events leading to killing of microorganisms, such as recruitment/chemotaxis, transmigration, phagocytosis, and activation, whereas post-phagocytosis sequelae were infrequently considered. In addition, it was widely accepted that human neutrophils possessed limited capacity for new gene transcription and thus, relatively little biosynthetic capacity. This notion has changed dramatically within the past decade. Further, there is now more effort directed to understand the events occurring in PMNs after killing of microbes. Herein we review the systems biology-level approaches that have been used to gain an enhanced view of the role of neutrophils during host-pathogen interaction. We anticipate that these and future systems-level studies will ultimately provide information critical to our understanding, treatment, and control of diseases caused by pathogenic microorganisms.

Keywords

Neutrophil; phagocytosis; microarray; inflammation; apoptosis

[Introduction]

Polymorphonuclear leukocytes (PMNs or neutrophils) are the most abundant cellular component of the host immune system and primary mediators of the innate immune response to invading microorganisms. The ability of neutrophils to rapidly kill invading microbes is indispensable for maintaining host health. Defects in neutrophil microbicidal processes or an overall decrease in PMN abundance are deleterious to human health and often result in severe and recurrent infections. In this regard, the neutrophil has garnered much respect and notoriety for its principal role as executioner. Although the net result of neutrophil function is killing of invading microorganisms, the steps leading up to the final act require careful orchestration and yield many options based on complex signal transduction events. Inasmuch as neutrophils have significant potential to damage host cells and tissues, PMNs ultimately have go-no-go decisions that are important for the resolution

of the inflammatory response. Therefore, an enhanced understanding of molecular signaling pathways induced in PMNs during these processes is critical for improving treatment and outcome of infectious diseases. To that end, genome-wide approaches have yielded significant insight into essential neutrophil processes such as granulopoiesis, host defense, and resolution of the inflammatory response (Fig. 1).

The inception of systems biology as an independent field of study is a relatively recent occurrence. The goal of systems level studies is to integrate comprehensive biological data sets from diverse experimental systems to understand complex interactions at the molecular level. In more simplistic terms, systems-level studies provide the prediction of phenotype changes in biological systems from a defined stimulus. In this regard, cells of the innate immune system (leukocytes) are highly amenable to systems biology approaches. Although leukocytes often overlap in function, there are distinct differences between cell types such as those pertaining to turnover and production of immunoregulators. Each type of leukocyte has a unique role in the immune response and generates lineage-specific gene expression patterns. Thus, a unified approach using a single representative cell type is unrealistic. Nevertheless, systems biology-level studies have provided important insight into the complex signal transduction pathways in both neutrophils and cells of the monocyte lineage. Herein, we review primary neutrophil functions and the genome-wide approaches used to better understand these processes. Collectively, these studies are an important first step toward developing a systems-level understanding of host-pathogen interactions.

Granulocyte development

Do human neutrophils possess capacity for new gene transcription?

Inasmuch as the primary function of neutrophils is to protect the host from invading pathogens, significant emphasis has been placed on processes relating to microbicidal activity. Neutrophils are able to initiate the processes of phagocytosis, degranulation, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent killing without new synthesis of proteins [1, 2]. However, Kasprisin and Harris demonstrated that new gene transcription and protein synthesis are required to maintain full capacity for human neutrophil phagocytosis and associated bactericidal activity [2, 3]. Despite these early studies, it is mistakenly assumed that mature neutrophils possess limited transcriptional potential—in fact, those isolated from venous blood constitutively express ~12,000 transcripts [4]. Transcriptional processes occurring during granulopoiesis have been investigated intensely. The transcriptional regulation of neutrophil development is mediated, in part, by the coordinated activities of several transcription factors and repressors. For example, CCAAT/enhancer binding protein alpha (C/EBP α), PU.1, RAR, CBF, and c-MYB are involved in early granulopoiesis; C/EBP ϵ , PU.1, SP1, CDP, HOXA10, signal transducer and activator of transcription (STAT)1, STAT3, STAT5 and GFI-1 are involved in terminal neutrophil differentiation [5, 6]. The PMN nucleus undergoes progressive chromatin condensation during the post-mitotic phase of neutrophil differentiation. In addition, mature cells contain significant quantities of heterochromatin [7], a property that suggests transcriptional repression. Conversely, early studies of RNA metabolism in mature PMNs demonstrated increased RNA biosynthesis following phagocytosis [8, 9]. It is now clear that mature neutrophils express several transcription factors that in turn regulate the transcription of numerous genes involved in a diversity of important cellular processes. Elucidation of mechanisms driving the transcriptional regulation of molecular pathways involved in PMN microbicidal activity, inflammation, and apoptosis are currently areas of intense investigation. Mature neutrophils are exposed to numerous stimuli in the context of the inflammatory milieu. In general, exposure of PMNs to pro-inflammatory cytokines such as interferon (IFN)- γ and granulocyte/macrophage-colony stimulating factor (GM-CSF) induces activation of STAT transcription factor members whereas tumor necrosis factor

(TNF)- α and interleukin (IL)-1 β induce those of the NF κ B family. NF κ B activation is also promoted by lipopolysaccharide (LPS), platelet-activating factor, N-formyl peptides (e.g., fMLF), leukotriene B₄, reactive oxygen species (ROS), phagocytosis, and apoptosis [6]. κ B-binding motifs are present in the promoter regions of several genes encoding inflammatory mediators including IL-1 α/β , TNF- α , IL-8, IL-12, CCL4, CCL20, and CXCL1. In addition, NF κ B inhibition of neutrophil apoptosis in hypoxic environments is further regulated by the hypoxia-inducible factor (HIF) transcriptional complex [10]. Inasmuch as apoptosis is a complex process, additional transcription factors are likely involved in regulating this critical neutrophil process. For example, the FOXO forkhead subfamily member FOXO3A has recently been shown to directly suppress neutrophil *FASL* transcription, thereby limiting the FAS-mediated death receptor pathway of apoptosis [11]. In addition, recent evidence suggests that epigenetic features and chromosomal organization may play a role in the transcriptional regulation of neutrophil development [12]. Although progress has been made, we are only beginning to appreciate the involvement of transcriptional regulation of the complexity of signal transduction networks that govern neutrophil function. Not surprisingly, several recent studies suggest that complex post-transcriptional / translational systems serve as additional checkpoints to regulate neutrophil participation in the innate immune response. For example microRNA-mediated RNA interference [13] and signal-dependent translation of constitutive mRNA [14, 15] are emerging as critical regulatory elements in neutrophil development and function. Thus, there is no question that neutrophils possess significant capacity for new gene transcription.

Transcriptome analyses of granulocyte development

Cells of the immune system originate from common hematopoietic progenitor cells in bone marrow. The greatest percentage of hematopoiesis is committed to production of neutrophils and granulocyte precursors comprise ~60% of the nucleated cells in bone marrow [7]. Human PMN turnover is $50\text{--}340 \times 10^7$ cells/kg/day ($\sim 0.4\text{--}3 \times 10^{11}$ cells per day in a 75 kg person) [16, 17]. The plasticity of the acute inflammatory response depends, in part, on the ability of the host to regulate neutrophil production based on demand. Thus, regulation and execution of granulopoiesis are highly structured and complex processes. Granulocyte differentiation and development are influenced by the concerted activities of myeloid colony stimulating factors such as granulocyte colony-stimulating factor (G-CSF) and GM-CSF [18]. PMN maturation occurs in distinct niches within the bone marrow microenvironment prior to migration towards venous sinuses. As myeloid precursors become mature neutrophils, they sequentially acquire features necessary for microbicidal activity including receptors for phagocytosis and signaling, granule components, and NADPH oxidase proteins. PMN maturation is also accompanied by morphological changes (nuclear segmentation) and increased motility and chemotactic responsiveness. This post-mitotic maturation time is approximately 6–8 days under basal conditions [19]. Mature PMNs are released into the bloodstream where they circulate with a typical half-life of 6–8 hours [16, 17, 20, 21]. Morphological analysis of circulating neutrophils indicates a low basal level of immature cells, e.g., band cells are ~1–3 % of peripheral leukocytes [20]. However, induction of neutrophilia during acute inflammation accelerates recruitment from bone marrow. Depletion of the stored basal reserves results in a decrease in post-mitotic maturation time to 3–4 days [22] and elicits additional release of functionally competent immature forms [23]. Granulopoiesis and PMN egress from bone marrow is enhanced by several immunomodulatory factors including GM-CSF, G-CSF, IL-8, and glucocorticoids [18, 24, 25]. Although progress has been made, the specific mechanisms for leukocytosis are not completely understood.

Early systematic studies of myelopoiesis focused on transcriptome and proteome analysis using in vitro-differentiated murine cell lines [26–28]. Importantly, these studies provided

the fundamental observation that a substantial proportion of neutrophil protein change is a consequence of mRNA transcription and serve as the premise for additional PMN microarray studies. More recently, transcriptome analyses have focused on immature neutrophils isolated from human bone marrow [29–31] or differentiating human leukemic HL-60 cells [32]. It is noteworthy that differentiation from common myeloid progenitor cells yield lineage-specific gene expression patterns [29, 33]. Here, we provide a few examples of systems-level studies on granulopoiesis and also refer the reader to a recent review on the topic [34]. In general, these studies demonstrate that PMN receptors for numerous inflammatory mediators are up-regulated throughout development, suggesting increased functional capacity upon maturation. In addition, the study by Theilgaard-Monch et al. identified 16 previously unknown granule protein candidates including protease inhibitors, proteases, signaling molecules, and acute-phase proteins [35]. These authors also found that there was temporal regulation of transcripts encoding granule proteins, consistent with the “targeting by timing” hypothesis put forth for proteins of granule subsets [36]. By contrast, gene expression patterns in mature PMNs indicate a decreased capacity for synthesis of granule proteins [29]. Functional studies with these proteins may provide additional insight into neutrophil microbicidal activity in mature cells and the process of granule exocytosis.

Neutrophils in the inflammatory response

Recruitment and priming

Following release from bone marrow, neutrophils circulate in the vasculature prior to extravasation, i.e., movement of cells out of circulation to peripheral tissue. Rapid recruitment of neutrophils to tissues is fundamentally important to the innate immune system. This multi-step process involves mobilization of PMNs from bone marrow reserves, accelerated hematopoiesis and recruitment from marginated pools in response to host- and pathogen-derived chemotactic stimuli. PMN migration from the vasculature to the extravascular milieu is dependent on receptor-mediated contact with endothelia of post capillary venules and signals received through soluble mediators. Marginating granulocytes slowly roll along the endothelial surface through interactions of a family of C-type lectin glycoproteins known as selectins [37]. L-selectin is constitutively expressed on the surface of neutrophils and mediates a low-affinity adhesive interaction. Stimulation of endothelial cells leads to the transient expression of both E- and P-selectin. In the presence of inflammatory mediators, adherence rapidly switches to a high-affinity interaction that is mediated by activation of PMN $\beta 2$ -integrins and endothelial cell intracellular adhesion molecule-1 (ICAM-1) and ICAM-2. Once firmly bound, several neutrophil surface molecules, including CD31 [38], CD54 [39], CD44 [40], and CD47 [41], facilitate transmigration through the endothelium into tissues.

The ability of neutrophils to localize to sites of infection is a key component of the acute inflammatory response. Neutrophil migration through tissue is influenced by chemoattractants produced by the host during inflammation and pathogen-derived factors. There are numerous host-derived factors that enhance the recruitment of neutrophils. One of the most potent neutrophil chemoattractants is IL-8 [42, 43]. This chemokine is produced in response to pro-inflammatory stimuli by a diversity of cell types including mononuclear phagocytes, neutrophils, mast cells, epithelial cells, keratinocytes, fibroblasts, and endothelial cells. Neutrophils are also recruited efficiently by leukotrienes and prostaglandins generated exogenously at sites of infection and by the complement component C5a [44]. Many products of bacteria, such as fMLF, peptidoglycan, and phenol-soluble modulins (PSMs), are chemoattractants and contribute directly to PMN recruitment [45, 46]. In addition to facilitating neutrophil migration, chemoattractants can also prime PMNs for enhanced function.

Given the high level of cytotoxic molecules produced by- or contained within PMNs, it is perhaps not surprising that these cells are intimately associated with the pathogenesis of tissue injury and trauma associated with inflammatory diseases [47, 48]. In order to moderate neutrophil-mediated tissue destruction, PMN activation most appropriately occurs in regions proximal to the infected tissue [49]. However, the ability of neutrophils to respond rapidly at the infection site is essential to host defense. Thus, it is reasonable to believe that the ability of the neutrophil to reside in a state intermediate to circulating quiescence and complete activation is optimal for plasticity. To that end, neutrophil priming is a reversible process that can enhance cell functions and limit the potential for indiscriminate host tissue damage [50]. The original descriptions of PMN priming indicated that a primary agonist, typically at sub-stimulatory concentration, enhances superoxide production triggered by a second stimulus [51]. Neutrophils can be primed by numerous host factors and processes including cytokines, chemokines, growth-factors, chemotactic factors, leukotrienes, ROS, adherence, and cellular contact [50]. Although priming classically implies augmentation of superoxide-generating potential, several studies have shown that PMN exposure to typical priming agents can also promote adherence, chemotaxis, cytokine secretion, phagocytosis, degranulation, and bactericidal activity (reviewed in [52]). Many priming agents produced by microorganisms are *Toll*-like receptor (TLR) agonists and may enable enhanced surveillance and clearance of pathogens from infection sites. The specific molecular mechanisms responsible for priming are unclear, and evidence suggests that multiple pathways converge on the common endpoint of enhanced PMN ROS production. Priming typically includes mobilization of secretory vesicles and some specific granules, and secretion of cytokines, but fails to induce complete degranulation or elicit production of superoxide [53]. Enhanced ROS production in primed PMNs is facilitated by partial assembly of the NADPH-oxidase complex. However, neutrophil exposure to different priming agents may result in heterogeneous changes in the structural organization of the oxidase (described below).

Systems biology-level approaches

Chemotaxis and transmigration—Active recruitment of neutrophils to the inflammatory milieu is a sequential process, resulting in the convergence of complex signals that ultimately enhance PMN function. Microarray analysis of PMNs exposed to the potent chemoattractant fMLF revealed a number of up-regulated transcripts encoding pro-inflammatory molecules, such as *CCL2*, *CCL3*, *CCL4*, *VEGF*, *CXCL1*, *CXCL2*, *IL1B*, *IL8*, and *TNF* [54]. In addition, several genes encoding factors involved in cytoskeletal regulation, adhesion, and motility were up-regulated following fMLF stimulation including *ICAM1*, *PLAU*, *PLAUR*, *TUBB*, *TUBB2*, *TUBB5*, *ACTG1*, *LAMB3*, *CD47*, and *TPM4*. Consistent with these findings, analysis of the neutrophil proteome following stimulation through the related formyl peptide receptor-like 1 (FPRL-1) identified several proteins involved in the remodeling of the cytoskeleton [55]. Differentially expressed or modified protein candidates such as the actin- and tubulin-interacting proteins, L-plastin, cofilin, moesin, and stathmin, were identified by 2-D difference gel electrophoresis (DIGE) analysis [55]. Thus, these in vitro studies demonstrate that selective stimulation of neutrophil formyl-peptide receptors induces a limited number of changes that likely enhance both chemotaxis and pro-inflammatory capacity. Two recent studies investigated neutrophil transcriptional changes following migration to aseptic skin lesions [34, 56] and LPS-induced alveolar transmigration [57]. Although neutrophil migration to sites of infection is a complex multifaceted process, the neutrophil transcriptomes delineated in these in vivo studies were remarkably similar to those observed in vitro using various PMN priming agents. In general, there were increases in transcripts encoding molecules of neutrophil pathways that regulate pro-inflammatory capacity, adherence, and migration. On the other hand, there was down-regulation of transcripts involved in pathways that promote apoptosis [56]. These findings

confirm and extend previous reports that indicate the pro-survival and pro-inflammatory effects of priming agents on neutrophil function.

Priming with G-CSF and GM-CSF—GM-CSF and G-CSF are multi-functional colony stimulating factors that not only participate in myelopoiesis, but emerging evidence suggests these important molecules enhance the cellular response at sites of inflammation. Similar to other neutrophil priming agents, both G-CSF and GM-CSF have been shown to delay neutrophil spontaneous apoptosis and this property is consistent with an overall role in promoting the inflammatory response. Microarray studies of the effects of G-CSF on mature human neutrophils demonstrated increased expression of genes encoding plasminogen activator urokinase (PLAU), IL-1 α , granulocyte chemotactic protein-2 (GCP-2), TLR2, and epithelial cell-derived neutrophil attractant-78 (ENA-78) [58]. ENA-78 was subsequently confirmed to enhance chemotaxis of mature neutrophils, thereby providing evidence that G-CSF stimulates mobilization of PMNs to inflammatory sites.

More recently, the effects of GM-CSF on neutrophil gene expression were determined by microarray analysis [29, 59]. Martinelli et al. focused on relative PMN transcription differences following 7 h of in vitro stimulation, whereas Kobayashi et al. performed an extended time course (24 h) of GM-CSF treatment to delineate effects on cell fate. Martinelli et al. noted a comparatively high level of IFN-regulated genes in mature PMNs (versus immature cells) that decreased significantly following GM-CSF treatment. Further investigation revealed that type I- and type II IFN signal transduction is more efficient in mature PMNs, and they proposed that IFN-signaling potentiates bactericidal activity through formation of neutrophil extracellular traps. Since relatively little is known about the role of IFN-signaling in neutrophil function, additional studies are needed to elucidate this interesting hypothesis. By comparison, Kobayashi et al. found a dramatic increase in transcripts encoding proteins that facilitate host defense or are central to the inflammatory response including *CD14*, *CD32*, *CD44*, *CD54*, *CD66A*, *CD69*, *CD74*, *CD89*, *CD119*, *CCR1* (encoding chemokine (C-C motif) receptor 1), *CYBB* (encoding gp91 $phox$), *IL1B*, *IL3RA*, *IL1R2*, and *TLR2* [59]. Although GM-CSF was known to enhance proinflammatory capacity, the number of host defense molecules found to be up-regulated by this cytokine is remarkable. Two other findings from this study were unexpected and made possible only by the availability of genome-wide approaches. First, transcripts encoding dozens of ribosomes and translation factors were increased 18–24 h after treatment with GM-CSF. This observation is consistent with the idea that human neutrophils have significant biosynthetic capacity. Second, there was an increase in mRNAs encoding major histocompatibility (MHC) class II proteins, which suggests that neutrophils have potential as antigen presenting cells [59]. Since GM-CSF extends the lifespan of PMNs significantly, the ability of human PMNs to function as antigen presenting cells could be important in this context.

In the absence of GM-CSF stimulation, the expression of dozens of genes encoding PMN proinflammatory molecules diminished over 24 h in culture and this phenomenon correlated well with induction of spontaneous neutrophil apoptosis. Neutrophil apoptosis was accompanied by significant loss of phagocytic capacity, a finding consistent with down-regulation of receptors [60] and diminished expression of transcripts encoding CD11b, CD16, CD18, CD32, CD35, and CD64 [59]. In addition, down-regulation of genes encoding antiapoptosis proteins, such as myeloid cell leukemia sequence 1 (MCL1), caspase 8 and FAS-associated via death domain-like apoptosis regulator (CFLAR), B cell chronic lymphocytic leukemia/lymphoma 2 (BCL2/adenovirus E1B 19 kDa-interacting protein 2 (BNIP2) and serum/glucocorticoid-regulated kinase (SGK) coincided with spontaneous apoptosis. In stark contrast, GM-CSF treatment delayed neutrophil apoptosis and prevented down-regulation of transcripts encoding pro-inflammatory factors [59]. These findings

provided a comprehensive view of the processes that regulate neutrophil survival and apoptosis.

Priming with LPS—Studies on the immunomodulatory effects of bacterial endotoxin are of historical importance for description of neutrophil function in general. In 1960, Cohn and Morse reported that LPS treatment enhanced neutrophil bactericidal activity [61]. In the early 1980's, Dahinden et al. demonstrated the ability of LPS to alter PMN adhesion, respiratory burst, degranulation, and motility [62]. These studies were followed by evidence that LPS is a potent neutrophil priming agent [51]. The discovery that a TLR4/MD2/CD14 complex recognized LPS facilitated further investigation into the pathways governing effects on neutrophil function [63]. Since then, several investigators have used transcriptome and proteome analyses to elucidate further the neutrophil LPS-mediated signal transduction pathways [54, 64–69]. Although there were variations in both microarray platforms used and LPS dose levels, there was a general concordance of gene expression patterns that support increased pro-inflammatory capacity of LPS-stimulated neutrophils. For example, Rel/NF κ B family members, *NFKB1*, *NFKB2*, and *RELA*, were up-regulated in response to LPS treatment. In addition, transcripts encoding IL-1 β , IL-6, MIP-1 β , MIP-3 α , MCP-1, GRO3, IL-10RA, TNF- α , and HM74 were up-regulated. Initial experiments LPS-stimulated PMNs performed by Worthen and colleagues revealed a surprising increase in expression of IFN-stimulated genes (ISG), such as *ISG15*, *MX1*, *IFI56*, *IFIT4*, *IFI54*, *IFI58*, and *IFP35* [67]. A follow-up study by the same laboratory investigated the mechanisms for induction of ISGs in LPS-stimulated neutrophils [69]. In contrast to monocytes, neutrophil expression levels of *IFNA* and *IFNB* remained unchanged and phosphorylation of STAT proteins was not detected. These authors concluded that neutrophil ISG expression following LPS-stimulation proceeds in an IFN-independent fashion. By contrast, a recent study by Tamassia et al. did not detect LPS-mediated induction of ISGs, although expression *IFNB* was similarly absent in PMNs and present in monocytes [68]. The reason for the discrepancy between studies is unclear. However, the divergence in TLR4 signaling pathways between neutrophils and monocytes is intriguing and warrants further investigation.

Neutrophil phagocytosis and activation

Neutrophils bind and ingest invading microorganisms at sites of infection through a process known as phagocytosis (Fig. 2). Bacteria contain a diversity of evolutionarily-conserved structures that facilitate direct recognition by neutrophils. Molecules such as LPS, lipoprotein, lipoteichoic acid (LTA), and flagellin comprise pathogen-associated molecular patterns (PAMPs) that interact with receptors on the surface of neutrophil membranes. Neutrophils express TLRs 1, 2, 4–10, and additional receptors such as the peptidoglycan recognition protein [70]. In general, ligation of the neutrophil pattern recognition receptors activates signal transduction pathways that ultimately prolong cell survival [71], facilitate adhesion [71] and phagocytosis [70], enhance release of cytokines, chemokines and ROS [70–72], and promote degranulation [73, 74], thereby contributing to innate host defense. PMN phagocytosis is efficiently promoted by opsonization of microbes with host proteins such as antibody and complement. Specific antibody recognizes epitopes on microbial surfaces and promotes the deposition of complement components through the classic pathway of complement activation. Antibodies bound to the surface of microbes are recognized by neutrophil receptors specific for the Fc-region of antibody, including CD64 (Fc γ RI, IgG receptor), CD32 (Fc γ RIIa, low-affinity IgG receptor) [75, 76], CD16 (Fc γ RIIIb, low-affinity IgG receptor) [75, 77], CD89 (Fc α R, IgA receptor) [78], and CD23 (Fc ϵ RI, IgE receptor) [79]. Microbes opsonized with complement are efficiently recognized by PMN surface receptors, such as C1qR [80], CD35 (CR1) [81, 82], CD11b/CD18 (CR3) [83, 84] and CD11c/CD18 (CR4) [85]. Ligation of these membrane-bound opsonin receptors initiates changes in the cytoskeleton that execute the physical process of phagocytosis.

Polymerization of PMN actin microfilaments proximal to receptor ligation facilitates plasma membrane flow around the microbial surface until completion of the engulfment process. Ingested microbes are thus sequestered within membrane-bound vacuoles known as phagosomes (Fig. 2).

PMN phagocytosis initiates sequential execution of the battery of neutrophil microbicidal mechanisms. Neutrophil antimicrobial activity is generated from two primary sources: 1) production of superoxide radicals and other secondarily-derived reactive oxygen species (ROS), and 2) granules containing antimicrobial peptides, proteins, and degradative enzymes (Fig. 2). Neutrophil activation triggers an abrupt increase in oxygen consumption, a process classically termed the respiratory burst. The generation of ROS is mediated by a multi-component membrane-bound complex, NADPH-dependent oxidase (reviewed in ref. [86]). In unactivated neutrophils, components of the NADPH oxidase are compartmentalized to either the cytosol (p40*phox*, p47*phox*, p67*phox*, and the GTPase Rac2) or membranes (flavocytochrome *b*₅₅₈, comprised of gp91*phox* and p22*phox*). The majority of flavocytochrome *b*₅₅₈ resides in the membranes of specific granules, gelatinase granules, and secretory vesicles, whereas the remainder is found in the plasma membrane. PMN priming stimuli induce varied degrees of intermediate structural changes within the NADPH oxidase complex. In general, the majority of neutrophil priming agents, including LPS [87], IL-8 [88], GM-CSF [89], and TNF- α [90], induce a modest increase in phosphorylation of p47*phox*. However, translocation of p47*phox* and both phosphorylation and translocation of additional cytosolic oxidase components are priming stimulus-dependent. PMN priming with LPS results in partial redistribution of flavocytochrome *b*₅₅₈ from the specific granules to the plasma membrane [87]. The assembly of NADPH oxidase is accomplished by translocation of the cytosolic components to the plasma- or phagosome membrane and association with flavocytochrome *b*₅₅₈. The oxidase mediates electron transfer from cytosolic NADPH to intraphagosomal molecular oxygen, thereby forming superoxide anion. Superoxide anion is short-lived and dismutates rapidly to hydrogen peroxide and forms other secondary reactive products, such as hypochlorous acid, hydroxyl radical, and singlet oxygen, which are effective microbicidal compounds [91–94].

Concomitant with assembly of the NADPH-oxidase, neutrophil phagocytosis triggers degranulation, which involves fusion of cytoplasmic granules with the plasma- and/or phagosome membrane. Peroxidase negative granules, i.e., secretory vesicles, gelatinase granules, and specific granules, serve as a reservoir of functionally important membrane proteins such as CR3, formyl peptide receptor, flavocytochrome *b*₅₅₈, and β 2-integrins. Fusion of primary/azurophilic granules (peroxidase-positive granules) with phagosomes enriches the vacuole lumen with antimicrobial agents including α -defensins, cathepsins, proteinase-3, elastase, azurocidin, lysozyme, and bactericidal-permeability-increasing protein. The cumulative antimicrobial activity of neutrophils is thus comprised of ROS and a broad range of antimicrobial peptides and enzymes. Inasmuch as many of these agents do not discriminate between host and microbe, and neutrophils also contain enzymes that degrade host tissues (e.g., elastase, gelatinase, and collagenase), a mechanism must exist to limit damage to host cells and tissues during and after the inflammatory response.

Transcriptome analyses of neutrophil phagocytosis—Although ingestion of microbes is mediated by interactions with neutrophil Fc- and complement receptors, the microbial surface contains a diversity of structures that are both phylogenetically conserved and species-specific. Pathogens are also capable of secreting putative virulence factors that interact with human cells, including those of the innate immune system. Thus, PMN response to bacterial pathogens involves complex signals induced by ligation of multiple receptors in addition to those participating in Fc- and complement-mediated phagocytosis.

Several studies have used microarray analysis to gain insight into molecular determinants that mediate events accompanying PMN phagocytosis and these findings have been reviewed previously [95]. Early transcriptome studies demonstrated that the process of PMN phagocytosis per se induces numerous transcriptional changes that are consistent with increased production of pro-inflammatory mediators not unlike that observed following priming [96, 97]. For example, PMNs up-regulate transcripts early that encode important inflammatory mediators, such as CCL3, CCL4, CCL20, oncostatin M, CXCL2, CXCL3, VEGF, IL-6, and TNF- α , following Fc- and complement receptor-mediated phagocytosis. However, it is also evident that binding and ingestion of microorganisms results in a complex series of neutrophil signal transduction events including TLR activation and the production of additional inflammatory mediators that are potentially pathogen-specific. Genome-wide PMN transcription analyses have been performed following phagocytosis of several microorganisms including *Escherichia coli* K12 [54, 96], attenuated *Yersinia pestis* KIM5 and KIM6 [96, 96], *Staphylococcus aureus* [98, 99], *Streptococcus pyogenes* [98], *Burkholderia cepacia* [98], *Listeria monocytogenes* [98], *Borrelia hermsii* [98], *Mycobacterium bovis* [100], *Candida albicans* [101, 102], and *Anaplasma phagocytophilum* [99, 103–105]. Inasmuch as differences in pathogen-induced PMN transcript levels occur in signal transduction mediators and prominent transcription factors, it is not surprising that the PMN response is not entirely conserved. Thus, identification of pathogen-specific PMN transcriptional profiles is a sound approach toward elucidation of mechanisms of bacterial and fungal pathogenesis. Although detailed dissection of pathogen-specific PMN profiles is beyond the scope of this review, specific examples are provided below.

Proteome analysis of neutrophil phagosomes—Compared with transcriptome-based studies of PMNs, there are a limited number of neutrophil proteome studies. Burlak et al. used high-resolution subcellular proteomics to generate a comprehensive view of proteins associated with human neutrophil phagosomes [106]. Many of the proteins found to be associated with neutrophil phagosomes are also known to be associated with phagosomes of macrophages [107]. These include proteins comprising the actin-based cytoskeleton or those involved in cell motility, such as actin, α -actinin, subunits of the actin-related protein 2/3 complex, myosin, tropomyosin, and vimentin. Somewhat unexpectedly, proteins typically found in the endoplasmic reticulum, including calnexin, ERp29, GRP58/ERp57, GRP78/Ig-heavy chain binding protein (BIP) and protein disulfide isomerase, were associated with neutrophil phagosomes. Previous studies by Garin et al. and Gagnon et al. were the first to demonstrate that molecular chaperones are associated with phagosomes of macrophages and proposed that the endoplasmic reticulum contributes to phagocytosis [107, 108]. The role of endoplasmic reticulum in macrophage phagocytosis was recently a topic of intense debate [109–111], and the precise function of these phagosomal chaperones remains to be determined. However, recent work by several laboratories indicates that antigens processed within phagosomes undergo cross presentation by MHC class I molecules [108, 112–114]. Based on these studies, it was proposed that protein processing and quality control machinery, which includes molecular chaperones and proteasome subunits, function within phagosomes to promote antigen presentation [112]. Inasmuch as we identified 11 molecular chaperones and 5 proteasome subunits associated with human neutrophil phagosomes [106], the function of these molecules on phagosomes is perhaps conserved between macrophages and neutrophils. Another unexpected finding from the proteomics analysis by Burlak et al. was that proteins typically found in the mitochondria, including F1 ATPase, prohibitin, and peroxiredoxin 3, were also associated with phagosomes [106]. Although the function of these proteins at neutrophil phagosomes is unknown, the results of these systems biology-level studies suggest that human PMNs have functions beyond killing of microorganisms, including potential for antigen processing.

Proteins of neutrophil granules—To better understand mechanisms of granule exocytosis, Lominadze et al. performed a comprehensive analysis of proteins comprising human neutrophil granule subsets [115]. These studies identified 286 proteins in gelatinase, specific and azurophil granules, and provided the first comprehensive view of proteins associated with these important neutrophil organelles. Subsequent work by Jethwaney et al. reported the proteomes of human neutrophil plasma membranes and secretory vesicles [116]. Each study identified proteins not previously known to be associated with the secretory vesicles (e.g., 5-lipoxygenase-activating protein and dysferlin) or neutrophil granules (e.g., calreticulin) [115, 116]. Taken together, the data are an important step toward developing a systems biology-level understanding of neutrophil function.

Neutrophil apoptosis

Apoptosis is an important mechanism for regulating homeostasis in cells of the immune system. Normal turnover of aging neutrophils occurs in the absence of activation through a process known as spontaneous apoptosis [117]. The intrinsic ability of aged neutrophils to undergo apoptosis is essential for maintaining appropriate cell numbers in circulation [118]. In addition, neutrophil apoptosis plays a central role in resolution of the acute inflammatory response. The indiscriminate release of highly toxic microbicidal components as a consequence of PMN lysis can lead to host tissue damage, which is exacerbated by prolonged inflammation. The association between prolonged neutrophil survival and clinically apparent inflammatory disorders is well documented [47, 48]. Apoptotic PMNs are recognized and cleared by macrophages, thereby preventing excessive host tissue damage by limiting inflammation at sites of infection [119]. Notwithstanding, the inflammatory milieu is a complex mixture of microbe- and host-derived factors and PMN apoptosis is not an immutable process. Determination of neutrophil fate in such a confounded environment requires simultaneous deciphering of both pro- and anti-apoptotic signals. Furthermore, stimuli that are potent inducers of apoptosis in other cell types can have the opposite effect on neutrophils. For example, exposure of neutrophils to corticosteroids [120], hypoxic environments [121], TNF- α [122], and conditions that elevate intracellular Ca^{2+} [123] and ATP levels [124] inhibit rather than promote neutrophil apoptosis. Nevertheless, it is increasingly clear that the extension of neutrophil survival promotes enhanced clearance of invading microbial pathogens, whereas induction of PMN apoptosis facilitates cell turnover and resolution of inflammation.

Constitutive (spontaneous) neutrophil apoptosis

PMN senescence induces characteristic morphological and physiological changes including cell shrinkage, nuclear condensation, vacuolated cytoplasm, DNA fragmentation, and mitochondrial depolarization [125] (Fig. 3). In addition, neutrophil apoptosis is associated with an overall diminished functional capacity including impaired chemotaxis, phagocytosis, respiratory burst, and degranulation [59, 60]. The overall decrease in neutrophil function is likely a result of down-regulation or shedding of neutrophil surface receptors [126–128]. Apoptotic neutrophils also expose phosphatidylserine on the cell surface, which triggers specific recognition and removal by macrophages [129]. Similar to other cells of the immune system, neutrophils are susceptible to both intrinsic and extrinsic pathways of apoptosis. The intrinsic 'stress-induced' pathway of apoptosis is accompanied by reduction in mitochondrial membrane potential, release of cytochrome c and activation of caspase 9. The extrinsic pathways of apoptosis are triggered by engagement of death receptors of the TNF family, which leads to the activation of caspase 8. The majority of studies demonstrate that spontaneous PMN apoptosis occurs primarily through intrinsic pathways and there is little evidence that death-receptors participate in this process. Although the mechanisms for initiation of PMN constitutive apoptosis are unknown, it is well accepted that neutrophil

apoptosis is a highly complex process and reports of PMN-specific variations in conventional pathways are common.

Mitochondria are a central component to intrinsic apoptosis pathways. However, neutrophil mitochondria are somewhat of an enigma. Neutrophil processes such as chemotaxis consume large quantities of ATP, which are almost exclusively generated through glycolysis [130]. Oxidative phosphorylation is virtually absent in human PMNs [131]. Early ultrastructural studies of neutrophil mitochondria reveal these organelles to be few in number and small in size [7]. However, more recent neutrophil imaging studies using fluorescent probes to measure transmembrane potential indicate PMNs contain a complex tubular network of mitochondria [132, 133]. Mitochondrial outer membrane permeabilization is an early event in the intrinsic apoptosis pathway and is facilitated by the activation of the pro-apoptotic BCL2-associated X protein (BAX) [133, 134]. During apoptosis, mitochondria release additional pro-apoptotic factors into the cytoplasm, such as cytochrome c, Smac/DIABLO [135], Omi/HtrA2 [135], EndoG [136], and apoptosis-inducing factor (AIF) [136]. Cytosolic cytochrome c, although significantly reduced in neutrophils [137], interacts with apoptotic protease-activating factor 1 (APAF-1) in the presence of dATP to trigger formation of a complex called the apoptosome [138]. The apoptosome recruits, binds, and activates the apoptosis initiator caspase-9. Caspase activation is additionally controlled by the X-linked inhibitor of apoptosis (XIAP) [135], which is rapidly recruited to the apoptosome complex. Displacement of XIAP by Smac/DIABLO potentiates caspase activation, including the apoptosis executioner caspase-3.

Activation of the apoptosis initiator caspase-8 is involved in neutrophil spontaneous apoptosis. Although activation of caspase-8 is commonly associated with apoptosis induced by ligation of death receptors, caspase-8 is peculiarly activated at times early (prior to caspase-3) in neutrophil spontaneous apoptosis [139–141]. Ligation of death receptors, such as TNF- α receptor [122], FAS [142], and TRAIL receptor [143], initiates the formation of a death-inducing signaling complex (DISC) and subsequent activation of caspase-8. However, caspase-8 activation during neutrophil spontaneous apoptosis occurs independently of death receptor ligation, which suggests the presence of an alternative activation pathway. Two distinct mechanisms have been proposed to account for the activation of caspase-8 during neutrophil spontaneous apoptosis. The first mechanism involves acid sphingomyelinase-induced clustering of death receptors in lipid rafts, spontaneous DISC assembly and subsequent activation of caspase-8 [144]. The second mechanism provides for the direct activation of caspase-8 by the neutrophil aspartic acid protease cathepsin D that is released from azurophilic granules [145]. Provocatively, ROS are important for both death receptor clustering and permeabilization of azurophilic granules during neutrophil spontaneous apoptosis. However, the mechanism for ROS production in non-activated neutrophils is unclear. ROS are clearly important for activation of caspase-8 during phagocytosis-induced neutrophil programmed cell death [146]. Caspase-8 is also involved in cleavage of the BCL-2 family protein BID that participates in mitochondrial membrane permeabilization by a mechanism similar to BAX [147]. The activity of caspases can also be impaired through direct modifications such as phosphorylation of caspase-8 and caspase-3 by p38-mitogen-activated protein kinase (MAPK) [148]. There are numerous additional pro- and anti-apoptotic factors that have been implicated in spontaneous apoptosis of neutrophils [149]. The existence of multiple pathways governing neutrophil cell fate highlights the importance of apoptosis as a regulatory rheostat between homeostasis and host defense. However, determination of the hierarchy for execution of distinct pathways in neutrophil apoptosis remains to be elucidated. Given the complexity of apoptosis pathways, studies of neutrophil apoptosis are ideally suited to interrogation by genome-wide approaches, as comprehensive understanding of these mechanisms using traditional single molecule studies would be time-consuming.

Extracellular factors

The ability of neutrophils to receive and respond to environmental signals is prerequisite for participation in host defense. Microbial infections are accompanied by important host responses, including release of pro-inflammatory cytokines, which alter neutrophil apoptosis. In general, the majority of inflammatory mediators are associated with delaying neutrophil spontaneous apoptosis and include factors, such as IL-1 β [150], IL-6 [151], IL-8 [152], GRO- α [153], platelet-activating factor [122], IFN- γ [154], G-CSF [150], C5a [155] and GM-CSF [150]. Notably, most of the host-derived cytokines that delay apoptosis are also neutrophil priming agents. However, reports on the effects of the potent PMN priming agent TNF- α on neutrophil survival are more variable. TNF- α has both pro- and anti-apoptotic effects on neutrophils. The divergent effects of TNF- α on neutrophil survival are related to concentration [156], duration of stimulus [122], and functional state of the cell [157]. High concentrations of TNF- α induce neutrophil spontaneous apoptosis associated with increased levels of ROS, whereas prolonged survival correlates with activation of NF κ B [158]. The in vivo relevance of this phenomenon is unclear. In addition to effects of pro-inflammatory cytokines on PMN fate, prolonged neutrophil survival is enhanced by neutrophil adhesion to ligands of β 2-integrin [159]. Ligation of CD11b with soluble fibrinogen delays neutrophil apoptosis and triggers NF κ B activation through an ERK1/2-dependent pathway [160]. Antibody cross-linking of β 2-integrin induces the activation of survival pathways through Akt and MAPK-ERK [159]. However, subsequent stimulation with TNF- α leads to decreased Akt activity through SH2-domain-containing inositol phosphatase (SHIP) activation and results in rapid progression of neutrophil apoptosis [161].

Bacterial-derived products such as LPS, LTA, and secreted toxins are known to delay neutrophil apoptosis [149]. Inasmuch as several TLR ligands are potent PMN priming agents, it follows that TLR-mediated signal transduction pathways influence neutrophil fate. However, the majority of evidence suggests that only TLR4 and TLR2 directly modulate PMN survival. Stimulation of neutrophils with either purified LPS (TLR4) [71] or LTA (TLR2) [74] delays spontaneous apoptosis. Inhibition of NF κ B activation is associated with enhanced TLR4-mediated neutrophil survival [71]. Studies on TLR-inhibition of neutrophil apoptosis in whole blood indicate that phosphatidylinositol 3-kinase (PI3K) activation may also be involved in enhanced survival [162]. The addition of monocytes to TLR4-stimulated PMNs also prolongs neutrophil survival [71], suggesting a possible synergy with cytokine-signaling pathways. Mononuclear cell-dependent enhancement of neutrophil survival has also been demonstrated following exposure to staphylococcal enterotoxins [163]. Several other bacterial toxins have been shown to directly promote neutrophil survival, such as *E. coli* verotoxin [164], Shiga toxin [165], and *Sta. epidermidis* PSMs [166]. Together, these observations suggest that enhanced neutrophil survival is a desirable consequence during early stages of inflammation and promotes the clearance of bacterial pathogens.

Influence of bacteria on PMN survival

The ability of inflammatory mediators and bacterial-derived products to support neutrophil survival preceding phagocytosis is important for maintenance of optimal microbicidal potential. In contrast, phagocytosis-induced cell death (PICD) is a mechanism to clear tissues of effete neutrophils containing killed microbes, thereby facilitating the resolution of infection. As with spontaneous neutrophil apoptosis, timely removal of neutrophils—although in this case after phagocytosis—would prevent release of cytotoxic molecules into surrounding host tissues, a phenomenon caused by necrotic lysis. The process of phagocytosis significantly accelerates the rate of apoptosis in human PMNs [98, 146, 167–169]. Consistent with this idea, neutrophil ingestion of *E. coli*, *Neisseria gonorrhoeae*, *Str. pneumoniae*, *Str. pyogenes*, *C. albicans*, *Sta. aureus*, *M. tuberculosis*, *Bur. cepacia*, *Bor. hermsii*, and *L. monocytogenes* significantly accelerates the rate of PMN apoptosis [149].

Importantly, phagocytosis significantly increases the rate of PMN apoptosis irrespective of any delay in cell fate imparted by cytokines or bacteria-derived factors [126, 169, 170]. Thus, PMN apoptosis plays a central role not only in regulation of cell turnover, but also in termination of the inflammatory process.

Although, PICD is desirable for the resolution of infection and prevention of prolonged inflammation, this process is not immutable. The relatively short life-span of neutrophils is not amenable to the long-term survival strategies employed by most intracellular pathogens; however, several bacterial pathogens are capable of exploiting apoptosis/PICD as a potential mechanism of pathogenesis. To that end, these microorganisms either accelerate or delay neutrophil apoptosis. Two bacterial pathogens have been shown conclusively to delay human neutrophil apoptosis. *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis, was the first bacterial pathogen reported to delay PMN apoptosis, which ultimately promotes replication within an endosomal compartment [171]. More recently, *Chlamydia pneumoniae* was shown to delay neutrophil spontaneous apoptosis [172] to facilitate intracellular replication. Conversely, bacterial pathogens such as *Sta. aureus* and *Str. pyogenes* cause direct PMN lysis and/or accelerate bacteria-induced apoptosis to the point of secondary necrosis [98, 173, 174]. The deleterious effects on neutrophil cell fate may be mediated in part by bacterial toxins. For example, some staphylococci produce leukotoxins that promote apoptosis at sublytic levels [175] or profoundly affect neutrophil integrity [173]. In addition, *Pseudomonas aeruginosa* pyocyanin [176] and *Aspergillus fumigatus* gliotoxin [177] have been shown to promote neutrophil apoptosis. It is likely that accelerated PMN lysis contributes to the overall levels of tissue necrosis associated with these infections.

Neutrophil apoptosis differentiation program

The importance of neutrophil spontaneous apoptosis in maintenance of cellular homeostasis is well recognized and considerable effort has been expended towards determining the molecular mechanisms governing this essential process. In addition, we now know that neutrophil activation has a direct impact on apoptosis and regulation of PICD differs from that of spontaneous neutrophil apoptosis. Reports of additional factors and conditions that modulate neutrophil fate have increased steadily over the past decade, but we are still far from a complete understanding of the specific pathways that regulate neutrophil apoptosis. That said, systems biology-level approaches have been instrumental in re-shaping our view of the role of neutrophils in the resolution of the inflammatory response. Notably, genome-wide studies have been a crucial guide in our investigation of the molecular determinants that regulate neutrophil PICD and have provided new insight into mechanisms by which bacteria exploit this important process.

Within a few hour hours after neutrophil phagocytosis, the number of differentially expressed apoptosis-related genes increases dramatically. For example, genes encoding apoptosis mediators, such as *BAX*, *BCL2A1*, *CFLAR*, and nuclear orphan receptors TR3 (*NR4A1*), NURR1 (*NR4A2*), and NOR1 (*NR4A3*), are up-regulated prior to initiation of PICD [97]. In addition, phagocytosis of bacterial pathogens up-regulates PMN transcripts encoding several key mediators of TNF- and TLR2-signaling, including *TNF*, *IRAK1*, and *TNFAIP8* [98]. Several lines of evidence indicate that proinflammatory capacity of human PMNs is regulated at the level of gene expression during PICD. For instance, the initial stages of PMN apoptosis are accompanied by changes in the expression of genes encoding important regulators of detoxification and redox pathways, such as those governing glutathione-, thioredoxin-, and heme metabolism [178]. Thus, transcriptional regulation of PMN detoxification pathways provides a secondary mechanism to limit inflammatory potential should neutrophils undergo premature lysis. Importantly, progression of neutrophil apoptosis correlates with down-regulation of genes encoding proinflammatory mediators,

phosphoinositide metabolism, and calcium signal transduction [128, 178]. Although it is appreciated that neutrophil apoptosis is characterized by an overall decrease in functional capacity, the sheer number of transcripts identified by genome-wide analyses as down-regulated during PICD was somewhat unexpected [97, 98, 128]. For example, dozens of transcripts encoding key proinflammatory mediators and signal transduction molecules were down-regulated during the initiation of phagocytosis-induced apoptosis [97, 98, 128, 178]. Among these, 42 genes encoded proteins critical to the inflammatory response, including receptors for IL-8 β , IL-10 α , IL-13 α 1, IL-15 α , IL-17, IL-18, C1q, formyl peptide, and CD31, CD32, and TLR6. In addition, the vast majority of genes encoding proinflammatory molecules that were up-regulated at times early following phagocytosis decreased significantly following induction of PICD. Based on these findings and the integration of functional studies using human neutrophils, we proposed that bacteria induce an apoptosis differentiation program in human neutrophils that facilitates PMN turnover and resolution of the inflammatory response [95] (Fig. 4). Thus, accelerated neutrophil apoptosis after phagocytosis and the accompanying down-regulation of inflammatory capacity is a desired consequence that contributes to the resolution of infection. Consistent with this hypothesis, PMN phagocytosis of *Bor. hermsii*, *Bur. cepacia*, *L. monocytogenes*, *Sta. aureus*, and *Str. pyogenes* elicit similar patterns of gene expression that collectively form a common transcriptome (apoptosis differentiation program) [98]. The neutrophil apoptosis differentiation program includes molecules involved in multiple biological processes, culminating with down-regulation of proinflammatory capacity and concomitant induction of apoptosis/PICD (Fig. 4).

Inasmuch as neutrophil apoptosis is a critical process for resolution of inflammation, it is not surprising that some bacterial pathogens alter the normal PMN apoptosis differentiation program as a mechanism of pathogenesis. For instance, the ability of *Str. pyogenes* to cause rapid PICD and ultimately PMN lysis is reflected by and/or results from the changes in neutrophil gene expression [98]. Ingestion of *Str. pyogenes* elicits unique transcriptional variations in cell death pathways that include up-regulation of activator protein-1 (AP-1) complex-related transcripts, such as *FOS*, *FOSL1*, *FOSB*, *JUNB*, and *TNFRSF5*, and down-regulation of transcripts encoding members of the NF- κ B signal transduction pathway [98]. These findings indicate that the ability of *Str. pyogenes* to exploit PMN fate pathways is likely an important component of streptococcal pathogenesis and is a potential contributor to fulminant tissue destruction observed in invasive disease [179]. It is important to note that ability of *Str. pyogenes* to induce comparatively more rapid PICD and neutrophil lysis was discovered with a transcriptome approach, as those results directed the subsequent in vitro studies. By stark contrast, the obligate intracellular pathogen *A. phagocytophilum* inhibits neutrophil apoptosis to survive and thereby cause disease. To investigate the molecular basis of *A. phagocytophilum* survival within neutrophils, Borjesson et al. used oligonucleotide microarrays to measure global changes in human PMN gene expression following infection with *A. phagocytophilum* [99]. Functional analysis of infected PMNs confirmed previous reports that *A. phagocytophilum* fails to trigger neutrophil production of ROS [180, 181] and the pathogen delays PMN spontaneous apoptosis [171]. In addition, PMN uptake of *A. phagocytophilum* occurs at a slow rate compared to that observed with other bacteria [99]. Consistent with the functional studies, infection of human PMNs with *A. phagocytophilum* delayed up-regulation of transcripts involved in the acute inflammatory response, such as *TNF*, *IL1B*, *CXCL1*, *CXCL2*, *CXCL3*, *CCL3*, *CCL4*, and *CD54* [99]. Similar gene expression profiles from *A. phagocytophilum*-infected neutrophils were reported in subsequent studies [103–105]. One key finding of these studies related to the levels of PMN transcripts encoding NADPH oxidase proteins. Previous studies using promyelocytic HL60 cells, an immortalized cell line sometimes used as a model for human neutrophils, had suggested that the ability of *A. phagocytophilum* to inhibit ROS production was due to down-regulation of transcripts encoding NADPH oxidase proteins [182, 183]. In contrast,

microarray using human neutrophils indicated that these transcripts remained unchanged or increased in expression following ingestion of *A. phagocytophilum* [99]. This finding resolved controversy in part about the mechanism underlying inhibition of ROS in *A. phagocytophilum*-infected neutrophils. In addition, *A. phagocytophilum*-infected PMNs increased expression levels of several anti-apoptosis genes including *BIRC2*, *BIRC3*, *CFLAR*, *TNFAIP8*, and *TNIP2*, and decreased expression of numerous apoptosis-inducing factors. Moreover, interaction of live or dead *A. phagocytophilum* with PMNs results in the inability to induce neutrophil apoptosis via ligation of the FAS death receptor [99], and indicate that pre-existing surface molecules facilitate survival during *A. phagocytophilum* infection, presumably to promote bacterial replication and persistence.

The ability of pathogens to alter neutrophil fate by either blocking apoptosis to facilitate intracellular pathogen survival or promoting rapid lysis to eliminate neutrophils represent plausible mechanisms of virulence. The genome-wide analyses with human neutrophils and bacteria, coupled with the results of many targeted studies using neutrophils and animal models, led to the hypothesis that there are two fundamental outcomes of PMN-bacteria interactions (Fig. 5). On one hand, phagocytosis activates neutrophils and ingested microorganisms are killed, thus leading to PICD and removal of effete PMNs by macrophages. This process leads to the resolution of infection and is healthy for the host. Alternatively, pathogens are ingested but not killed, and neutrophils either lyse or have delayed turnover that promotes pathogen replication (Fig. 5). Either of these latter outcomes is counterproductive for the resolution of infection and potentially results in disease. Although systems biology-level approaches have been critical to enhancing our understanding of the molecular mechanisms of neutrophil PICD and bacterial pathogenesis, the complex association of neutrophils, macrophages, and secreted pathogen and host factors at infection sites complicate this rather simplistic model. Furthermore, it has been suggested recently that bacterial pathogens alter the process of efferocytosis by influencing secretion of TNF- α from macrophages [184], which may in turn impact the resolution of inflammation [185, 186]. The development of complex *in vitro* and *in vivo* model systems, combined with genome-wide approaches, will be the next important step towards a complete understanding of the role of neutrophil apoptosis in bacterial pathogenesis.

Disease processes

Neutropenia and inherent deficiencies in neutrophil function are important predisposing risk factors for development of life-threatening bacterial and fungal infections. The majority of congenital neutrophil disorders are relatively rare, thereby confounding characterization of disease. Genome-wide expression analyses have provided new insight into the pathophysiology of disease or served as an important first step toward identifying the molecular genetic basis of disease.

Chronic granulomatous disease

Leukocytes from patients with X-linked chronic granulomatous disease (XCGD) are defective in their ability to produce ROS due to defined heterogeneous mutations that preclude assembly of the NADPH-oxidase [187–191]. Although the genetic and cellular basis of XCGD has long been known (reviewed in ref. [192]), the molecular basis of associated pathophysiologies, such as formation of granulomas, remains undetermined. Recently, we used gene expression profiling to obtain a comprehensive global view of the impact of PMN-derived oxidants on the resolution of inflammation [193]. Analysis of PMNs from XCGD patients revealed that these cells had increased levels of transcripts encoding proinflammatory molecules and decreased expression of genes encoding anti-inflammation mediators, consistent with a previous report showing excessive IL-8 production by CGD neutrophils [194]. In addition, neutrophil transcripts involved in PICD are differentially

expressed between activated PMNs from healthy control subjects and XCGD patients and coincide with a delay in apoptosis. Taken together, these findings provide support to the hypothesis that reduced or absent PICD in XCGD neutrophils results in delayed resolution of inflammation, which could contribute to the formation of granulomas in XCGD patients.

Specific granule deficiency

Neutrophil specific granule deficiency (SGD) is a rare congenital disorder characterized by susceptibility to recurrent pyogenic infections. Neutrophils from SGD patients have impaired bactericidal activity due to deficiencies in specific granule contents, in addition to selective absence of azurophilic- and tertiary granule constituents. The molecular basis of SGD was recently shown to involve germ-line mutations in the transcription factor, C/EBP ϵ [195]. Preceding characterization of the human defect, a mice deficient in C/EBP ϵ demonstrated a defined role in hematopoiesis [196], and mature neutrophils are severely functionally impaired [197]. Recently, gene expression profiling was used to provide additional insight into the pathophysiology of SGD in a mouse model of disease [198]. This study demonstrated myeloid cell transcriptional changes in genes corresponding to a broad range of cellular processes. Dysregulation of gene expression was appropriately observed in key inflammatory response regulators, such as *CCL2*, *CCL4*, *CCL7*, *IL6*, *IL1B*, *CCR1*, *IL8RB*, and *CSF3R*. In addition, there was dysregulation of genes encoding cytoskeletal structural and regulatory proteins, potentially explaining neutrophil defects in chemotaxis, phagocytosis and superoxide production. Further investigation into the role of C/EBP ϵ in regulating the transcription of genes identified in those studies will likely provide additional insight into the pathophysiology of SGD.

Hyper-IgE Syndrome (HIES)/Job's Syndrome

HIES or Job's syndrome is a rare immunological disorder characterized by dermatitis, recurrent staphylococcal skin abscesses, cyst-forming pneumonias, elevated serum IgE levels, and multiple developmental abnormalities. Although HIES was originally described by Davis et al. in 1966 [199], the molecular genetic basis of the disease was identified only recently [4, 200]. As a first step toward identifying the cause of HIES and to gain insight into the molecular processes that contribute to the pathophysiology of the disease, Holland et al. compared global gene expression in leukocytes from HIES patients and healthy control individuals [4]. Transcriptome analysis of unstimulated and stimulated PMNs from HIES patients revealed that there were elevated levels of transcripts encoding proinflammatory molecules in patient cells, including at least thirty transcripts encoding proteins related to IFN signal transduction (Fig. 6). Collectively, the PMN transcriptome data suggested that signal transduction involving IFNs and STATs was altered in HIES patients (Fig. 6). Consistent with those results, HIES leukocytes had increased production of IFN- γ and TNF- α following stimulation. However, leukocytes from these patients also displayed impaired stimulus-dependent signaling through the IL-6 receptor [4]. DNA sequence analysis revealed distinct mutations in the DNA-binding region and SH2 domain of *STAT3* in HIES patients [4]. These studies clearly demonstrate that autosomal dominant and sporadic forms of HIES are caused by mutations in the gene encoding STAT3, a key transcription activator following cytokine- or growth factor stimulation, thereby explaining the diverse nature of the disease. Given the involvement of STAT3-SOCS3 in recruitment of myeloid cells and execution of anti-inflammatory responses in mature immune cells [201], the finding is of significant importance for increased understanding of the regulation of inflammation in general. Moreover, the studies strong provide support to the idea that microarray-based studies can be used as a first-tier approach for the identification of the genetic basis of hereditary immune disorders.

Conclusions

The advent of systems biology technologies, such as microarray and proteome analyses, has provided a means to globally interrogate the molecular events governing host-pathogen interactions. Because of these genome-wide approaches, we now know that neutrophils have significant biosynthetic potential and therefore, their role in the innate immune system is being reconsidered more broadly. Systems biology-level approaches have enabled molecular dissection of key neutrophil processes or functions, including post-phagocytosis sequelae and many new discoveries have been made (Table 1). The idea that neutrophils actively participate in processes beyond phagocytosis and killing of microbes is a relatively recent concept and there is now considerable effort directed to understand the role of PMNs in the resolution of inflammation.

Current genome-wide approaches with neutrophils primarily involve analysis of transcriptome or proteome. However, future systems-level studies will likely include analysis of lipid mediators, such as lipoxins, resolvins, and protectins, which have an essential role in the resolution of inflammation [202]. Indeed, recent metabolome studies by Serhan and colleagues revealed that human PMNs convert resolvin E1 to novel metabolic products that have potent anti-inflammatory activities [203]. This line of investigation is especially intriguing for system-level analyses, since it is possible to predict synthesis of these novel compounds using lipidomics and then test function [204, 205]. It will be important to investigate relative production of these mediators during interaction of neutrophils with bacterial pathogens.

There is little doubt that whole-genome sequencing will play a prominent role in future studies directed to elucidate the molecular genetic basis of immune disorders, including those that involve neutrophils and host defense. This systems-level approach has already been implemented for discovery of tumor-specific somatic mutations, as Ley et al. used whole-genome sequencing to identify eight new mutations associated with acute myeloid leukemia [206]. For a true systems biology analysis of the role of neutrophils in innate immunity, it will be important to develop tools that integrate data sets from multiple laboratories into a user-friendly platform from which to ask important questions. There is a significant challenge in the integration of enormous data sets generated across a diversity of platforms. Comparison of systems-level studies in neutrophils is further confounded by apparent functional discrepancies between primary human neutrophils, in vitro cell lines (e.g. HL-60), and experimental animal model systems. Despite these caveats, significant progress has been toward a comprehensive understanding of neutrophil functions and the role of these cells in innate immunity.

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References

1. Homburg CH, Roos D. Apoptosis of neutrophils. *Curr. Opin. Hematol.* Jan; 1996 3(1):94–99. [PubMed: 9372057]
2. Kasprisin DO, Harris MB. The role of RNA metabolism in polymorphonuclear leukocyte phagocytosis. *J. Lab. Clin. Med.* Jul; 1977 90(1):118–124. [PubMed: 874363]
3. Kasprisin DO, Harris MB. The role of protein synthesis in polymorphonuclear leukocyte phagocytosis II. *Exp. Hematol.* Aug; 1978 6(7):585–589. [PubMed: 211044]

4. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C, Schaffer AA, Puck JM, Grimbacher B. STAT3 Mutations in the Hyper-IgE Syndrome. *N. Engl. J. Med.* Sep; 2007 357(16):1608–1619. [PubMed: 17881745]
5. Friedman AD. Transcriptional regulation of granulocyte and monocyte development. *Oncogene.* May; 2002 21(21):3377–3390. [PubMed: 12032776]
6. McDonald PP. Transcriptional regulation in neutrophils: teaching old cells new tricks. *Adv. Immunol.* 2004:821–48.
7. Bainton DF, Ulliyot JL, Farquhar MG. The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. *J. Exp. Med.* Oct; 1971 134(4):907–934. [PubMed: 4106490]
8. Cline MJ. Phagocytosis and synthesis of ribonucleic acid in human granulocytes. *Nature.* Jan.1966 : 2121431–1433.
9. Cline MJ. Ribonucleic acid biosynthesis in human leukocytes. Effects of phagocytosis on RNA metabolism. *Blood.* Aug; 1966 28(2):188–200. [PubMed: 5222583]
10. Walmsley SR, Print C, Farahi N, Peyssonnaud C, Johnson RS, Cramer T, Sobolewski A, Condliffe AM, Cowburn AS, Johnson N, Chilvers ER. Hypoxia-induced neutrophil survival is mediated by HIF-1 α -dependent NF- κ B activity. *J. Exp. Med.* Jan; 2005 201(1):105–115. [PubMed: 15630139]
11. Jonsson H, Allen P, Peng SL. Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. *Nat. Med.* Jun; 2005 11(6):666–671. [PubMed: 15895074]
12. Kosak ST, Scalzo D, Alworth SV, Li F, Palmer S, Enver T, Lee JS, Groudine M. Coordinate gene regulation during hematopoiesis is related to genomic organization. *PLoS. Biol.* Nov.2007 5(11):e309. [PubMed: 18031200]
13. Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature.* Feb; 2008 451(7182):1125–1129. [PubMed: 18278031]
14. Lindemann SW, Yost CC, Denis MM, McIntyre TM, Weyrich AS, Zimmerman GA. Neutrophils alter the inflammatory milieu by signal-dependent translation of constitutive messenger RNAs. *Proc. Natl. Acad. Sci. USA.* May; 2004 101(18):7076–7081. [PubMed: 15118085]
15. Yost CC, Denis MM, Lindemann S, Rubner FJ, Marathe GK, Buerke M, McIntyre TM, Weyrich AS, Zimmerman GA. Activated polymorphonuclear leukocytes rapidly synthesize retinoic acid receptor- α : a mechanism for translational control of transcriptional events. *J. Exp. Med.* Sep; 2004 200(5):671–680. [PubMed: 15337793]
16. Cline, MJ. Production and distribution of neutrophils. In: Cline, Martin J., editor. *The White Cell.* Harvard University Press; Cambridge, Massachusetts: 1975. p. 22–38.
17. Athens JW, Haab OP, Raab SO, Mauer AM, Ashenbrucker H, Cartwright GE, Wintrobe MM. Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. *J. Clin. Invest.* Jun; 1961 40(6):989–995. [PubMed: 13684958]
18. Dale DC, Liles WC, Llewellyn C, Price TH. Effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) on neutrophil kinetics and function in normal human volunteers. *Am. J. Hematol.* Jan; 1998 57(1):7–15. [PubMed: 9423810]
19. Dancy JT, Deubelbeiss KA, Harker LA, Finch CA. Neutrophil kinetics in man. *J. Clin. Invest.* Sep; 1976 58(3):705–715. [PubMed: 956397]
20. Cronkite EP, Flidner TM. Granulocytopoiesis. *N. Engl. J. Med.* Jun.1964 :2701403–1408.
21. Flidner TM, Cronkite EP, Robertson JS. Granulocytopoiesis. I. Senescence and random loss of neutrophilic granulocytes in human beings. *Blood.* Oct.1964 :24402–414.
22. Flidner TM, Cronkite EP, Killmann SA, Bond VP. Granulocytopoiesis. II. Emergence and pattern of labeling of neutrophilic granulocytes in humans. *Blood.* Dec; 1964 24(6):683–700. [PubMed: 14235351]
23. Orr Y, Wilson DP, Taylor JM, Bannon PG, Geczy C, Davenport MP, Kritharides L. A kinetic model of bone marrow neutrophil production that characterizes late phenotypic maturation. *Am. J. Physiol Regul. Integr. Comp Physiol.* Apr; 2007 292(4):R1707–R1716. [PubMed: 17185405]

24. Aglietta M, Piacibello W, Sanavio F, Stacchini A, Apra F, Schena M, Mossetti C, Carnino F, Caligaris-Cappio F, Gavosto F. Kinetics of human hemopoietic cells after in vivo administration of granulocyte-macrophage colony-stimulating factor. *J. Clin. Invest.* Feb; 1989 83(2):551–557. [PubMed: 2643633]
25. Laterveer L, Lindley IJ, Heemskerk DP, Camps JA, Pauwels EK, Willemze R, Fibbe WE. Rapid mobilization of hematopoietic progenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. *Blood.* Jan; 1996 87(2):781–788. [PubMed: 8555503]
26. Lian Z, Wang L, Yamaga S, Bonds W, Beazer-Barclay Y, Kluger Y, Gerstein M, Newburger PE, Berliner N, Weissman SM. Genomic and proteomic analysis of the myeloid differentiation program. *Blood.* Aug; 2001 98(3):513–524. [PubMed: 11468144]
27. Lian Z, Kluger Y, Greenbaum DS, Tuck D, Gerstein M, Berliner N, Weissman SM, Newburger PE. Genomic and proteomic analysis of the myeloid differentiation program: global analysis of gene expression during induced differentiation in the MPRO cell line. *Blood.* Nov; 2002 100(9):3209–3220. [PubMed: 12384419]
28. Iida S, Kohro T, Kodama T, Nagata S, Fukunaga R. Identification of CCR2, flotillin, and gp49B genes as new G-CSF targets during neutrophilic differentiation. *J. Leukoc. Biol.* Aug; 2005 78(2):481–490. [PubMed: 15894583]
29. Martinelli S, Urosevic M, Daryadel A, Oberholzer PA, Baumann C, Fey MF, Dummer R, Simon HU, Yousefi S. Induction of genes mediating interferon-dependent extracellular trap formation during neutrophil differentiation. *J. Biol. Chem.* Oct; 2004 279(42):44123–44132. [PubMed: 15302890]
30. Theilgaard-Mönch K, Knudsen S, Follin P, Borregaard N. The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. *J. Immunol.* Jun; 2004 172(12):7684–7693. [PubMed: 15187151]
31. Ferrari F, Bortoluzzi S, Coppe A, Basso D, Bicciato S, Zini R, Gemelli C, Danieli GA, Ferrari S. Genomic expression during human myelopoiesis. *BMC. Genomics.* 2007:8264.
32. Mollinedo F, Lopez-Perez R, Gajate C. Differential gene expression patterns coupled to commitment and acquisition of phenotypic hallmarks during neutrophil differentiation of human leukaemia HL-60 cells. *Gene.* Aug; 2008 419(1–2):16–26. [PubMed: 18547747]
33. Kluger Y, Tuck DP, Chang JT, Nakayama Y, Poddar R, Kohya N, Lian Z, Ben NA, Halaban HR, Krause DS, Zhang X, Newburger PE, Weissman SM. Lineage specificity of gene expression patterns. *Proc. Natl. Acad. Sci. U. S. A.* Apr; 2004 101(17):6508–6513. [PubMed: 15096607]
34. Theilgaard-Monch K, Porse BT, Borregaard N. Systems biology of neutrophil differentiation and immune response. *Curr. Opin. Immunol.* Feb; 2006 18(1):54–60. [PubMed: 16343884]
35. Theilgaard-Monch K, Jacobsen LC, Borup R, Rasmussen T, Bjerregaard MD, Nielsen FC, Cowland JB, Borregaard N. The transcriptional program of terminal granulocytic differentiation. *Blood.* Feb; 2005 105(4):1785–1796. [PubMed: 15514007]
36. Le C, V, Cowland JB, Calafat J, Borregaard N. Targeting of proteins to granule subsets is determined by timing and not by sorting: The specific granule protein NGAL is localized to azurophil granules when expressed in HL-60 cells. *Proc. Natl. Acad. Sci. U. S. A.* Jun; 1996 93(13):6454–6457. [PubMed: 8692836]
37. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell.* May; 1991 65(5):859–873. [PubMed: 1710173]
38. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J. Exp. Med.* Aug; 1993 178(2):449–460. [PubMed: 8340753]
39. Diamond MS, Staunton DE, de Fougères AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA. ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). *J. Cell Biol.* Dec; 1990 111(6):3129–3139. [PubMed: 1980124]
40. Khan AI, Kerfoot SM, Heit B, Liu L, Andonegui G, Ruffell B, Johnson P, Kubes P. Role of CD44 and hyaluronan in neutrophil recruitment. *J. Immunol.* Dec; 2004 173(12):7594–7601. [PubMed: 15585887]

41. Cooper D, Lindberg FP, Gamble JR, Brown EJ, Vadas MA. Transendothelial migration of neutrophils involves integrin-associated protein (CD47). *Proc. Natl. Acad. Sci. USA.* Apr; 1995 92(9):3978–3982. [PubMed: 7732016]
42. Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem. Biophys. Res. Commun.* Dec; 1987 149(2):755–761. [PubMed: 3322281]
43. Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc. Natl. Acad. Sci. U. S. A.* Dec; 1987 84(24):9233–9237. [PubMed: 3480540]
44. Ehrengreuer MU, Geiser T, Deranleau DA. Activation of human neutrophils by C3a and C5A: Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst. *FEBS Lett.* Jun; 1994 346(2–3):181–184. [PubMed: 8013630]
45. Showell HJ, Freer RJ, Zigmond SH, Shiffmann E, Aswanikumar S, Corcoran B, Becker EL. The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J. Exp. Med.* May; 1976 143(5):1154–1169. [PubMed: 1262785]
46. Schmeling DJ, Peterson PK, Hammerschmidt DE, Kim Y, Verhoef J, Wilkinson BJ, Quie PG. Chemotaxis by cell surface components of *Staphylococcus aureus*. *Infect. Immun.* Oct; 1979 26(1):57–63. [PubMed: 500215]
47. Boxer LA, Axtell R, Suchard S. The role of the neutrophil in inflammatory diseases of the lung. *Blood Cells.* 1990; 16(1):25–42. [PubMed: 2190649]
48. Edwards SW, Hallett MB. Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. *Immunol. Today.* Jul; 1997 18(7):320–324. [PubMed: 9238834]
49. Swain SD, Rohn TT, Quinn MT. Neutrophil priming in host defense: role of oxidants as priming agents. *Antioxid. Redox. Signal.* Feb; 2002 4(1):69–83. [PubMed: 11970845]
50. Sheppard FR, Kelher MR, Moore EE, McLaughlin NJ, Banerjee A, Silliman CC. Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. *J. Leukoc. Biol.* Nov; 2005 78(5):1025–1042. [PubMed: 16204621]
51. Guthrie LA, McPhail LC, Henson PM, Johnston RB Jr. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J. Exp. Med.* Dec; 1984 160(6):1656–1671. [PubMed: 6096475]
52. Kobayashi SD, Voyich JM, Burlak C, DeLeo FR. Neutrophils in the innate immune response. *Arch. Immunol. Ther. Exp. (Warsz.).* Nov; 2005 53(6):505–517. [PubMed: 16407783]
53. DeLeo FR, Renee J, McCormick S, Nakamura M, Apicella M, Weiss JP, Nauseef WM. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J. Clin. Invest.* Jan; 1998 101(2):455–463. [PubMed: 9435318]
54. Zhang XQ, Kluger Y, Nakayama Y, Poddar R, Whitney C, DeTora A, Weissman SM, Newburger PE. Gene expression in mature neutrophils: early responses to inflammatory stimuli. *J. Leukoc. Biol.* Feb; 2004 75(2):358–372. [PubMed: 14634056]
55. Boldt K, Rist W, Weiss SM, Weith A, Lenter MC. FPRL-1 induces modifications of migration-associated proteins in human neutrophils. *Proteomics.* Sep; 2006 6(17):4790–4799. [PubMed: 16892486]
56. Theilgaard-Monch K, Knudsen S, Follin P, Borregaard N. The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. *J. Immunol.* Jun; 2004 172(12):7684–7693. [PubMed: 15187151]
57. Coldren CD, Nick JA, Poch KR, Woolum MD, Fouty BW, O'Brien JM, Gruber MP, Zamora MR, Svetkauskaite D, Richter DA, He Q, Park JS, Overdier KH, Abraham E, Geraci MW. Functional and genomic changes induced by alveolar transmigration in human neutrophils. *Am. J. Physiol. Lung Cell Mol. Physiol.* Dec; 2006 291(6):L1267–L1276. [PubMed: 16861384]
58. Suzuki S, Kobayashi M, Chiba K, Horiuchi I, Wang J, Kondoh T, Hashino S, Tanaka J, Hosokawa M, Asaka M. Autocrine production of epithelial cell-derived neutrophil attractant-78 induced by granulocyte colony-stimulating factor in neutrophils. *Blood.* Mar; 2002 99(5):1863–1865. [PubMed: 11861308]

59. Kobayashi SD, Voyich JM, Whitney AR, DeLeo FR. Spontaneous neutrophil apoptosis and regulation of cell survival by granulocyte macrophage-colony stimulating factor. *J. Leukoc. Biol.* Dec; 2005 78(6):1408–1418. [PubMed: 16204629]
60. Whyte MK, Meagher LC, MacDermot J, Haslett C. Impairment of function in aging neutrophils is associated with apoptosis. *J. Immunol.* Jun; 1993 150(11):5124–5134. [PubMed: 8388425]
61. Cohn ZA, Morse SI. Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin. *J. Exp. Med.* May; 1960 111(5):689–704. [PubMed: 13694492]
62. Dahinden CA, Galanos C, Fehr J. Granulocyte activation by endotoxin. I. Correlation between adherence and other granulocyte functions, and role of endotoxin structure on biologic activity. *J. Immunol.* Feb; 1983 130(2):857–862. [PubMed: 6848599]
63. Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK. Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. *J. Immunol.* May; 2002 168(9):4701–4710. [PubMed: 11971020]
64. Tsukahara Y, Lian Z, Zhang XQ, Whitney C, Kluger Y, Tuck D, Yamaga S, Nakayama Y, Weissman SM, Newburger PE. Gene expression in human neutrophils during activation and priming by bacterial lipopolysaccharide. *J. Cell. Biochem.* Jul; 2003 89(4):848–861. [PubMed: 12858349]
65. O'Neill AJ, Doyle BT, Molloy E, Watson C, Phelan D, Greenan MC, Fitzpatrick JM, Watson RW. Gene expression profile of inflammatory neutrophils: alterations in the inhibitors of apoptosis proteins during spontaneous and delayed apoptosis. *Shock.* Jun; 2004 21(6):512–518. [PubMed: 15167679]
66. Silva E, Arcaroli J, He Q, Svetkauskaite D, Coldren C, Nick JA, Poch K, Park JS, Banerjee A, Abraham E. HMGB1 and LPS induce distinct patterns of gene expression and activation in neutrophils from patients with sepsis-induced acute lung injury. *Intensive Care Med.* Oct; 2007 33(10):1829–1839. [PubMed: 17581740]
67. Fessler MB, Malcolm KC, Duncan MW, Worthen GS. A genomic and proteomic analysis of activation of the human neutrophil by lipopolysaccharide and its mediation by p38 mitogen-activated protein kinase. *J. Biol. Chem.* Aug; 2002 277(35):31291–31302. [PubMed: 11943771]
68. Tamassia N, Le M, V, Calzetti F, Donini M, Gasperini S, Ear T, Cloutier A, Martinez FO, Fabbri M, Locati M, Mantovani A, McDonald PP, Cassatella MA. The MyD88-independent pathway is not mobilized in human neutrophils stimulated via TLR4. *J. Immunol.* Jun; 2007 178(11):7344–7356. [PubMed: 17513785]
69. Malcolm KC, Arndt PG, Manos EJ, Jones DA, Worthen GS. Microarray analysis of lipopolysaccharide-treated human neutrophils. *Am. J. Physiol. Lung Cell. Mol. Physiol.* Apr; 2003 284(4):L663–L670. [PubMed: 12495940]
70. Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. *Blood.* Oct; 2003 102(7):2660–2669. [PubMed: 12829592]
71. Sabroe I, Prince LR, Jones EC, Horsburgh MJ, Foster SJ, Vogel SN, Dower SK, Whyte MK. Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. *J. Immunol.* May; 2003 170(10):5268–5275. [PubMed: 12734376]
72. Kurt-Jones EA, Mandell L, Whitney C, Padgett A, Gosselin K, Newburger PE, Finberg RW. Role of toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin 8 responses in neutrophils. *Blood.* Sep; 2002 100(5):1860–1868. [PubMed: 12176910]
73. Bellocchio S, Moretti S, Perruccio K, Fallarino F, Bozza S, Montagnoli C, Mosci P, Lipford GB, Pitzurra L, Romani L. TLRs govern neutrophil activity in aspergillosis. *J. Immunol.* Dec; 2004 173(12):7406–7415. [PubMed: 15585866]
74. Lotz S, Aga E, Wilde I, van ZG, Hartung T, Solbach W, Laskay T. Highly purified lipoteichoic acid activates neutrophil granulocytes and delays their spontaneous apoptosis via CD14 and TLR2. *J. Leukoc. Biol.* Mar; 2004 75(3):467–477. [PubMed: 14673018]
75. Mantovani B. Different roles of IgG and complement receptors in phagocytosis by polymorphonuclear leukocytes. *J. Immunol.* Jul; 1975 115(1):15–17. [PubMed: 1151058]

76. Nauseef, WM.; Clark, RA. Granulocytic phagocytes. In: Mandell, Gerald L.; Bennett, John E.; Dolin, Raphael, editors. *Principles and Practica of Infectious Diseases*. Churchill Livingstone; New York: 2000. p. 89-112.
77. Fleit HB, Wright SD, Unkeless JC. Human neutrophil Fc gamma receptor distribution and structure. *Proc. Natl. Acad. Sci. U. S. A.* May; 1982 79(10):3275–3279. [PubMed: 6808506]
78. Albrechtsen M, Yeaman GR, Kerr MA. Characterization of the IgA receptor from human polymorphonuclear leucocytes. *Immunology*. Jun; 1988 64(2):201–205. [PubMed: 3292406]
79. Gounni AS, Lamkhioed B, Koussih L, Ra C, Renzi PM, Hamid Q. Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. *FASEB J.* Apr; 2001 15(6):940–949. [PubMed: 11292654]
80. Eggleton P, Ghebrehiwet B, Sastry KN, Coburn JP, Zaner KS, Reid KB, Tauber AI. Identification of a gC1q-binding protein (gC1q-R) on the surface of human neutrophils. Subcellular localization and binding properties in comparison with the cC1q-R. *J. Clin. Invest.* Apr; 1995 95(4):1569–1578. [PubMed: 7706463]
81. Rabellino EM, Ross GD, Polley MJ. Membrane receptors of mouse leukocytes. I. Two types of complement receptors for different regions of C3. *J. Immunol.* Mar; 1978 120(3):879–885. [PubMed: 632590]
82. Ross GD, Jarowski CI, Rabellino EM, Winchester RJ. The sequential appearance of Ia-like antigens and two different complement receptors during the maturation of human neutrophils. *J. Exp. Med.* Mar; 1978 147(3):730–744. [PubMed: 273062]
83. Dana N, Todd RF III, Pitt J, Springer TA, Arnaout MA. Deficiency of a surface membrane glycoprotein (Mo1) in man. *J. Clin. Invest.* Jan; 1984 73(1):153–159. [PubMed: 6361068]
84. Hickstein DD, Ozols J, Williams SA, Baenziger JU, Locksley RM, Roth GJ. Isolation and characterization of the receptor on human neutrophils that mediates cellular adherence. *J. Biol. Chem.* Apr; 1987 262(12):5576–5580. [PubMed: 3553180]
85. Myones BL, Dalzell JG, Hogg N, Ross GD. Neutrophil and monocyte cell surface p150,95 has iC3b-receptor (CR4) activity resembling CR3. *J. Clin. Invest.* Aug; 1988 82(2):640–651. [PubMed: 2969921]
86. Quinn MT, Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with non-phagocyte oxidases. *J. Leukoc. Biol.* Oct; 2004 76(4):760–781. [PubMed: 15240752]
87. DeLeo FR, Renee J, McCormick S, Nakamura M, Apicella M, Weiss JP, Nauseef WM. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J. Clin. Invest.* Jan; 1998 101(2):455–463. [PubMed: 9435318]
88. Guichard C, Pedruzzi E, Dewas C, Fay M, Pouzet C, Bens M, Vandewalle A, Ogier-Denis E, Gougerot-Pocidalo MA, Elbim C. Interleukin-8-induced priming of neutrophil oxidative burst requires sequential recruitment of NADPH oxidase components into lipid rafts. *J. Biol. Chem.* Nov; 2005 280(44):37021–37032. [PubMed: 16115878]
89. Dang PMC, Dewas C, Gaudry M, Fay M, Pedruzzi E, Gougerot-Pocidalo MA, El Benna J. Priming of human neutrophil respiratory burst by granulocyte macrophage colony-stimulating factor (GM-CSF) involves partial phosphorylation of p47^{phox}. *J. Biol. Chem.* Jul; 1999 274(29):20704–20708. [PubMed: 10400704]
90. Brown GE, Stewart MQ, Bissonnette SA, Elia AEH, Wilker E, Yaffe MB. Distinct ligand-dependent roles for p38 MAPK in priming and activation of the neutrophil NADPH oxidase. *J. Biol. Chem.* Jun; 2004 279(26):27059–27068. [PubMed: 15102856]
91. Klebanoff SJ. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *J. Bacteriol.* Jun; 1968 95(6):2131–2138. [PubMed: 4970226]
92. Klebanoff SJ. Role of the superoxide anion in the myeloperoxidase-mediated antimicrobial system. *J. Biol. Chem.* Jun; 1974 249(12):3724–3728. [PubMed: 4366184]
93. Rosen H, Klebanoff SJ. Formation of singlet oxygen by the myeloperoxidase-mediated antimicrobial system. *J. Biol. Chem.* Jul; 1977 252(14):4803–4810. [PubMed: 194894]
94. Rosen H, Klebanoff SJ. Bactericidal activity of a superoxide anion-generating system. A model for the polymorphonuclear leukocyte. *J. Exp. Med.* Jan; 1979 149(1):27–39. [PubMed: 216766]

95. Kobayashi SD, DeLeo FR. An apoptosis differentiation programme in human polymorphonuclear leucocytes. *Biochem. Soc. Trans.* Jun; 2004 32(3):474–476. [PubMed: 15157164]
96. Subrahmanyam YVBK, Yamaga S, Prashar Y, Lee HH, Hoe NP, Kluger Y, Gerstein M, Goguen JD, Newburger PE, Weissman SM. RNA expression patterns change dramatically in human neutrophils exposed to bacteria. *Blood.* Apr; 2001 97(8):2457–2468. [PubMed: 11290611]
97. Kobayashi SD, Voyich JM, Buhl CL, Stahl RM, DeLeo FR. Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. *Proc. Natl. Acad. Sci. U. S. A.* May; 2002 99(10):6901–6906. [PubMed: 11983860]
98. Kobayashi SD, Braughton KR, Whitney AR, Voyich JM, Schwan TG, Musser JM, DeLeo FR. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. *Proc. Natl. Acad. Sci. USA.* Sep; 2003 100(19):10948–10953. [PubMed: 12960399]
99. Borjesson DL, Kobayashi SD, Whitney AR, Voyich JM, Argue CM, DeLeo FR. Insights into pathogen immune evasion mechanisms: *Anaplasma phagocytophilum* fails to induce an apoptosis differentiation program in human neutrophils. *J. Immunol.* May; 2005 174(10):6364–6372. [PubMed: 15879137]
100. Suttman H, Lehan N, Bohle A, Brandau S. Stimulation of neutrophil granulocytes with *Mycobacterium bovis* bacillus Calmette-Guerin induces changes in phenotype and gene expression and inhibits spontaneous apoptosis. *Infect. Immun.* Aug; 2003 71(8):4647–4656. [PubMed: 12874345]
101. Mullick A, Elias M, Harakidas P, Marcil A, Whiteway M, Ge B, Hudson TJ, Caron AW, Bourget L, Picard S, Jovceviski O, Massie B, Thomas DY. Gene expression in HL60 granulocytoids and human polymorphonuclear leukocytes exposed to *Candida albicans*. *Infect. Immun.* Jan; 2004 72(1):414–429. [PubMed: 14688123]
102. Fradin C, Mavor AL, Weindl G, Schaller M, Hanke K, Kaufmann SH, Mollenkopf H, Hube B. The early transcriptional response of human granulocytes to infection with *Candida albicans* is not essential for killing but reflects cellular communications. *Infect. Immun.* Mar; 2007 75(3):1493–1501. [PubMed: 17145939]
103. Sukumaran B, Carlyon JA, Cai JL, Berliner N, Fikrig E. Early transcriptional response of human neutrophils to *Anaplasma phagocytophilum* infection. *Infect. Immun.* Dec; 2005 73(12):8089–8099. [PubMed: 16299303]
104. Lee HC, Kioi M, Han J, Puri RK, Goodman JL. *Anaplasma phagocytophilum*-induced gene expression in both human neutrophils and HL-60 cells. *Genomics.* Sep; 2008 92(3):144–151. [PubMed: 18603403]
105. Lee HC, Goodman JL. *Anaplasma phagocytophilum* causes global induction of antiapoptosis in human neutrophils. *Genomics.* Oct; 2006 88(4):496–503. [PubMed: 16876385]
106. Burlak C, Whitney AR, Mead DJ, Hackstadt T, DeLeo FR. Maturation of Human Neutrophil Phagosomes Includes Incorporation of Molecular Chaperones and Endoplasmic Reticulum Quality Control Machinery. *Mol. Cell Proteomics.* Apr; 2006 5(4):620–634. [PubMed: 16415295]
107. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M. The phagosome proteome: insight into phagosome functions. *J. Cell Biol.* Jan; 2001 152(1):165–180. [PubMed: 11149929]
108. Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, Paiement J, Bergeron JJ, Desjardins M. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell.* Jul; 2002 110(1):119–131. [PubMed: 12151002]
109. Touret N, Paroutis P, Terebiznik M, Harrison RE, Trombetta S, Pypaert M, Chow A, Jiang A, Shaw J, YIP C, Moore HP, van der WN, Houben D, Peters PJ, de CC, Mellman I, Grinstein S. Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. *Cell.* Oct; 2005 123(1):157–170. [PubMed: 16213220]
110. Touret N, Paroutis P, Grinstein S. The nature of the phagosomal membrane: endoplasmic reticulum versus plasmalemma. *J. Leukoc. Biol.* Jun; 2005 77(6):878–885. [PubMed: 15728715]
111. Gagnon E, Bergeron JJ, Desjardins M. ER-mediated phagocytosis: myth or reality? *J. Leukoc. Biol.* Jun; 2005 77(6):843–845. [PubMed: 15845646]

112. Houde M, Bertholet S, Gagnon E, Brunet S, Goyette G, Laplante A, Princiotta MF, Thibault P, Sacks D, Desjardins M. Phagosomes are competent organelles for antigen cross-presentation. *Nature*. Sep; 2003 425(6956):402–406. [PubMed: 14508490]
113. Ackerman AL, Kyritsis C, Tampe R, Cresswell P. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. *Proc. Natl. Acad. Sci. U. S. A.* Oct; 2003 100(22):12889–12894. [PubMed: 14561893]
114. Guermonprez P, Saveanu L, Kleijmeer M, Davoust J, van EP, Amigorena S. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature*. Sep; 2003 425(6956):397–402. [PubMed: 14508489]
115. Lominadze G, Powell DW, Luerman GC, Link AJ, Ward RA, McLeish KR. Proteomic analysis of human neutrophil granules. *Mol. Cell Proteomics*. Oct; 2005 4(10):1503–1521. [PubMed: 15985654]
116. Jethwaney D, Islam MR, Leidal KG, de Bernabe DB, Campbell KP, Nauseef WM, Gibson BW. Proteomic analysis of plasma membrane and secretory vesicles from human neutrophils. *Proteome. Sci.* 2007:512.
117. Savill J. Apoptosis in resolution of inflammation. *J. Leukoc. Biol.* 1997; 61(4):375–380. [PubMed: 9103222]
118. Shi J, Gilbert GE, Kokubo Y, Ohashi T. Role of the liver in regulating numbers of circulating neutrophils. *Blood*. Aug; 2001 98(4):1226–1230. [PubMed: 11493474]
119. Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J. Clin. Invest.* Mar; 1989 83(3):865–875. [PubMed: 2921324]
120. Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J. Immunol.* Jun; 1996 156(11):4422–4428. [PubMed: 8666816]
121. Hannah S, Mecklenburgh K, Rahman I, Bellingan GJ, Greening A, Haslett C, Chilvers ER. Hypoxia prolongs neutrophil survival in vitro. *FEBS Lett.* Sep; 1995 372(2–3):233–237. [PubMed: 7556675]
122. Murray J, Barbara JA, Dunkley SA, Lopez AF, Van O,X, Condliffe AM, Dransfield I, Haslett C, Chilvers ER. Regulation of neutrophil apoptosis by tumor necrosis factor- α : requirement for TNFR55 and TNFR75 for induction of apoptosis in vitro. *Blood*. Oct; 1997 90(7):2772–2783. [PubMed: 9326245]
123. Whyte MK, Hardwick SJ, Meagher LC, Savill JS, Haslett C. Transient elevations of cytosolic free calcium retard subsequent apoptosis in neutrophils in vitro. *J. Clin. Invest.* Jul; 1993 92(1):446–455. [PubMed: 8392090]
124. Bours MJ, Swennen EL, Di VF, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol. Ther.* Nov; 2006 112(2):358–404. [PubMed: 16784779]
125. Savill JS, Henson PM, Haslett C. Phagocytosis of aged human neutrophils by macrophages is mediated by a novel “charge-sensitive” recognition mechanism. *J. Clin. Invest.* Nov; 1989 84(5):1518–1527. [PubMed: 2553775]
126. Hart SP, Ross JA, Ross K, Haslett C, Dransfield I. Molecular characterization of the surface of apoptotic neutrophils: Implications for functional downregulation and recognition by phagocytes. *Cell Death Differ.* May; 2000 7(5):493–503. [PubMed: 10800083]
127. Homburg CH, de HM, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. *Blood*. Jan; 1995 85(2):532–540. [PubMed: 7812008]
128. Kobayashi SD, Voyich JM, Braughton KR, DeLeo FR. Down-regulation of proinflammatory capacity during apoptosis in human polymorphonuclear leukocytes. *J. Immunol.* Mar; 2003 170(6):3357–3368. [PubMed: 12626596]
129. Fadok VA, Savill JS, Haslett C, Bratton DL, Doherty DE, Campbell PA, Henson PM. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. *J. Immunol.* Dec; 1992 149(12):4029–4035. [PubMed: 1281199]

130. Lane TA, Lamkin GE. A reassessment of the energy requirements for neutrophil migration: adenosine triphosphate depletion enhances chemotaxis. *Blood*. Nov; 1984 64(5):986–993. [PubMed: 6487808]
131. Peachman KK, Lyles DS, Bass DA. Mitochondria in eosinophils: functional role in apoptosis but not respiration. *Proc. Natl. Acad. Sci. U. S. A.* Feb; 2001 98(4):1717–1722. [PubMed: 11172017]
132. Fossati G, Moulding DA, Spiller DG, Moots RJ, White MR, Edwards SW. The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. *J. Immunol.* Feb; 2003 170(4):1964–1972. [PubMed: 12574365]
133. Maianski NA, Mul FP, Van Buul JD, Roos D, Kuijpers TW. Granulocyte colony-stimulating factor inhibits the mitochondria-dependent activation of caspase-3 in neutrophils. *Blood*. Jan; 2002 99(2):672–679. [PubMed: 11781253]
134. Pryde JG, Walker A, Rossi AG, Hannah S, Haslett C. Temperature-dependent arrest of neutrophil apoptosis. Failure of Bax insertion into mitochondria at 15 degrees C prevents the release of cytochrome c. *J. Biol. Chem.* Oct; 2000 275(43):33574–33584. [PubMed: 10896657]
135. Maianski NA, Geissler J, Srinivasula SM, Alnemri ES, Roos D, Kuijpers TW. Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. *Cell Death. Differ.* Feb; 2004 11(2):143–153. [PubMed: 14576767]
136. El KD, Jozsef L, Khreiss T, Filep JG. Inhibition of K⁺ efflux prevents mitochondrial dysfunction, and suppresses caspase-3-, apoptosis-inducing factor-, and endonuclease G-mediated constitutive apoptosis in human neutrophils. *Cell Signal.* Dec; 2006 18(12):2302–2313. [PubMed: 16806822]
137. Murphy BM, O'Neill AJ, Adrain C, Watson RW, Martin SJ. The apoptosome pathway to caspase activation in primary human neutrophils exhibits dramatically reduced requirements for cytochrome C. *J. Exp. Med.* Mar; 2003 197(5):625–632. [PubMed: 12615903]
138. Hill JM, Vaidyanathan H, Ramos JW, Ginsberg MH, Werner MH. Recognition of ERK MAP kinase by PEA-15 reveals a common docking site within the death domain and death effector domain. *EMBO J.* Dec; 2002 21(23):6494–6504. [PubMed: 12456656]
139. Khwaja A, Tatton L. Caspase-mediated proteolysis and activation of protein kinase Cdelta plays a central role in neutrophil apoptosis. *Blood*. Jul; 1999 94(1):291–301. [PubMed: 10381525]
140. Brown SB, Savill J. Phagocytosis triggers macrophage release of Fas ligand and induces apoptosis of bystander leukocytes. *J. Immunol.* Jan; 1999 162(1):480–485. [PubMed: 9886423]
141. Scheel-Toellner D, Wang K, Craddock R, Webb PR, McGettrick HM, Assi LK, Parkes N, Clough LE, Gulbins E, Salmon M, Lord JM. Reactive oxygen species limit neutrophil life span by activating death receptor signaling. *Blood*. Oct; 2004 104(8):2557–2564. [PubMed: 15238425]
142. Liles WC, Kiener PA, Ledbetter JA, Aruffo A, Klebanoff SJ. Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: Implications for the regulation of apoptosis in neutrophils. *J. Exp. Med.* 1996; 184(2):429–440. [PubMed: 8760796]
143. Renshaw SA, Parmar JS, Singleton V, Rowe SJ, Dockrell DH, Dower SK, Bingle CD, Chilvers ER, Whyte MK. Acceleration of human neutrophil apoptosis by TRAIL. *J. Immunol.* Jan; 2003 170(2):1027–1033. [PubMed: 12517970]
144. Scheel-Toellner D, Wang K, Assi LK, Webb PR, Craddock RM, Salmon M, Lord JM. Clustering of death receptors in lipid rafts initiates neutrophil spontaneous apoptosis. *Biochem. Soc. Trans.* Nov; 2004 32(5):679–681. [PubMed: 15493986]
145. Conus S, Perozzo R, Reinheckel T, Peters C, Scapozza L, Yousefi S, Simon HU. Caspase-8 is activated by cathepsin D initiating neutrophil apoptosis during the resolution of inflammation. *J. Exp. Med.* Mar; 2008 205(3):685–698. [PubMed: 18299403]
146. Zhang B, Hirahashi J, Cullere X, Mayadas TN. Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross-talk between caspase 8, reactive oxygen species, and MAPK/ERK activation. *J. Biol. Chem.* Aug; 2003 278(31):28443–28454. [PubMed: 12736263]
147. Baumann R, Casaulta C, Simon D, Conus S, Yousefi S, Simon HU. Macrophage migration inhibitory factor delays apoptosis in neutrophils by inhibiting the mitochondria-dependent death pathway. *FASEB J.* Dec; 2003 17(15):2221–2230. [PubMed: 14656984]

148. varado-Kristensson M, Melander F, Leandersson K, Ronnstrand L, Wernstedt C, Andersson T. p38-MAPK signals survival by phosphorylation of caspase-8 and caspase-3 in human neutrophils. *J. Exp. Med.* Feb; 2004 199(4):449–458. [PubMed: 14970175]
149. DeLeo FR. Modulation of phagocyte apoptosis by bacterial pathogens. *Apoptosis.* Jul; 2004 9(4): 399–413. [PubMed: 15192322]
150. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood.* Oct; 1992 80(8):2012–2020. [PubMed: 1382715]
151. Biffl WL, Moore EE, Moore FA, Barnett CC Jr. Interleukin-6 suppression of neutrophil apoptosis is neutrophil concentration dependent. *J. Leukoc. Biol.* Nov; 1995 58(5):582–584. [PubMed: 7595060]
152. Kettritz R, Gaido ML, Haller H, Luft FC, Jennette CJ, Falk RJ. Interleukin-8 delays spontaneous and tumor necrosis factor- α -mediated apoptosis of human neutrophils. *Kidney Int.* Jan; 1998 53(1):84–91. [PubMed: 9453003]
153. Dunican AL, Leuenroth SJ, Grutkoski P, Ayala A, Simms HH. TNF α -induced suppression of PMN apoptosis is mediated through interleukin-8 production. *Shock.* Sep; 2000 14(3):284–288. [PubMed: 11028544]
154. Sakamoto E, Hato F, Kato T, Sakamoto C, Akahori M, Hino M, Kitagawa S. Type I and type II interferons delay human neutrophil apoptosis via activation of STAT3 and up-regulation of cellular inhibitor of apoptosis 2. *J. Leukoc. Biol.* Jul; 2005 78(1):301–309. [PubMed: 15845643]
155. Lee A, Whyte MK, Haslett C. Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J. Leukoc. Biol.* Oct; 1993 54(4):283–288. [PubMed: 8409750]
156. van den Berg JM, Weyer S, Weening JJ, Roos D, Kuijpers TW. Divergent effects of tumor necrosis factor α on apoptosis of human neutrophils. *J. Leukoc. Biol.* Mar; 2001 69(3):467–473. [PubMed: 11261795]
157. Avdi NJ, Nick JA, Whitlock BB, Billstrom MA, Henson PM, Johnson GL, Worthen GS. Tumor necrosis factor- α activation of the c-Jun N-terminal kinase pathway in human neutrophils. *J. Biol. Chem.* Jan; 2001 276(3):2189–2199. [PubMed: 11053415]
158. Ward C, Chilvers ER, Lawson MF, Pryde JG, Fujihara S, Farrow SN, Haslett C, Rossi AG. NF- κ B activation is a critical regulator of human granulocyte apoptosis in vitro. *J. Biol. Chem.* Feb; 1999 274(7):4309–4318. [PubMed: 9933632]
159. Whitlock BB, Gardai S, Fadok V, Bratton D, Henson PM. Differential roles for α (M) β (2) integrin clustering or activation in the control of apoptosis via regulation of akt and ERK survival mechanisms. *J. Cell Biol.* Dec; 2000 151(6):1305–1320. [PubMed: 11121444]
160. Rubel C, Gomez S, Fernandez GC, Isturiz MA, Caamano J, Palermo MS. Fibrinogen-CD11b/CD18 interaction activates the NF- κ B pathway and delays apoptosis in human neutrophils. *Eur. J. Immunol.* May; 2003 33(5):1429–1438.
161. Gardai S, Whitlock BB, Helgason C, Ambruso D, Fadok V, Bratton D, Henson PM. Activation of SHIP by NADPH oxidase-stimulated Lyn leads to enhanced apoptosis in neutrophils. *J. Biol. Chem.* Feb; 2002 277(7):5236–5246. [PubMed: 11724799]
162. Francois S, El BJ, Dang PM, Pedruzzi E, Gougerot-Pocidalo MA, Elbim C. Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF- κ B signaling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J. Immunol.* Mar; 2005 174(6):3633–3642. [PubMed: 15749901]
163. Moulding DA, Walter C, Hart CA, Edwards SW. Effects of staphylococcal enterotoxins on human neutrophil functions and apoptosis. *Infect. Immun.* May; 1999 67(5):2312–2318. [PubMed: 10225889]
164. Liu J, Akahoshi T, Sasahana T, Kitasato H, Namai R, Sasaki T, Inoue M, Kondo H. Inhibition of neutrophil apoptosis by verotoxin 2 derived from *Escherichia coli* O157:H7. *Infect. Immun.* Nov; 1999 67(11):6203–6205. [PubMed: 10531291]
165. Brigotti M, Carnicelli D, Ravanelli E, Barbieri S, Ricci F, Bontadini A, Tozzi AE, Scavia G, Caprioli A, Tazzari PL. Interactions between Shiga toxins and human polymorphonuclear leukocytes. *J. Leukoc. Biol.* Oct; 2008 84(4):1019–1027. [PubMed: 18625912]

166. Liles WC, Thomsen AR, O'Mahony DS, Klebanoff SJ. Stimulation of human neutrophils and monocytes by staphylococcal phenol-soluble modulin. *J. Leukoc. Biol.* Jul; 2001 70(1):96–102. [PubMed: 11435491]
167. Coxon A, Rieu P, Barkalow FJ, Askari S, Sharpe AH, von Adrian UH, Arnaout MA, Mayadas TN. A novel role for the $\beta 2$ integrin CD11b/CD18 in neutrophil apoptosis: A homeostatic mechanism in inflammation. *Immunity.* 1996; 5(6):653–666. [PubMed: 8986723]
168. Gamberale R, Giordano M, Trevani AS, Andonegui G, Geffner JR. Modulation of human neutrophil apoptosis by immune complexes. *J. Immunol.* Oct; 1998 161(7):3666–3674. [PubMed: 9759891]
169. Watson RW, Redmond HP, Wang JH, Bouchier-Hayes D. Bacterial ingestion, tumor necrosis factor- α , and heat induce programmed cell death in activated neutrophils. *Shock.* Jan; 1996 5(1):47–51. [PubMed: 8821103]
170. Engelich G, White M, Hartshorn KL. Neutrophil survival is markedly reduced by incubation with influenza virus and *Streptococcus pneumoniae*: role of respiratory burst. *J. Leukoc. Biol.* Jan; 2001 69(1):50–56. [PubMed: 11200067]
171. Yoshiie K, Kim HY, Mott J, Rikihisa Y. Intracellular infection by the human granulocytic ehrlichiosis agent inhibits human neutrophil apoptosis. *Infect. Immun.* Mar; 2000 68(3):1125–1133. [PubMed: 10678916]
172. van ZG, Gieffers J, Kothe H, Rupp J, Bollinger A, Aga E, Klinger M, Brade H, Dalhoff K, Maass M, Solbach W, Laskay T. Chlamydia pneumoniae multiply in neutrophil granulocytes and delay their spontaneous apoptosis. *J. Immunol.* Feb; 2004 172(3):1768–1776. [PubMed: 14734760]
173. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat. Med.* Dec; 2007 13(12):1510–1514. [PubMed: 17994102]
174. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR. Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *J. Immunol.* Sep; 2005 175(6):3907–3919. [PubMed: 16148137]
175. Genestier AL, Michallet MC, Prevost G, Bellot G, Chalabreysse L, Peyrol S, Thivolet F, Etienne J, Lina G, Vallette FM, Vandenesch F, Genestier L. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J. Clin. Invest.* Nov; 2005 115(11):3117–3127. [PubMed: 16276417]
176. Usher LR, Lawson RA, Geary I, Taylor CJ, Bingle CD, Taylor GW, Whyte MK. Induction of neutrophil apoptosis by the *Pseudomonas aeruginosa* exotoxin pyocyanin: a potential mechanism of persistent infection. *J. Immunol.* Feb; 2002 168(4):1861–1868. [PubMed: 11823520]
177. Ward C, Dransfield I, Chilvers ER, Haslett C, Rossi AG. Pharmacological manipulation of granulocyte apoptosis: potential therapeutic targets. *Trends Pharmacol. Sci.* Dec; 1999 20(12):503–509. [PubMed: 10603493]
178. Kobayashi SD, Voyich JM, Somerville GA, Braughton KR, Malech HL, Musser JM, DeLeo FR. An apoptosis-differentiation program in human polymorphonuclear leukocytes facilitates resolution of inflammation. *J. Leukoc. Biol.* Feb; 2003 73(2):315–322. [PubMed: 12554809]
179. Musser JM, DeLeo FR. Toward a genome-wide systems biology analysis of host-pathogen interactions in group A *Streptococcus*. *Am. J. Pathol.* Dec; 2005 167(6):1461–1472. [PubMed: 16314461]
180. Mott J, Rikihisa Y. Human granulocytic ehrlichiosis agent inhibits superoxide anion generation by human neutrophils. *Infect. Immun.* Dec; 2000 68(12):6697–6703. [PubMed: 11083784]
181. Carlyon JA, Latif DA, Pypaert M, Lacy P, Fikrig E. *Anaplasma phagocytophilum* utilizes multiple host evasion mechanisms to thwart NADPH oxidase-mediated killing during neutrophil infection. *Infect. Immun.* Aug; 2004 72(8):4772–4783. [PubMed: 15271939]
182. Carlyon JA, Chan WT, Galan J, Roos D, Fikrig E. Repression of *rac2* mRNA expression by *Anaplasma phagocytophilum* is essential to the inhibition of superoxide production and bacterial proliferation. *J. Immunol.* Dec; 2002 169(12):7009–7018. [PubMed: 12471136]

183. Banerjee R, Anguita J, Roos D, Fikrig E. Cutting edge: infection by the agent of human granulocytic ehrlichiosis prevents the respiratory burst by down-regulating gp91phox. *J. Immunol.* Apr; 2000 164(8):3946–3949. [PubMed: 10754283]
184. Persson YA, Blomgran-Julinder R, Rahman S, Zheng L, Stendahl O. Mycobacterium tuberculosis-induced apoptotic neutrophils trigger a pro-inflammatory response in macrophages through release of heat shock protein 72, acting in synergy with the bacteria. *Microbes. Infect.* Mar; 2008 10(3):233–240. [PubMed: 18328761]
185. McPhillips K, Janssen WJ, Ghosh M, Byrne A, Gardai S, Remigio L, Bratton DL, Kang JL, Henson P. TNF-alpha inhibits macrophage clearance of apoptotic cells via cytosolic phospholipase A2 and oxidant-dependent mechanisms. *J. Immunol.* Jun; 2007 178(12):8117–8126. [PubMed: 17548650]
186. Michlewska S, Dransfield I, Megson IL, Rossi AG. Macrophage phagocytosis of apoptotic neutrophils is critically regulated by the opposing actions of pro-inflammatory and anti-inflammatory agents: key role for TNF- α . *FASEB J.* Oct.2008
187. Volpp BD, Nauseef WM, Clark RA. Two cytosolic neutrophil oxidase components absent in autosomal chronic granulomatous disease. *Science.* Dec; 1988 242(4883):1295–1297. [PubMed: 2848318]
188. Curnutte JT, Scott PJ, Mayo LA. Cytosolic components of the respiratory burst oxidase: resolution of four components, two of which are missing in complementing types of chronic granulomatous disease. *Proc. Natl. Acad. Sci. USA.* Feb; 1989 86(3):825–829. [PubMed: 2915980]
189. Nunoi H, Rotrosen D, Gallin JI, Malech HL. Two forms of autosomal chronic granulomatous disease lack distinct neutrophil cytosol factors. *Science.* Dec; 1988 242(4883):1298–1301. [PubMed: 2848319]
190. Hamers MN, deBoer M, Meirhof LJ, Weening RS, Roos D. Complementation in monocyte hybrids revealing genetic heterogeneity in chronic granulomatous disease. *Nature.* Feb; 1984 307(5951):553–555. [PubMed: 6320013]
191. Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, Curnutte JT, Orkin SH. Cloning the gene for an inherited human disorder--chronic granulomatous disease--on the basis of its chromosomal location. *Nature.* Jul; 1986 322(6074):32–38. [PubMed: 2425263]
192. Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. *N. Engl. J. Med.* Dec; 2000 343(23):1703–1714. [PubMed: 11106721]
193. Kobayashi SD, Voyich JM, Braughton KR, Whitney AR, Nauseef WM, Malech HL, DeLeo FR. Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. *J. Immunol.* Jan; 2004 172(1):636–643. [PubMed: 14688376]
194. Lekstrom-Himes JA, Kuhns DB, Alvord WG, Gallin JI. Inhibition of human neutrophil IL-8 production by hydrogen peroxide and dysregulation in chronic granulomatous disease. *J. Immunol.* Jan; 2005 174(1):411–417. [PubMed: 15611265]
195. Lekstrom-Himes J, Xanthopoulos KG. Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J. Biol. Chem.* Oct; 1998 273(44):28545–28548. [PubMed: 9786841]
196. Yamanaka R, Barlow C, Lekstrom-Himes J, Castilla LH, Liu PP, Eckhaus M, Decker T, Wynshaw-Boris A, Xanthopoulos KG. Impaired granulopoiesis, myelodysplasia, and early lethality in CCAAT/enhancer binding protein epsilon-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* Nov; 1997 94(24):13187–13192. [PubMed: 9371821]
197. Lekstrom-Himes J, Xanthopoulos KG. CCAAT/enhancer binding protein epsilon is critical for effective neutrophil-mediated response to inflammatory challenge. *Blood.* May; 1999 93(9):3096–3105. [PubMed: 10216107]
198. Gombart AF, Krug U, O'Kelly J, An E, Vegesna V, Koeffler HP. Aberrant expression of neutrophil and macrophage-related genes in a murine model for human neutrophil-specific granule deficiency. *J. Leukoc. Biol.* Nov; 2005 78(5):1153–1165. [PubMed: 16204633]
199. Davis SD, Schaller J, Wedgwood RJ. Job's Syndrome. Recurrent, "cold", staphylococcal abscesses. *Lancet.* May; 1966 1(7445):1013–1015. [PubMed: 4161105]

200. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, Pasic S, Stojkovic O, Metin A, Karasuyama H. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature*. Aug; 2007 448(7157):1058–1062. [PubMed: 17676033]
201. O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. *Immunity*. Apr; 2008 28(4):477–487. [PubMed: 18400190]
202. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* May; 2008 8(5):349–361. [PubMed: 18437155]
203. Hong S, Porter TF, Lu Y, Oh SF, Pillai PS, Serhan CN. Resolvin E1 metabolome in local inactivation during inflammation-resolution. *J. Immunol.* Mar; 2008 180(5):3512–3519. [PubMed: 18292578]
204. Serhan CN, Lu Y, Hong S, Yang R. Mediator lipidomics: search algorithms for eicosanoids, resolvins, and protectins. *Methods Enzymol.* 2007:432275–317.
205. Serhan CN, Hong S, Lu Y. Lipid mediator informatics-lipidomics: novel pathways in mapping resolution. *AAPS. J.* 2006; 8(2):E284–E297. [PubMed: 16796379]
206. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, Dooling D, Dunford-Shore BH, McGrath S, Hickenbotham M, Cook L, Abbott R, Larson DE, Koboldt DC, Pohl C, Smith S, Hawkins A, Abbott S, Locke D, Hillier LW, Miner T, Fulton L, Magrini V, Wylie T, Glasscock J, Conyers J, Sander N, Shi X, Osborne JR, Minx P, Gordon D, Chinwalla A, Zhao Y, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson M, Baty J, Ivanovich J, Heath S, Shannon WD, Nagarajan R, Walter MJ, Link DC, Graubert TA, DiPersio JF, Wilson RK. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature*. Nov; 2008 456(7218):66–72. [PubMed: 18987736]

Fig. 1. Overview of neutrophil functions / processes that have been investigated using proteomics (indicated by 2-D gel) or transcriptomics (indicated with a cDNA microarray). See text for details.

Fig. 2. Neutrophil phagocytosis and microbicidal process. Panel A illustrates binding and phagocytosis of a microbe opsonized with antibody or serum complement. Phagocytosis triggers production of superoxide ($O_2^{\bullet -}$) from which other secondarily derived ROS are formed, including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Panel B is a transmission electron micrograph of a human neutrophil that has phagocytosed numerous *Staphylococcus aureus* (Microbe).

Fig. 3. Neutrophil apoptosis. Human neutrophils were isolated from venous blood and then processed for transmission electron microscopy or stained with Wright-Giemsa (inset) after purification (0 h) or 24 h in culture.

Fig. 4.

Neutrophil apoptosis differentiation program. Post-phagocytosis sequelae identified using microarray-based approaches followed by in vitro assays with human neutrophils. The neutrophil apoptosis differentiation program represents a final stage of transcriptionally regulated PMN maturation that is accelerated by phagocytosis.

Fig. 5.
A schematic that illustrates two fundamental outcomes of microbe-neutrophil interaction.
See text for details.

Fig. 6. Comparison of transcript levels in PMNs and peripheral blood mononuclear cells (PBMCs) from patients with HIES and control subjects. Principal component analysis of baseline transcript levels in PMNs (A) or PBMCs (B) from control subjects and patients. C) Transcripts increased (yellow) or decreased (blue) in patient leukocytes. D) A modified Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com) of the PMN microarray data indicating relative changes in transcripts encoding molecules involved in interferon and STAT signal transduction. Yellow nodes indicate transcripts that are increased and blue nodes indicate transcripts that are decreased in HIES patient PMNs. Adapted from Fig. 2 of the *Supplementary Appendix* of Holland et al. [4].

Table 1

Selected processes or functions of neutrophils discovered using genome-wide approaches.

Function or process	Discovery or conclusion	Ref.
Granulopoiesis	Positive correlation between protein and mRNA levels during neutrophil development	[26–28]
	Temporal regulation of transcripts encoding granule proteins during neutrophilic differentiation of human primary bone marrow progenitors.	[35]
	Lineage specificity in gene expression among hematopoietic cells.	[33]
	Mature neutrophils have increased capacity to respond to interferon-gamma	[29]
	Coordinate gene regulation during cellular differentiation is influenced by chromosomal organization.	[12]
Recruitment		
Priming	Comprehensive view of the similarities and differences between priming agents and phagocytic stimuli	[54]
	Lipopolysaccharide elicits changes in expression of interferon-stimulated genes (<i>ISGs</i>)	[67, 69]
Transmigration	Regulates cell fate and promotes wound healing	[30]
Spontaneous apoptosis	Comprehensive view of molecules involved in the loss of neutrophil function during apoptosis. This study reveals the remarkable number of host defense-related molecules that are down-regulated during constitutive (spontaneous) neutrophil apoptosis.	[59]
Phagocytosis		
Phagocytosis-induced cell death (PICD)	Phagocytosis induces a neutrophil apoptosis differentiation program, a final stage of transcriptionally regulated PMN maturation. Neutrophil programmed cell death is regulated at the level of gene expression, and thus PMN gene regulation facilitates resolution of inflammation.	[97, 98, 128]
	Comprehensive view of the down-regulation of proinflammatory capacity during PICD. The study highlights the sheer magnitude of change in proinflammatory molecules during apoptosis.	[128]
	Bacterial pathogens modulate the neutrophil apoptosis differentiation program. This work forms the basis of a new paradigm for the resolution of infection. The discovery is that there are two possible outcomes of neutrophil-bacteria interactions; one leads to resolution of infection and the other to disease (see Fig. 4).	[98]
	PICD is delayed in neutrophils from chronic granulomatous disease patients and ROS are critical for PICD.	[193]
	Neutrophil phagosomes contain proteins typically associated with the endoplasmic reticulum. This finding suggests neutrophil phagosomes have potential for antigen processing	[106]
Delayed apoptosis	<i>A. phagocytophilum</i> fails to induce the apoptosis differentiation program in human neutrophils.	[99, 103–105]
	GM-CSF enhances neutrophil pro-inflammatory capacity by up-regulating dozens of molecules involved in host defense. Neutrophils have potential for antigen presentation.	[59]