Multiple Phenotypic Changes Define Neutrophil Priming

Irina Miralda 1, Silvia M. Uriarte 1,2 and Kenneth R. McLeish 2,3*

1 Department of Microbiology, University of Louisville School of Medicine, Louisville, KY, United States, 2 Department of Medicine, University of Louisville School of Medicine, Louisville, KY, United States, 3 Robley Rex VA Medical Center, Louisville, KY, United States

Exposure to pro-inflammatory cytokines, chemokines, mitochondrial contents, and bacterial and viral products induces neutrophils to transition from a basal state into a primed one, which is currently defined as an enhanced response to activating stimuli. Although, typically associated with enhanced generation of reactive oxygen species (ROS) by the NADPH oxidase, primed neutrophils show enhanced responsiveness of exocytosis, NET formation, and chemotaxis. Phenotypic changes associated with priming also include activation of a subset of functions, including adhesion, transcription, metabolism, and rate of apoptosis. This review summarizes the breadth of phenotypic changes associated with priming and reviews current knowledge of the molecular mechanisms behind those changes. We conclude that the current definition of priming is too restrictive. Priming represents a combination of enhanced responsiveness and activated functions that regulate both adaptive and innate immune responses.

Keywords: neutrophils, priming, cytokines, chemotaxis, apoptosis, phagocytosis, respiratory burst, exocytosis

INTRODUCTION

Polymorphonuclear leukocytes, or neutrophils, account for 40–60% of peripheral blood leukocytes in humans (Summers et al., 2010). They play an essential role in the innate immune response, as demonstrated by the development of life-threatening infections or uncontrolled inflammation in individuals with severe neutropenia or genetic disruption of neutrophil anti-microbial capabilities (Kannengiesser et al., 2008; van de Vijver et al., 2012; Moutsopoulos et al., 2014; Nauseef and Borregaard, 2014). Figure 1 shows the multistep process of neutrophil recruitment in response to microbial invasion, including adhesion to vascular endothelium, transmigration into the interstitial space, chemotaxis/chemokinesis toward the site of infection, phagocytosis of pathogens, destruction of microbes within phagosomes by release of antimicrobial granule contents following granule fusion and ROS generation at the phagosomal membrane, and amplification and organization of the inflammatory response. Uncontrolled or prolonged neutrophil activation uses antimicrobial responses to injure normal host cells, leading to pathologic changes to tissues and organs in autoimmune and inflammatory diseases (Nathan, 2006). Consequently, neutrophil activation is normally tightly regulated.

Circulating neutrophils exist in a basal state, characterized by non-adherence, a round morphology, minimal transcriptional activity, and a limited capacity to respond to activating stimuli. That limited response protects against unwarranted inflammatory responses and tissue injury (Sheppard et al., 2005). To effectively clear invading organisms, neutrophils must be capable of mounting rapid, vigorous responses to activating stimuli. The transition to a state of enhanced...
responsiveness has been termed priming (Condliffe et al., 1998; El-Benna et al., 2008; Wright et al., 2013). It occurs in vitro following neutrophil exposure to pro-inflammatory lipids and cytokines, chemokines, mitochondrial contents, and bacterial and viral products (El-Benna et al., 2008). Neutrophil priming in vitro represents an in vivo phenomena, as primed neutrophils have been identified in humans with infections, rheumatoid arthritis, chronic kidney disease, traumatic injury, and acute respiratory distress syndrome (Bass et al., 1986; McLeish et al., 1996; Ogura et al., 1999; Naegele et al., 2012). Although, substantial circumstantial evidence suggests that primed neutrophils participate in a number of human diseases, direct evidence is lacking. The relative contribution of neutrophil priming to the severity of human inflammatory diseases is an important gap in knowledge that needs to be addressed.

Historically, the term “priming” was primarily used to describe the augmented reactive oxygen species (ROS) generation upon neutrophil stimulation because of the depth of knowledge of molecular mechanisms of NADPH oxidase complex assembly, the ease of measurement of ROS generation, and the importance of ROS to anti-microbial activity. Figure 1 illustrates that primed neutrophils demonstrate a number of phenotypic changes in addition to enhanced NADPH oxidase activation, including granule release, cytokine and lipid synthesis, adhesion and transmigration, enhanced chemotaxis, and delayed apoptosis. Thus, neutrophil priming is not just a transition state in which neutrophils become more responsive to activating stimuli. We believe a new definition of priming is required to include the activation of a subset of neutrophil functions as opposed solely to a heightened state of responsiveness. In this review of the recent advances in neutrophil priming, we will highlight the functional evidence for the activation of a subset of neutrophil functions during priming and review the current state of knowledge of the molecular basis for those phenotypic changes to illustrate this new definition. Our goal is to encourage research that will provide a more complete understanding of priming, leading to identification of new targets for treatment of inflammatory and infectious diseases. Much of our discussion focuses on the effects of TNFα, as studies frequently use that cytokine as a model priming agent. The large number of agents capable of initiating priming of neutrophil respiratory burst activity was recently reviewed (El-Benna et al., 2016). We compare current state of knowledge of the effects of
those priming agents on the various phenotypic changes to those induced by TNFα in Table 1.

PHENOTYPIC CHANGES DURING PRIMING

Respiratory Burst Activity

For decades, enhanced respiratory burst activity has defined a primed neutrophil. The respiratory burst generates ROS through conversion of molecular oxygen to superoxide by the multi-component NADPH oxidase complex. The oxidase is comprised of three membrane subunits (gp91phox/NOX2, p22phox, and Rap1A) and four cytosolic proteins (p47phox, p67phox, p40phox, and Rac2). Spatial separation of the membrane and cytosolic components maintains enzymatic inactivity in resting neutrophils. Upon stimulation, the cytosolic components translocate to the membrane to form the catalytically active enzyme complex. Phosphorylation of cytosolic NADPH oxidase components is necessary for translocation of those components to the plasma membrane. One of the major targets of phosphorylation is the p47phox subunit. Phosphorylation of a number of serines (Ser303–Ser379) early in the activation process facilitates p47phox docking to membrane and cytosolic oxidase components, leading to assembly of the functional oxidase (El-Benna et al., 1994, 1996; Groemping et al., 2003).

Non-receptor tyrosine kinases and p38 mitogen-activated protein kinase (MAPK) are signaling molecules that participate in priming respiratory burst activity by TNFα (El-Benna et al., 1996; McLeish et al., 1998; Forsberg et al., 2001; Dewas et al., 2003; Boussetta et al., 2010). Inhibition of tyrosine kinase activity blocks the activation of p38 MAPK by TNFα (McLeish et al., 1998), indicating that tyrosine kinases participate in priming by activating p38 MAPK. TNFα-mediated activation of the p38 MAPK pathway contributes to priming by enhancing plasma membrane translocation of the cytosolic components of the NADPH oxidase and by increasing expression of the plasma membrane oxidase components. Enhanced translocation of cytosolic components results from p38 MAPK-dependent phosphorylation of Ser345 on p47phox. Phosphorylation of Ser345 initiates a series of conformational changes in p47phox that result in hyperactivation of the NADPH oxidase. The initial event is binding of the prolyl isomerase Pin1 to the phospho-Ser345 site (Boussetta et al., 2010). This produces a conformational change in p47phox that exposes additional amino acids for phosphorylation by protein kinase C (PKC). Phosphorylation by PKC produces a second conformational change that promotes p47phox binding to p22phox. That interaction leads to translocation and assembly of all the cytosolic oxidase components with the membrane NADPH oxidase components. Pin1 is also involved in priming by GM-CSF and CL097, a TLR8 agonist (Makni-Maalej et al., 2012, 2015). Unlike TNFα, GM-CSF induces phosphorylation of Ser345 on p47phox through activation of ERK1/2, not p38 MAPK (Boussetta et al., 2010; Makni-Maalej et al., 2015). This observation indicates that multiple signal transduction pathways induce the same molecular events required for priming. Those redundant signal transduction pathways are unlikely to serve as effective therapeutic targets.

Over a decade ago, it was suggested that TNFα and LPS play a role in respiratory burst priming by influencing membrane trafficking (DeLeo et al., 1998; Ward et al., 2000). Direct confirmation was provided recently by selectively blocking exocytosis prior to priming through the use of cell-permeable, peptide inhibitors of SNARE protein interactions (Uriarte et al., 2011; McLeish et al., 2013). Those studies determined that exocytosis of secretory vesicles and gelatinase granules is required for priming by TNFα and platelet activating factor. Exocytosis could be contributing to priming by increasing plasma membrane expression of receptors, signaling molecules, and/or NADPH oxidase membrane components. The role of receptor and signaling molecule expression in priming was examined by measuring the activation of p38 MAPK and ERK1/2 in neutrophils primed during inhibition of exocytosis (Uriarte et al., 2011). The absence of granule exocytosis had no effect on activation of either MAPK, indicating that increased expression of receptors and signaling molecules does not contribute to priming (Uriarte et al., 2011). Inhibition of Pin1 activity had no effect on neutrophil granule exocytosis (McLeish et al., 2013). We interpret those studies to indicate that enhanced translocation of cytosolic oxidase components and increased expression of membrane oxidase components are independent events, both of which are required for priming.

A second membrane trafficking event that participates in priming respiratory burst activity is clathrin-mediated endocytosis. Moreland and colleagues reported that the NADPH oxidase assembles on endosomes, and the subsequent H2O2 production was required for neutrophil priming by endotoxin (Moreland et al., 2007; Volk et al., 2011; Lamb et al., 2012). We have confirmed those observations and determined that endocytosis is an upstream event in neutrophil granule exocytosis.

Neutrophil Granule Release

Neutrophil granules are divided into four classes based on granule density and contents (Borregaard and Cowland, 1997; Lominadze et al., 2005; Rørvig et al., 2013). Secretory vesicles are created by endocytosis, while gelatinase (tertiary), specific (secondary), and azurophilic (primary) granules are formed from the trans-Golgi network during neutrophil maturation (Borregaard, 2010). Granule subsets undergo an ordered release based on stimulus intensity, termed graded exocytosis (Sengelov et al., 1993, 1995). Secretory vesicles undergo exocytosis more easily and completely than gelatinase granules. Specific and azurophilic granules, which contain toxic anti-microbial components, undergo the most limited exocytosis. An in vivo study showed that neutrophils migrating into a skin blister created in normal human subjects release nearly 100% of their secretory vesicles, 40% of gelatinase granules, 20% of specific granules, and <10% of azurophilic granules (Sengelov et al., 1995).

We recently reported that TNFα directly stimulated exocytosis of secretory vesicles and gelatinase granules (McLeish et al., 2017). Those results support previous studies showing that
Table 1 shows the phenotypic changes induced by agents known to prime neutrophil respiratory burst activity. All the priming agents listed in this table are known inducers of enhanced NADPH oxidase activity. ↑ refers to an increase in activity compared to unprimed neutrophils, ↓ refers to a decrease in activity, and ? indicates an unknown effect of priming agent.

| Priming Agent | Adhesion | Chemotaxis | Phagocytosis | Granule Release | NET formation | Apoptosis | Inflammatory Mediators |
|---------------|----------|------------|--------------|----------------|--------------|-----------|-----------------------|
| Chemoattractants | IMLF | ↑ (El Azreq et al., 2011) | ◊ Halpert et al., 2011 | ↑ Richardson and Patel, 1995 | ↑ Uriarte et al., 2011 | ? | No change |
| C5a | ↑ Jagels et al., 2000 | ↑ Halpert et al., 2011 | ↑/i Morris et al., 2011; Tsuboi et al., 2011 | ↑ DScipio et al., 2006 | ? | ? | ↑ Finsterbusch et al., 2014 |
| LTB4 | ↑ Eun et al., 2011 | ↑ Alonzo et al., 2012 | ↑ Mancuso et al., 2001 | ↑ Kannan, 2002 | ? | ↓ Klein et al., 2001 | ↑ Finsterbusch et al., 2014 |
| PAF | ↑ Kulkarni et al., 2007 | ↑ Shalit et al., 1987 | ↑ Rosales and Brown, 1991 | ↑ Andreasson et al., 2013 | ? | ↑ Khreiss et al., 2004 | ↑ Aquino et al., 2016 |
| Cytokines | TNF-α | ↑ Bouaouina et al., 2004 | ↑ Montecucco et al., 2008 | ↑ Della Bianca et al., 1995 | ↑ McLeish et al., 2013, 2017 | ↑ Hazeldine et al., 2014 | ↑/↓ Murray et al., 1997 |
| | GM-CSF | ↑ Yue et al., 1990 | ↑ Cheng et al., 2001 | ↑ Ketter et al., 1989 | ↑ Kowanko et al., 1991 | ↑ Yousefi et al., 2009 | ↑ Klein et al., 2000 |
| | IFN-γ | ↑ Klebanoff et al., 1992 | ↑ Asa et al., 1996 | ↑ Melby et al., 1982 | ↑ Cassatella et al., 1988 | ↑ Protazi et al., 2016 | ↑ Perussia et al., 1987 |
| | IL-1β | ↑ Brandolini et al., 1997 | ↑ Brandolini et al., 1997 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Baggiolini and Clark-Lewis, 1992 |
| | IL-6 | ↑ Delmers et al., 1990 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Richardson and Patel, 1995 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Baggiolini and Clark-Lewis, 1992 | ↑ Baggiolini and Clark-Lewis, 1992 |
| | IL-15 | ↑ Mastroianni et al., 2000 | ↑ Musso et al., 1998 | ↑ | ↑ | ↑ | ↑ |
| | IL-18 | ↑ Wyman et al., 2002 | ↑ Le et al., 2012 | ↑ Lan et al., 2016 | ↑ | | ↑ |
| | IL-33 | ↑ | ↑ | ↑ | ↑ | | |
| Adhesion proteins | Adhesion | Adhesion | Adhesion | Adhesion | Adhesion | Adhesion | Adhesion |
| Microbial Products | LPS | ↑ Hayashi et al., 2003; Sabroe et al., 2003 | ↑/↑ Fan and Malik, 2003; Hayashi et al., 2003 | ↑ Hayashi et al., 2003 | ↑ Fittschen et al., 1988; Ward et al, 2000 | ↑ Hazeldine et al., 2014 | ↓ Klein et al., 2001 |
| | LAMs | ? | No change | ↑ Hayashi et al., 2003 | ↑ Faldt et al., 2001 | ? | ? |
| | Lipopeptide | ↑ Hayashi et al., 2003; Sabroe et al., 2003 | ↑/↑ Aomatsu et al., 2008 | ↑ Hayashi et al., 2003 | ↑/↓ Whitmore et al., 2016 | ? | Minimal effect |
| | Flagellin | ↑ Hayashi et al., 2003 | ↑ Hayashi et al., 2003 | ↑ Hayashi et al., 2003 | ↑ Hayashi et al., 2003 | ? | ↑/↓ Hayashi et al., 2003 |
| Others | ATP | ? | ↑ Ding et al., 2016 | ↑ Azz et al., 1997; Meshki et al., 2004 | ? | ? | ? |
| | Substance P | ↑ Dianzani et al., 2003 | ↑ Marasco et al., 1981; Perianin et al., 1989 | ↑ Marasco et al., 1981 | ↑ Marasco et al., 1981 | ? | ↑ Bockmann et al., 2001 |
| | CL097 | ? | ? | ↑ Makhi-Maalal et al., 2012 | ? | ? | ↑ Perianin et al., 1989; Wozniak et al., 1989 |
| | CL075 | ? | ? | ? | ? | ? | ? |
| Table 1 shows the phenotypic changes induced by agents known to prime neutrophil respiratory burst activity. All the priming agents listed in this table are known inducers of enhanced NADPH oxidase activity. ↑ refers to an increase in activity compared to unprimed neutrophils, ↓ refers to a decrease in activity, and ? indicates an unknown effect of priming agent. |
exocytosis of secretory vesicles and gelatinase granule is required for TNFα-induced priming (McLeish et al., 2013). Neither TNFα nor fMLF, alone, stimulated exocytosis of specific and azurophilic granules. However, TNFα primed the release of both granule subsets upon subsequent stimulation by fMLF (McLeish et al., 2017). The ability of TNFα to prime exocytosis of azurophilic granules was also reported by Potera et al. (2016). Thus, differential regulation of exocytosis of the four granule subsets by TNFα primes the two major neutrophil antimicrobial defense mechanisms for enhanced release of ROS and toxic granule contents, while protecting against cell injury from inappropriate release of those toxic products. On the other hand, Ramadass et al. showed that GM-CSF both stimulated and primed exocytosis of gelatinase, specific, and azurophilic granules in mouse neutrophils (Ramadass et al., 2017). The basis for differences between TNFα and GM-CSF could be due to disparate capabilities of priming agents or to species differences.

Proteins that control priming by regulating exocytosis have only recently been identified. As pharmacologic inhibition of p38 MAPK prevents TNF-α stimulated exocytosis (Mocsai et al., 1999; Uriarte et al., 2011; McLeish et al., 2013), we employed a phosphoproteomic analysis by mass spectrometry to identify proteins phosphorylated by the p38 MAPK pathway during TNFα stimulation (McLeish et al., 2017). Four of the proteins identified, Raf1, MARCKS, ABI1, and myosin VI, were previously shown to be involved in exocytosis in various cells. We confirmed that Raf1 participates in TNFα-stimulated exocytosis. Catz and colleagues used neutrophils from transgenic mice to identify Rab27a and its target, Munc13-4, as mediators of neutrophil exocytosis stimulated by GM-CSF (Ramadass et al., 2017). They showed that Rab27a, but not Munc13-4, was required for GM-CSF priming of exocytosis to subsequent stimulation by TLR agonists or formyl peptides. Thus, the mechanisms that control neutrophil exocytosis during priming offer potential targets for intervention in inflammatory processes in which neutrophil priming is involved.

Adhesion, Chemotaxis, and Phagocytosis

As shown in Figure 1, microbial invasion or tissue injury releases pathogen-associated molecular pattern (PAMPs) or damage-associated molecular pattern (DAMPs) molecules that induce sentinel immune cells to release pro-inflammatory cytokines. Those cytokines modify both endothelial cell and neutrophil adhesion molecule expression to facilitate the capture of circulating neutrophils and to mediate their migration into tissues. As shown in Table 1, all priming agents for which there are data directly activate neutrophil adhesion. However, differential regulation of adhesion molecule expression and activation by different priming agents may produce different rates of neutrophil adhesion and migration efficiency. For example, neutrophil exposure to TNFα increases plasma membrane expression of the β2 integrin receptor, CD11b/CD18, through exocytosis of secretory vesicles; decreases expression of the selectin receptor CD62-L through receptor shedding; and induces sustained activation of CD11b/CD18 through inside-out signaling (Condliffe et al., 1996; Swain et al., 2002). On the other hand, PAF increases surface expression of the CD11b/CD18, has no effect on selectin expression, and induces only transient activation of CD11b/CD18 (Berends et al., 1997; Khreiss et al., 2004). The in vivo significance of those differences in adhesion molecule expression and activation remains to be determined.

With the exception of IFNγ, neutrophil chemotaxis is enhanced by all priming agents for which there are data (Table 1). In addition to increased expression of adhesion molecules and receptors resulting from exocytosis, priming agents increase actin reorganization (Borgquist et al., 2002), and enhances chemokinesis and chemotaxis (Montecucco et al., 2008; Yao et al., 2015). For example, treatment of neutrophils with PAF, IL-8, or TNFα, alone, induces chemokinesis, while subsequent exposure to an fMLF gradient leads to enhanced neutrophil chemotaxis (Drost and MacNee, 2002). Additionally, TNFα-primed neutrophils gain the ability to migrate toward the chemokine CCL3, which is found in inflammatory sites, but is normally not a neutrophil chemoattractant (Montecucco et al., 2008).

Neutrophil adhesion through both the engagement of neutrophil β2 integrin receptors with endothelial cell adhesion molecules and the binding of neutrophil receptors with extracellular matrix proteins primes respiratory burst activity (Stanislawski et al., 1990; Dapino et al., 1993; Liles et al., 1995). Neutrophil adhesion induces other priming phenotypes, including exocytosis of secretory vesicles and gelatinase granules and a reduced rate of apoptosis (Hu et al., 2004; McGetrick et al., 2006; Paulsson et al., 2010). Thus, transmigration of neutrophils into the extravascular space can be expected to directly induce some of the features of priming.

When neutrophils arrive at the site of infection, they demonstrate increased phagocytosis due to upregulation in the number and affinity of phagocytic receptors (Condliffe et al., 1998; Rainard et al., 2000; Le et al., 2012). Table 1 lists the effects of specific priming agents on phagocytosis. Exposure of bovine neutrophils to the combination of two priming agents, TNFα and C5a each at suboptimal concentrations, enhanced both the rate of phagocytosis and the killing capacity toward serum opsonized Staphylococcus aureus (Rainard et al., 2000); and incubation of human neutrophils with insulin-like growth factor I (IGF-I) results in a significant increase in phagocytosis of both IgG-opsonized S. aureus and serum-opsonized Candida albicans (Bjerknes and Aarskog, 1995). Increased neutrophil phagocytosis is dependent on the concentration and incubation time with IGF-1, and is due to increased complement receptor (CR) 1 and CR3 expression. IGF-1 enhances Fcy receptor-dependent phagocytosis through increased receptor function and activation, while Fcy receptor expression is unchanged (Bjerknes and Aarskog, 1995). Thus, neutrophil exposure to the complex milieu of priming agents in vivo is likely to produce additive or synergistic changes in functional responses. Defining neutrophil responses in that complex environment will require application of systems biology methodologies.

Neutrophil Extracellular Trap (NET) Formation

Since their first description in 2004, neutrophil extracellular traps (NETs) have received intense investigation. Although, the
majority of studies have measured NET formation by resting neutrophils, neutrophils from normal subjects primed by TNFα in vitro demonstrated robust NET formation following a 3 h exposure to anti-neutrophil cytoplasmic antibodies (Kessenbrock et al., 2009). Enhanced NET formation in primed neutrophils is supported by other in vitro studies using GM-CSF and TNFα (Yousefi et al., 2009; Hazeldine et al., 2014). The effect of priming agents on NET formation is listed in Table 1.

Despite their original classification as the third bacterial killing mechanism, current opinion leans toward NETs being important contributors to autoimmunity and tissue injury, rather than antibacterial activity (Sorensen and Borregaard, 2016). In vivo, enhanced NET formation following a systemic change in levels of inflammatory cytokines has been described in cancers, multiple sclerosis, and diabetes (Chechlinska et al., 2010; Naegle et al., 2012; Fadini et al., 2016). Using a chronic myelogenous leukemia mouse model, Demers and colleagues reported that non-malignant neutrophils showed enhanced NET formation, leading to increased coagulation and thrombosis (Demers et al., 2012). Priming of NET formation was reproduced in control mice by sequential administration of granulocyte colony-stimulating factor (G-CSF) and LPS. The authors suggested that priming NET formation by systemic cytokines plays a role in cancer progression. While the current literature indicates that enhanced NET formation is a component of neutrophil priming, the functional consequences of that response remain to be determined.

**Secretion of Lipid and Cytokine Mediators**

As summarized in Table 1, primed neutrophils demonstrate increased metabolic and transcriptional activity that leads to synthesis of a number of pro- and anti-inflammatory chemokines, cytokines, and lipids. Although, the ability of neutrophils to synthesize those products is less than that of macrophages, the large number of neutrophils present at sites of inflammation is postulated to influence both innate and adaptive immune responses through release of those inflammatory mediators.

Pro-inflammatory lipid mediators like leukotriene B4 (LTB4) can be produced de novo by the arachidonate 5-lipoxygenase (5-LO) pathway in neutrophils and play important roles in aggregation, degranulation, and chemotaxis (O’Flaherty et al., 1979; Flamand et al., 2000). The production of these lipid mediators occurs through a series of biochemical events that primarily take place in the perinuclear region where membrane phospholipids are first converted to arachidonic acid (AA) by the calcium–dependent enzyme phospholipase A2 (PLA2) (Luo et al., 2003; Leslie, 2004). The newly synthesized AA is then converted by 5-LO into leukotriene A4 (LTA4), which is the immediate precursor of LTB4. Neutrophil production of LTB4 is responsible for a second wave of neutrophil recruitment during inflammation, a process termed “swarming” (Lammermann et al., 2013). This is one of many examples of amplification loops initiated by neutrophils (Nemeth and Mocsai, 2016).

Direct activation of neutrophils by fMLF does not lead to the detectable release of leukotrienes, but priming with GM-CSF, LPS, or TNFα followed by fMLF stimulation significantly increases LTB4 release (see Table 1; DiPersio et al., 1988a; Schatz-Mundung and Ullrich, 1992; Palmantier et al., 1994; Seeds et al., 1998; Zarini et al., 2006). All three of these priming agents activate PLA2 and increase AA release without increasing intracellular Ca2+ (DiPersio et al., 1988b; Schatz-Mundung and Ullrich, 1992; Zarini et al., 2006). The elevation in available AA substrate leads to prolonged activation of 5-LO and enhanced production of downstream lipid mediators (Surette et al., 1993, 1998; Doerrler et al., 1994). Once produced, LTB4 can exert autocrine effects. It primes neutrophil responses to toll-like-receptor (TLR) agonists, resulting in enhanced cytokine (IL-8, TNFα) secretion (Gaudreault et al., 2012). TLR9 mRNA levels are upregulated upon priming with LTB4, but there is no increase in surface expression of TLR2, TLR4, or the co-receptors TLR1 and TLR6 following LTB4 exposure (Gaudreault and Gosselin, 2009; Gaudreault et al., 2012). Instead, neutrophil LTB4-induced hyper-responsiveness is mediated by the potentiation of TLR-induced intracellular signaling. TAK1 and p38 MAPK, which are essential in TLR-activated cytokine release, are phosphorylated and activated following LTB4 interaction with its seven transmembrane-spanning receptor.

PAF is another lipid inflammatory mediator whose production is primed in neutrophils. Both LPS and GM-CSF enhance PAF synthesis in response to activating stimuli (Aiglietta et al., 1990; Surette et al., 1998). After priming with GM-CSF, there is increased enzymatic activity of acetyltransferase, the enzyme responsible for the synthesis of PAF (Aiglietta et al., 1990). However, the pattern of PAF synthesis after LPS priming is attributed to a biphasic, autocrine response. The early peak in production is due to the direct effect of LPS, while the delayed peak is a result of LPS-induced IL-8 and TNF-α release (Bussolati et al., 1997).

Neutrophils modulate inflammation through the release of stored or newly produced cytokines and chemokines (Cassatella, 1999). Exposure of neutrophils to priming agents leads to an increase in synthesis and release of IL-1α, IL-1β, IL-6, IL-8, TNFα, CXCL1, CXCL2, CCL3 (MIP-1α), CCL4 (MIP-1β) (Roberge et al., 1998; Zallen et al., 1999; Jablonska et al., 2002b; Choi et al., 2008; Wright et al., 2013). The inducible synthesis of the majority of cytokines and chemokines results from increased gene transcription (Marucha et al., 1991; Cassatella et al., 1995; Cassatella, 1996, 1999; Fernandez et al., 1996). TNFα, LPS, and GM-CSF increase intra-nuclear translocation of NF-κB, C/EBP, or CREB transcription factors (Cloutier et al., 2007, 2009; Mayer et al., 2013). LPS induces a biphasic production of IL-8. For the first few hours (2–6 h) of exposure, LPS directly stimulates IL-8 synthesis, but the second wave of sustained IL-8 release (up to 18 h) is due to the endogenous release of TNFα and IL-1β (Cassatella et al., 1993).

**Release of Neutrophil Extracellular Vesicles**

Cell-derived vesicles represent a mechanism for cell-cell communication. Exosomes are 50–100 nm vesicles released from multivesicular bodies that are involved in antigen presentation and cell-to-cell transfer of receptors or RNA (Gyorgy et al.,
Larger vesicles, called microvesicles or microparticles, express tissue factors on their surface that are capable of initiating coagulation. Neutrophils undergoing apoptosis or stimulated by chemotactic agents, opsonic receptors, or TNFα release microparticles. However, the microparticles have varying compositions and functional capabilities, depending on the stimulus (Dalli et al., 2013; Johnson et al., 2014; Lorincz et al., 2015). Microparticles obtained from neutrophils stimulated by chemotactic agents or phorbol esters activate cytokine (IL-6) secretion from endothelial cells and platelets (Mesri and Altieri, 1998; Pluskota et al., 2008). Chemotactic peptide-induced microparticles increase secretion of the anti-inflammatory cytokine transforming growth factor-β and interfere with the maturation of monocyte-derived dendritic cells (Gasser and Schifferli, 2004; Eken et al., 2010). Auto-antibody-stimulated release of neutrophil microparticles was suggested to be involved in the pathogenesis of vasculitis (Hong et al., 2012). Additional activities ascribed to neutrophil microparticles include suppression of bacterial growth, activation of endothelial cell cytokine production, altered cytokine profile of natural killer cells and monocytes, and increased coagulation (Mesri and Altieri, 1998; Timar et al., 2013a,b; Pliyev et al., 2014). An understanding of the stimuli and signal transduction pathways leading to formation and release of neutrophil extracellular vesicles and their roles in inflammation remains to be developed.

**Rate of Apoptosis**

Table 1 indicates that neutrophil apoptosis is variably affected in response to priming agents. While LPS, GM-CSF, IL-8, and LTβ4 have been found to extend neutrophil lifespan in vitro, PAF, fMLF, and IL-6 show no effect, and TNFα shows a biphasic response where it promotes apoptosis during the first 8 h of exposure, followed by a delayed rate of apoptosis at later times (Klein et al., 2000, 2001; Cowburn et al., 2002; Liu et al., 2005; Wright et al., 2014). Primed neutrophils from patients with multiple sclerosis, ANCA-associated vasculitis, and liver cirrhosis show increased apoptosis (Harper et al., 2001; Klimchenko et al., 2011; Naegle et al., 2012), while neutrophils from patients at risk of multiple-organ failure and individuals presenting with septic peritonitis, severe trauma, or septic trauma show a decrease in apoptosis (Ertel et al., 1998; Biffl et al., 1999, 2001; Nolan et al., 2000; Feterowski et al., 2001). Those conflicting reports of the effect of inflammation on apoptosis in vivo are likely due to different priming agents involved in different diseases, different responses during the time course of disease, and differences in the neutrophil micro-environment, such as cell density (Hannah et al., 1998).

The mechanisms underlying the effects of priming on neutrophil apoptosis have been partially characterized. As for TNFα, increased rates of apoptosis during the first hours of exposure are associated with activation of caspase cascades (Murray et al., 1997). TNFα also induces an early, PI-3K-mediated increase in mRNA levels for Bad, a member of the BCL2 family that regulates apoptosis. On the other hand, decreased neutrophil apoptosis observed at later time points is associated with a reduction in Bad mRNA levels (Cowburn et al., 2002). GM-CSF, IL-8, LPS, and LTβ4 decrease the rate of neutrophil apoptosis through activation of ERK1/2 and/or PI-3K/Akt pathways (Klein et al., 2000, 2001). Incubation of neutrophils with fMLF had no effect on the rate of apoptosis, despite activation of both ERK1/2 and Akt (Klein et al., 2001). GM-CSF was also shown to decrease mRNA levels of Bad, while increasing its phosphorylation (Cowburn et al., 2002). A RNA seq study comparing TNFαs and GM-CSF priming pathways showed that out of 580 genes differentially expressed between both agents, 58 were implicated in the delay of apoptosis. Thus, each priming agent produced a distinct profile of pro- and anti-apoptotic genes (Wright et al., 2013). The varying rates of neutrophil apoptosis may serve different functions in the inflammatory response. For example, a reduced rate of apoptosis early in the recruitment of neutrophils results in a brisk accumulation of primed neutrophils. On the other hand, an enhanced rate of apoptosis at later time points promotes resolution through loss of active neutrophils and a change in phenotype of monocytes engulfing apoptotic neutrophils.

**CONCLUSIONS**

The altered neutrophil functions described in this review indicate that priming is a complex phenomenon. Priming involves enhanced respiratory burst, exocytosis, NET formation, and chemotaxis in response to a second stimulus. Priming, however, is not just preparation for an enhanced response to a second stimulus. Priming involves activation of a subset of neutrophil responses, including adhesion, transcription, cytoskeletal reorganization, translocation and expression of receptors, and other molecules, the rate of constitutive apoptosis, metabolic activity, and phagocytosis. The altered neutrophil responses associated with priming primarily result in amplification of the inflammatory response. Although, recruitment of primed neutrophils improves the clearance of invading microbes, the risk of directly injuring surrounding cells is increased. Moreover, the increased synthesis and release of cytokines and lipids by primed neutrophils, combined with increased neutrophil recruitment and life-span, result in an increased local concentration of pro-inflammatory agents. Those agents recruit and prime additional neutrophils, leading to an enhanced innate immune response. Neutrophil-dependent recruitment and activation of dendritic cells and various lymphocyte subsets also enhances the adaptive immune response.

We propose that the current definition of priming, which focuses on a transition state to an enhanced responsiveness to a second stimulus, is too restrictive. Neutrophil priming also results in activation of a subset of neutrophil responses that regulate innate and adaptive immunity. Additionally, neutrophil responses to priming agents vary depending on concentration of the priming agent, time of exposure, and the specific priming agent (Potera et al., 2016; McLeish et al., 2017). It seems likely that neutrophils are exposed to graded concentrations of priming agents as they progress through the multistep process of recruitment, as occurs with chemoattractants. This leads to the hypothesis that, similar to graded granule exocytosis, priming...
occurs in a graded manner during a neutrophil's journey to the site of inflammation. This graded response allows neutrophils to acquire functions in an ordered manner, as required during recruitment. A fully primed neutrophil that releases a maximal amount of toxic chemicals would occur when an optimal concentration of a priming stimulus is encountered. Combining knowledge of the molecular events with an understanding of priming at a systems level will identify therapeutic targets for neutrophil functions that exacerbate individual diseases, while preserving the functions that participation in host defense.

REFERENCES

Aas, V., Lappéregard, K. T., Siebbe, E. M., and Benestad, H. B. (1996). Modulation by interferons of human neutrophil granulocyte migration. J. Interferon Cytokine Res. 16, 929–935. doi: 10.1089/jir.1996.16.929

Acori, M. J., Dias-Melicio, L. A., Golim, M. A., Bordon-Gracián, A. P., Percalli, M. T., and Soares, A. M. (2009). Inhibition of human neutrophil apoptosis by Paracoccidioides brasiliensis: role of interleukin-8. Scand. J. Immunol. 69, 73–79. doi: 10.1111/j.1365-3038.2008.02199.x

Afonso, P. V., Janka-Junttila, M., Lee, Y. J., McCann, C. P., Oliver, C. M., Aamer, K. M., et al. (2012). LTβ4 is a signal-relay molecule during neutrophil chemotaxis. Dev. Cell 22, 1079–1091. doi: 10.1016/j.devcel.2012.02.003

Aglietta, M., Monzeglio, C., Apra, F., Mossetti, C., Stern, A. C., Giribaldi, G., et al. (1990). In vivo priming of human normal neutrophils by granulocyte-macrophage colony stimulating factor: effect on the production of platelet activating factor. Br. J. Haematol. 75, 333–339. doi: 10.1111/j.1365-2141.1990.tb04345.x

Andreonssen, E., Onnheim, K., and Forsman, H. (2013). The subcellular localization of the receptor for platelet-activating factor in neutrophils affects signaling and activation characteristics. Clin. Dev. Immunol. 2013:546047. doi: 10.1155/2013/456047

Aomatsu, K., Kato, T., Fujita, H., Hato, F., Oshitani, N., Kamaeta, N., et al. (2008). Toll-like receptor agonists stimulate human neutrophil migration via activation of mitogen-activated protein kinases. Immunology 123, 171–180. doi: 10.1111/j.1365-2567.2007.02684.x

Aquino, E. N., Neves, A. C., Santos, K. C., Uribe, C. E., Souza, P. E., Cawley, J. C., et al. (2016). Protein degradation in neutrophil priming by PAF. Protein Pept. Lett. 23, 142–151. doi: 10.2174/0929866X23666151202210604

Aziz, K. A., Casley, J. C., Treweeke, A. T., and Zuzel, M. (1995). The production of cytokines by polymorphonuclear neutrophils during stimulation with GM-CSF, TNF and IL-1. J. Immunol. 154, 63, 499–506.

Baggiolini, M., and Clark-Lewis, I. (1992). Interleukin-8, a chemotactic and inflammatory cytokine. FEBS Lett. 307, 97–101. doi: 10.1016/0014-5793(92)80090-Z

Bass, D. A., Olbrantz, P., Szejda, P., Seeds, M. C., and McCall, C. E. (1986). A novel molecular switch for TNF-alpha-induced priming of the NADPH oxidase in human neutrophils. Blood 116, 5795–5802. doi: 10.1182/blood-2010-03-273094

Bouaouina, M., Blouin, E., Halbwachs-Mecarelli, L., Lesavre, P., and Rieu, P. (2004). TNF-induced beta2 integrin activation involves Src kinases and a redox-regulated activation of p38 MAPK. J. Immunol. 173, 1313–1320. doi: 10.4049/jimmunol.173.2.1313

Boussada, T., Gougerot-Pocidalo, M. A., Hayem, G., Ciappelloni, S., Raad, H., Araki Derkawi, R., et al. (2010). The prolyl isomerase Pin1 acts as a novel molecular switch for TNF-alpha-induced priming of the NADPH oxidase in human neutrophils. Blood 116, 5795–5802. doi: 10.1182/blood-2010-03-273094

Braggioni, M., and Clark-Lewis, I. (1992). Interleukin-8: a redox-regulated activation of p38 MAPK. J. Immunol. 154, 661–670. doi: 10.4049/jimmunol.154.2.661

Browning, D. D., Pan, Z. K., Prossnitz, E. R., and Ye, R. D. (1997). Cell type- and developmental stage-specific activation of NF-kappaB by bFGF-Leu-Phe in myeloid cells. J. Biol. Chem. 272, 7995–8001. doi: 10.1074/jbc.272.12.7995

Bussolati, B., Mariano, F., Montrucchio, G., Piccoli, G., and Camussi, G. (1997). Modulatory effect of interleukin-10 on the production of platelet-activating factor and superoxide anions by human leucocytes. Immunology 90, 440–447. doi: 10.1111/j.1365-2567.1997.00440.x

Castellana, M. A. (1995). The production of cytokines by polymorphonuclear neutrophils. Immunol. Today 16, 21–26. doi: 10.1016/0167-5699(95)80066-2

Castellana, M. A. (1996). Interferon-gamma inhibits the lipopolysaccharide-induced macrophage inflammatory protein-1 alpha gene transcription in human neutrophils. Immunol. Lett. 49, 79–82. doi: 10.1016/0165-2478(95)02484-0

Castellana, M. A. (1999). Neutrophil-derived proteins: selling cytokines by the pound. Adv. Immunol. 73, 369–509. doi: 10.1016/S0065-2776(08)60791-9

Castellana, M. A., Cappelli, R., Della Bianca, V., Trinchieri, G., and Berton, G. (1988). Interferon-gamma activates human neutrophil oxygen metabolism and exocytosis. Immunology 63, 499–506.

Castellana, M. A., Gasperini, S., Cazzulli, F., McDonald, P. P., and Trinchieri, G. (1995). Lipopolysaccharide-induced interleukin-8 gene expression in human granulocytes: transcriptional inhibition by interferon-gamma. Biochem. J. 310(Pt 3), 751–755. doi: 10.1042/bj3100751

AUTHOR CONTRIBUTIONS

Contributed to the writing and editing of the manuscript IM, KM, SU; designed and illustrated the figure IM.

FUNDING

The authors were supported by a Merit Review Award (BX001838) from the Department of Veterans Affairs (KM) and by the National Institute of Dental and Craniofacial Research (DE024509 to SU).
Feterowski, C., Weighardt, H., Emmanuëlidis, K., Hartung, T., and Holzmann, B. (2001). Immune protection against septic peritonitis in endotoxin-primed mice is related to reduced neutrophil apoptosis. *Eur. J. Immunol.* 31, 1268–1277. doi: 10.1002/1521-4141(200104)31:4<1268:AID-IMMU1268>3.0.CO;2-C

Fietta, A., Francioli, C., and Gialdroni Grassi, G. (2000). Mycobacterial lipopolysaccharide affects human polymorphonuclear and mononuclear phagocyte functions differently. *Haematologica* 85, 11–18.

Finsterbusch, M., Voisin, M. B., Beyrau, M., Williams, T. J., and Nourshargh, S. (2014). Neutrophils recruited by chemoattractants in vivo induce microvascular plasma protein leakage through secretion of TNF. *J. Exp. Med.* 211, 1307–1314. doi: 10.1084/jem.20132413

Fittschen, C., Sandhaus, R. A., Worthen, G. S., and Henson, P. M. (1988). Bacterial lipopolysaccharide enhances chemoattractant-induced elastase secretion by human neutrophils. *J. Leukoc. Biol.* 43, 547–556.

Flamand, N., Boudreault, S., Picard, S., Austin, M., Surette, M. E., Plante, H., et al. (2000). Adenosine, a potent natural suppressor of arachidonic acid release and leukotriene biosynthesis in human neutrophils. *Am. J. Respir. Crit. Care Med.* 161 (2 Pt 1), S88–S94. doi: 10.1164/ajccm.161.supplement_1.11a-18

Forsberg, M., Löfgren, R., Zheng, L., and Stendahl, O. (2001). Tumour necrosis factor-alpha potentiates CR3-induced respiratory burst by activating p38 MAP kinase in human neutrophils. *Immunology* 103, 465–472. doi: 10.1046/j.1365-2567.2001.01270.x

Francis, S., El Benna, J., Dang, P. M., Pedruzzi, E., Gougerot-Pocidalo, M. A., and Elbim, C. (2005). Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF-kappaB signalling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J. Immunol.* 174, 3633–3642. doi: 10.4049/jimmunol.174.6.3633

Gasser, O., and Schifferli, J. A. (2004). Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood* 104, 2543–2548. doi: 10.1182/blood-2004-01-0361

Gaudreault, E., and Gosselin, J. (2009). Leukotriene B4 potentiates CPG signaling for enhanced cytokine secretion by human leukocytes. *J. Immunol.* 183, 2650–2658. doi: 10.4049/jimmunol.0804135

Gaudreault, E., Paquet-Boucher, C., Fiola, S., Le Bel, M., Lacerte, P., Shio, M. T., et al. (2012). TAK1 contributes to the enhanced responsiveness of LTBR(4)-treated neutrophils to Toll-like receptor ligands. *Int. Immunol.* 24, 693–704. doi: 10.1093/intimm/dxs074

Groemping, Y., Lapouge, K., Smerdon, S. J., and Rittinger, K. (2003). Molecular basis of phosphorylation-induced activation of the NADPH oxidase. *Cell* 113, 343–355. doi: 10.1016/S0092-8674(03)00314-3

Gyorgy, B., Szabo, T. G., Pasztoi, M., Pal, Z., Misjak, P., Arad, I., et al. (2011). LTB(4) derived microvesicles: emerging role of a key mediator to the immune response. *Endocr. Metab. Immune Disord. Drug Targets* 14, 210–217. doi: 10.2174/18715303146610222083717

Humphreys, J. M., Hughes, V., and Edwards, S. W. (1989). Stimulation of protein synthesis in human neutrophils by gamma-interferon. *Biochem. Pharmacol.* 38, 1241–1246. doi: 10.1016/0006-2952(89)90329-8

Jablonska, E., Izycza, A., and Wawrusiewicz, N. (2002a). Effect of IL-18 on IL-1beta and IL-1R1II production by human neutrophils. *Arch. Immunol. Ther. Exp.* 50, 139–141.

Jablonska, E., Kiluk, M., Markiewicz, W., and Jablonski, J. (2002b). Priming effects of GM-CSF, IFN-gamma and TNF-alpha on human immunological inflammatory cytokine production. *Melanoma Res.* 12, 123–128. doi: 10.1097/00008390-200204000-00004

Jablonska, E., Piotrowski, L., Kiluk, M., Jablonski, J., Grabowska, Z., and Markiewicz, W. (2001). Effect of IL-15 on the secretion of IL-1beta, IL-1ra and sIL-1R1II by PMN from cancer patients. *Cytokine* 16, 173–177. doi: 10.1016/cyto.2001.0931

Jayne, D., et al. (2012). Anti-neutrophil cytoplasmic antibodies stimulate neutrophil priming and potentiates neutrophil antibacterial activity and chemotaxis in response to inflammatory cytokine production. *Kidney Int.* 85, 11–18.

Kasorn, A., Alcaide, P., Jia, Y., Subramanian, K. K., Sarrai, B., Li, Y., et al. (2009). Focal adhesion kinase regulates pathogen-killling capability and life span of neutrophils via mediating both adhesion-dependent and -independent cellular signals. *J. Immunol.* 183, 1032–1043. doi: 10.4049/jimmunol.0802984

Klebanoff, S. J., Olszowski, S., Van Voorhis, W. C., Ledbetter, J. A., Waltersdorph, A. M., and Elbim, C. (2005). Inhibition of neutrophil apoptosis by granulocyte-macrophage colony-stimulating factor delays and abrogates the increase in neutrophil phagocytosis and degranulation induced by granulocyte-macrophage colony-stimulating factor. *Blood* 106, 2660–2669. doi: 10.1182/blood-2004-03-0478

Kletter, Y., Bleiberg, I., Golde, D. W., and Fabian, I. (1989). Antibody to Mol is related to reduced neutrophil apoptosis. *Kidney Int.* 59, 1729–1738. doi: 10.1046/j.1523-1755.2001.0590051729.x

Hu, M., Miller, E. J., Lin, X., and Simms, H. H. (2004). Transmigration across a lung epithelial monolayer delays apoptosis of polymorphonuclear leukocytes. *Surgery* 135, 87–98. doi: 10.1016/S0039-6060(03)00347-7

Kidney Int.* 59, 1729–1738. doi: 10.1046/j.1523-1755.2001.0590051729.x

Klimek, O., Di Stefano, A., Georger, B., Hamidi, S., Opolon, P., Robert, T., et al. (2011). Monocytic cells derived from human embryonic stem cells and fetal liver share common differentiation pathways and homeostatic functions. *Blood* 117, 3065–3075. doi: 10.1182/blood-2010-07-295246

Kowanko, I. C., Ferrante, A., Harvey, D. P., and Carman, K. L. (1991). Granulocyte-macrophage colony-stimulating factor augments neutrophil killing of Torulopsis glabrata and stimulates neutrophil
respiratory burst and degranulation. Clin. Exp. Immunol. 83, 225–230. doi: 10.1111/j.1365-2249.1991.tb05619.x

Kulkarni, S., Woolard, K. J., Thomas, S., Osley, D., and Jackson, S. P. (2007). Conversion of platelets from a proaggregatory to a proinflammatory adhesive phenotype role of PAF in spatially regulating neutrophil adhesion and spreading. Blood 109(18), 7699–7706. doi: 10.1182/blood-2006-08-040980

Lamb, F. S., Hook, J. S., Hilkin, B. M., Huber, J. N., Volk, A. P., and Moreland, J. G. (2012). Endotoxin priming of neutrophils requires endocytosis and NADPH oxidase-dependent endosomal reactive oxygen species. J. Biol. Chem. 287, 12395–12404. doi: 10.1074/jbc.M111.306530

Lammermann, T., Afonso, P. V., Angermann, B. R., Wang, J. M., Kastenmüller, W., Parent, C. A., et al. (2013). Neutrophil swarms require LTβ4 and integrins at sites of cell death in vivo. Nature 498, 371–375. doi: 10.1038/nature12175

Lan, F., Yuan, B., Liu, T., Luo, X., Huang, P., Liu, Y., et al. (2016). Interleukin-33 facilitates neutrophil recruitment and bacterial clearance in S. aureus-caused peritonitis. Mol. Immunol. 72, 74–80. doi: 10.1016/j.molimm.2016.03.004

Le, H. T., Tran, V. G., Kim, W., Cho, H. R., and Kwon, B. (2012). IL-1β induces granzyme B and perforin release in human neutrophils and promotes their cytotoxic activity. J. Immunol. 189, 287–295. doi: 10.4049/jimmunol.1103564

Leslie, C. C. (2004). Regulation of arachidonic acid availability for eicosanoid production. Biochem. Cell Biol. 82, 1–17. doi: 10.1139/e03-080

Liles, W. C., Ledbetter, J. A., Waltersdorpf, A. W., and Klebanoff, S. J. (1995). Cross-linking of CD18 primes human neutrophils for activation of the respiratory burst in response to specific stimuli: implications for adhesion-dependent physiological responses in neutrophils. J. Leukoc. Biol. 58, 690–697

Lindemann, A., Riedel, D., Oster, W., Meuer, S. C., Blohm, D., Mertelsmann, R. H., et al. (1988). Granulocyte/macrophage colony-stimulating factor induces interleukin 1 production by human polymorphonuclear neutrophils. J. Immunol. 140, 837–839

Liu, J. J., Song, C. W., Yue, Y., Duan, C. G., Yang, J., He, T., et al. (2005). Quercetin inhibits LPS-induced delay in spontaneous apoptosis and activation of neutrophils. Inflamm. Res. 54, 500–507. doi: 10.1007/s00011-005-1358-2

Lominadze, G., Powell, D. W., Luerman, G. C., Link, A. J., Ward, R. A., and McLeish, K. R. (2005). Proteomic analysis of human neutrophil granules. Mol. Cell. Proteomics 4, 1503–1521. doi: 10.1074/mcp.M500143-MCP200

Lorincz, A. M., Schutte, M., Timar, C. I., Veres, D. S., Kittel, A., McLeish, K. R., et al. (2015). Functionally and morphologically distinct populations of extracellular vesicles produced by human neutrophil granulocytes. J. Leukoc. Biol. 98, 583–589. doi: 10.1189/jlb.3A0114-514R

Luo, M., Jones, S. M., Peters-Golden, M., and Brock, T. G. (2003). Nuclear localization of 5-lipoxygenase as a determinant of leukotriene B4 synthetic capacity. Proc. Natl. Acad. Sci. U.S.A. 100, 12161–12167. doi: 10.1073/pnas.2133253100

Makni-Maalej, K., Marzaioli, V., Boussetta, T., Hurtado-Nedelec, M., Belambri, S. A., Gougerot-Pocidalo, M. A., and El-Benna, J. (2012). The TLR7/8 agonist CL097 primes N-mycolate-induced primary granule release in human neutrophils. J. Immunol. 189, 4657–4665. doi: 10.4049/jimmunol.1201007

Makni-Maalej, K., Marzaio, V., Boussetta, T., Belambri, S. A., Gougerot-Pocidalo, M. A., Hurtado-Nedelec, M., et al. (2015). TLIR8, but not TLIR7, induces the priming of the NADPH oxidase activation in human neutrophils. J. Leukoc. Biol. 97, 1081–1087. doi: 10.1189/jlb.2A1214-632R

Mancuso, P., Nano-Sinkam, P., and Peters-Golden, M. (2001). Leukotriene B4 augments neutrophil phagocytosis of Klebsiella pneumoniae. Infect. Immun. 69, 2011–2016. doi: 10.1128/IAI.69.4.2011-2016.2001

Marasco, W. A., Showell, H. J., and Becker, E. L. (1981). Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. Biochem. Biophys. Res. Commun. 99, 1065–1072. doi: 10.1016/0006-291X(81)90727-0

Maruca, P. T., Zeff, R. A., and Kreutzer, D. L. (1991). Cytokine-induced IL-1 beta gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and IL-1. J. Immunol. 147, 2603–2608

Mastroianni, C. M., d’Ettorre, G., Forcina, G., Lichtner, M., Mengoni, F., D’Agostino, C., et al. (2000). Interleukin-15 enhances neutrophil functional activity in patients with human immunodeficiency virus infection. Blood 96, 1979–1984.

Mayadas, T. N., and Callere, X. (2005). Neutrophil beta2 integrins: moderators of life or death decisions. Trends Immunol. 26, 388–395. doi: 10.1016/j.ti.2005.05.006

Mayer, T. Z., Simard, F. A., Cloutier, A., Vardhan, H., Dubois, C. M., and McDonald, P. P. (2013). The p38-MSK1 signaling cascade influences cytokine production through CREB and C/EBP factors in human neutrophils. J. Immunol. 191, 4299–4307. doi: 10.4049/jimmunol.1301117

McGettrick, H. M., Lord, J. M., Wang, K. Q., Rainger, G. E., Buckley, C. D., and Nash, G. B. (2006). Chemokine- and adhesion-dependent survival of neutrophils after transmigration through cytokine-stimulated endothelium. J. Leukoc. Biol. 79, 779–788. doi: 10.1189/jlb.0605350

McLeish, K. R., Klein, J. B., Lederer, E. D., Head, K. Z., and Ward, R. A. (1996). Azoxetin, TNF alpha, and LPS prime the human neutrophil oxidative burst by distinct mechanisms. Kidney Int. 50, 407–416

McLeish, K. R., Knall, C., Ward, R. A., Gerwins, P., Coxon, P. Y., Klein, J. B., et al. (1998). Activation of mitogen-activated protein kinase cascades during priming of human neutrophils by TNF-alpha and GM-CSF. J. Leukoc. Biol. 64, 537–545

Muirhead, S. M., Peters-Golden, M., and Brock, T. G. (2003). Nuclear factor kappa B activation of neutrophils. Cell. Signal. 15, 875–883. doi: 10.1016/j.cellsig.2003.05.003

Murray, J., Barbara, J. A., Dunkley, S. A., Lopez, A. F., Van Ostade, X., Condliffe, A., et al. (2014). Defective neutrophil recruitment in leukocyte adhesion deficiency type 1 disease causes loss of IL-17-driven inflammatory bone loss. Sci. Transl. Med. 6, 229ra40. doi: 10.1126/scitranslmed.3007696

Murray, J., Barbara, J. A., Dunkley, S. A., Lopez, A. F., Van Ostade, X., Condiffe, A. M., et al. (1997). Regulation of neutrophil apoptosis by tumor necrosis factor-alpha: requirement for TNFR55 and TNFR75 for induction of apoptosis in vitro. Blood 90, 2772–2783

Musso, T., Calosso, L., Zucca, M., Millesimo, M., Puliti, M., Bifulone-Paus, S., et al. (1998). Interleukin-15 activates proinflammatory and antimicrobial functions in polymorphonuclear cells. Infect. Immun. 66, 2640–2647

Nagel, M., Tillack, K., Reinhardt, S., Schipling, S., Martin, R., and Sospenda, M. (2012). Neutrophils in multiple sclerosis are characterized by a primed phenotype. J. Neuroimmunol. 242, 60–71. doi: 10.1016/j.neuroim.2011.11.009
Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. Nat. Rev. Immunol. 6, 173–182. doi: 10.1038/nri1785
Nauseef, W. M., and Borregaard, N. (2014). Neutrophils at work. Nat. Immunol. 15, 602–611. doi: 10.1038/ni.2921
Nemeth, T., and Mocsai, A. (2016). Feedback amplification of neutrophil function. Trends Immunol. 37, 412–424. doi: 10.1016/j.it.2016.04.002
Nolan, B., Collette, H., Baker, S., Duffy, A., De, M., Miller, C., et al. (2000). Inhibition of neutrophil apoptosis after severe trauma is NFKappai dependent. J. Trauma 48, 599–604; discussion: 604–595.
O’Flaherty, J. T., Showell, H. J., Becker, E. L., and Ward, P. A. (1979). Neutrophil priming for the synthesis of 5-lipoxygenase products in human blood ex vivo by granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha. Lab. Invest. 70, 696–704.
Ogura, H., Tanaka, H., Koh, T., Hashiguchi, N., Kugotsubo, H., et al. (1999). Priming, second-hit priming, and apoptosis in leukocytes from trauma patients. J. Trauma 46, 774–781; discussion: 781–773. doi: 10.1097/00005373-199905000-00004
Palmantier, R., Surette, M. E., Sanchez, A., Braquet, P., and Borgeat, P. (1994). Priming of the synthesis of 5-lipoxygenase products in human blood ex vivo by polymorphonuclear leukocytes from granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha. J. Immunol. 158, 765–774.
Paulsson, J. M., Jacobson, S. H., and Lundahl, J. (2010). Neutrophil priming for the synthesis of 5-lipoxygenase products in human blood ex vivo by increasing calcium mobilisation. Eur. J. Biochem. 204, 705–712. doi: 10.1111/j.1432-1327.1992.tb06685.x
Seeds, M. C., Jones, D. F., Chilton, F. H., and Bass, D. A. (1998). Secretory and cytosolic phospholipases A2 are activated during TNF priming of human neutrophils. Biochim. Biophys. Acta 1389, 273–284. doi: 10.1016/S0006-2765(97)00151-3
Sengelov, H., Bolin, P., Kjeldsen, L., Lillevik, K., Dahlgren, C., and Borregaard, N. (1995). Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J. Immunol. 154, 4157–4165.
Sengelov, H., Kjeldsen, L., and Borregaard, N. (1993). Control of exocytosis in early neutrophil activation. J. Immunol. 150, 1535–1543.
Sheppard, F. R., Kelher, M. R., Moore, E. E., McLaughlin, N. J., Banerjee, A., and Silliman, C. C. (2005). Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. J. Leukoc. Biol. 78, 1025–1042. doi: 10.1189/jlb.0804442
Sorensen, O. E., and Borregaard, N. (2016). Neutrophil extracellular traps - the dark side of neutrophils. J. Clin. Invest. 126, 1612–1620. doi: 10.1172/JCI84538
Surette, M. E., Palmantier, R., and Borgeat, P. (1998). Mechanisms of the priming effect of lipopolysaccharides on the biosynthesis of leukotriene B4 synthesis by human neutrophils (PMN) is synergistically enhanced by tumour necrosis factor alpha and low dose diacylglycerol. Int. J. Biochem. Cell Biol. 28, 771–776. doi: 10.1016/S0013-7323(03)00105-3
Timar, C. I., Lorincz, A. M., Csepanyi-Komi, R., Valyi-Nagy, A., Nagy, S., et al. (2003). Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. J. Immunol. 170, 5268–5275. doi: 10.4049/jimmunol.170.10.5268
Sorensen, O. E., and Borregaard, N. (2016). Neutrophil extracellular traps - the dark side of neutrophils. J. Clin. Invest. 126, 1612–1620. doi: 10.1172/JCI84538
Sorensen, O. E., and Borregaard, N. (2016). Neutrophil extracellular traps - the dark side of neutrophils. J. Clin. Invest. 126, 1612–1620. doi: 10.1172/JCI84538
Steadman, R., Petersen, M. M., and Williams, J. D. (1996). CD11b/CD18-dependent stimulation of leukotriene biosynthesis in human neutrophils (PMN) is synergistically enhanced by tumour necrosis factor alpha and low dose diacylglycerol. Int. J. Biochem. Cell Biol. 28, 771–776. doi: 10.1016/S0013-7323(03)00105-3
Summers, C., Rankin, S. M., Condiffe, A. M., Singh, N., Peters, A. M., and Chivers, E. R. (2010). Neutrophil kinetics in health and disease. Trends Immunol. 31, 318–324. doi: 10.1016/j.it.2010.05.006
Surette, M. E., Dallaire, N., Jean, N., Picard, S., and Borgeat, P. (1998). Mechanisms of the priming effect of lipopolysaccharides on the biosynthesis of leukotriene B4 in chemotactic peptide-stimulated human neutrophils. FASEB J. 12, 1521–1531.
Surette, M. E., Palmantier, R., Gosselin, J., and Borgeat, P. (1993). Lipopolysaccharides prime whole human blood and isolated neutrophils for the increased synthesis of 5-lipoxygenase products by enhancing arachidonic acid availability: involvement of the CD14 antigen. J. Exp. Med. 178, 1347–1355. doi: 10.1084/jem.178.4.1347
Swain, S. D., Rohn, T. T., and Quinn, M. T. (2002). Neutrophil priming in host defense: role of oxidants as priming agents. Antioxid. Redox Signal. 4, 69–83. doi: 10.1089/153448402753625870
Timar, C. I., Lorincz, A. M., Csepnyi-Komi, R., Valyi-Nagy, A., Nagy, G., Buzas, E. I., et al. (2013a). Antibacterial effect of microvesicles released from human neutrophil granulocytes. Blood 121, 510–518. doi: 10.1182/blood-2012-05-341114
Timar, C. I., Lorincz, A. M., and Ligeti, E. (2013b). Changing world of neutrophils. Pflugers Arch. 465, 1521–1533. doi: 10.1007/s00424-013-1285-1
Tsuboi, N., Ermendaz, T., Li, X., Nishi, H., Cullere, X., Mekala, D., et al. (2011). Regulation of human neutrophil Fc gamma receptor IIa by Csa receptor promotes inflammatory arthritis in mice. Arthritis Rheum. 63, 467–478. doi: 10.1002/art.30141

Frontiers in Cellular and Infection Microbiology | www.frontiersin.org 12 May 2017 | Volume 7 | Article 217
Miraïda et al. Neutrophil Priming
