CHAPTER 3

THE NEUTROPHIL

DAVID H. DOCKRELL, EMMET E. MCGRATH, MOIRA K. B. WHYTE, AND IAN SABROE

Academic Unit of Respiratory Medicine, Division of Genomic Medicine, University of Sheffield, M Floor, Royal Hallamshire Hospital, Sheffield S10 2JF

Abstract: The neutrophil provides a crucial defence against fungal infections. This numerous phagocyte is recruited rapidly to sites of infection, where it detects pathogens through a range of pathogen-recognition receptors. Phagocytosis and the generation of a range of microbicidal molecules neutralises the pathogen, following which neutrophil death by apoptosis triggers an injury-limiting resolution process facilitating the restoration of normal tissue architecture

1. GENERAL INTRODUCTION

Neutrophils are our most numerous professional phagocyte, whose role is host defence against infections, principally those caused by bacteria and fungi. The importance of the neutrophil in fungal infection is demonstrated in patients rendered acutely neutropaenic through chemotherapy or following bone marrow transplantation, when susceptibility to, and mortality from, fungal infections dramatically increases.

1.1. The History of the Neutrophil

In the 18th century, light microscopes were able to identify some of the basic constituents of blood, namely the red cells and the white cells, or white corpuscles, as they were first described. Further progress was made in the 19th century when Paul Ehrlich used staining techniques to identify different types of corpuscles that he termed the acidophile, basophile and neutrophile because of their acidic, alkaline and negative staining respectively. It was at this time that Erhlich first described the neutrophil as a polynuclear cell. Later, evidence pointed to a single nucleus, but with many lobes, and Metchnikoff described the cell as a polymorphonuclear leukocyte. This term became popular with scientists and

G.D. Brown and M.G. Netea (eds.), Immunology of Fungal Infections, 51–73.

book authors at the time, despite the fact Metchnikoff himself preferred the term microphage to distinguish it from its similar, yet larger, phagocytosing relative, the macrophage.

As time progressed, investigators described these polymorphonuclear leukocytes leaving the circulation through a process of diapedesis. Observing infected sites led Waller to speculate that pus cells assisted bacteria in their transport, growth and survival. This theory was further strengthened by the fact that bacteria were seen in white corpuscles, presumably being transported to new sites of infection. Metchnikoff disagreed with Waller's theory, believing the white corpuscles were in fact the bacteria's enemy.

Many pathologists wondered how the bacteria gained access to these cells. Metchnikoff observed starfish larvae contained specialised cells that appeared to attempt to ingest and destroy foreign tissue (in his case, a rose thorn inserted into a starfish gastrula). He also noted that the *Amoeba*, a unicellular organism, engulfed its prey and digested it intracellularly, its appearance closely resembling a leukocyte ingesting bacteria. As a result, Metchnikoff proposed that neutrophils patrolled the circulation and mobilised to infected tissues and engaged in a process of phagocytosis of bacteria. From these seminal early studies, our understanding of the neutrophil has moved forward to develop a detailed understanding of its crucial role in host defence, its potential to cause disease, and the exquisite regulation of its life and function. The importance of the neutrophil in microbial defence is shown by those congenital syndromes in which neutrophil function is impaired or neutrophil numbers are reduced, and where microbial infection is a major cause of morbidity and mortality. In the Chediak-Higashi syndrome, an autosomal recessive condition, granules are unable to fuse with the phagosome and thus cannot exert their functional role. This condition is associated with recurrent microbial infections, albinism, hepatoslenomegaly, and lymphoproliferative malignancy. Giant cytoplasmic inclusions of granules in leukocytes are observed under light microscopy. Disorders with marked abnormalities of neutrophil function tend to be rare, presumably because of a substantial negative pressure for their persistence across the generations.

1.2. The Structure of the Neutrophil

Neutrophils mature in the bone marrow over a 7–14 day period. They evolve through six morphological stages: myeloblast, promyeloblast, myelocyte, metamyelocyte, non-segmented neutrophil and segmented neutrophil. In the myeloblast stage, the nucleus is very large and round, taking up much of the intracellular space and allowing only a small amount of cytoplasm, which does not contain any granules. Several nucleoli are present. The promyeloblast stage is characterised by a larger cell with a round or oval nucleus, in which the nucleoli are less obvious, and during this stage the azurophilic granules begin to appear within the cytoplasm. The secondary granules appear in the myelocyte stage. These contain large amounts of

glycoprotein which give rise to the pink colouration upon staining with classical H&E based stains. The nucleus becomes almost horseshoe shaped in the metamyelocyte stage. The chromatin becomes denser. The final stages of development give rise to the segmented neutrophil. Strand bridges join two or more nuclear lobes. The cytoplasm stains pink due to the presence of primary (azurophilic), secondary (specific) and tertiary granules. Primary (Azurophil) granules contain cationic proteins, myeloperoxidase (MPO), matrix metalloproteinases (MMPs) and hydrolases. Secondary (specific) granules contain collagenase, lactoferrin and histaminase. Immature neutrophils have vast reserves dedicated to the synthesis of granules with a large and active Golgi apparatus and endoplasmic reticulum that disappear as the neutrophils age rendering the mature neutrophil unable to synthesis new granules.

1.3. Regulation of Neutrophil Numbers

The neutrophil is our most numerous professional phagocyte, with $2.5 - 7.5 \times 10^9$ cells/litre in the circulation. Once the neutrophil is released from the cytokine rich environment of the bone marrow, their inactivated state allows them a lifespan of between six to ten hours in the circulation. The bone marrow manufactures 1×10^{11} neutrophils per day in healthy adults (Figure 1). The capacity to dramatically increase these numbers exists within the functional system of the marrow. At any given time, approximately 60% of the marrow activity is dedicated to neutrophil production with approximately 20% of activity reserved for erythrocyte production. The bone marrow has a large store of neutrophils that can be mobilised rapidly to target infections as soon as they are detected. Retention and release are regulated by adhesion molecule expression, with evidence for roles for both CD11b/CD18 and VLA-4 in the control of these processes (Burdon et al., 2005). Additional roles in these processes are evident for chemoattractant cytokines (chemokines), with evidence that chemokines acting on CXCR2, the major receptor regulating neutrophil recruitment from the microcirculation into tissues, also cause mobilisation of neutrophils from the marrow into the circulation (Burdon et al., 2005). Interestingly, chemokines acting on the receptor CXCR4 also appear to be important in regulating neutrophil release from the marrow, but in contrast to CXCR2 ligands,

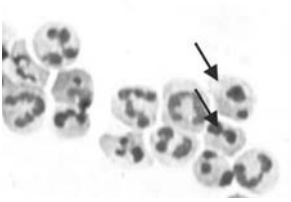

Figure 1. Neutrophils in culture. Healthy viable neutrophils exhibit the classical polymorphonuclear phenotype. As they age, they undergo constitutive apoptosis, with shrinking of the nucleus (pyknosis). Two apoptotic neutrophils, showing nuclear condensation, are marked by arrows (See Color Section.)

activation of CXCR4 serves to firstly retain immature cells in the marrow, and secondly to direct the return of senescent neutrophils from the circulation to the marrow for destruction (Martin et al., 2003). Once released by the bone marrow, neutrophils appear to be retained in the circulation unless recruited to inflammatory sites, though a substantial proportion of cells may be held, at least transiently, in a marginating pool in capillary beds such as those of the lung. Without an inflammatory stimulus, circulating senescent neutrophils upregulate expression of CXCR4, which provides a directional cue moving cells back to the bone marrow for removal, with analogous events likely to occur in the phagocytic systems in the spleen and liver.

1.4. Regulation of Neutrophil Recruitment

Neutrophil recruitment to sites of infection is rapid and efficient. Migration from the microvasculature occurs along gradients of chemotactic factors that are generated by the host and potentially the pathogen. Host-generated chemotactic mediators include chemotactic cytokines (chemokines) and complement fragments, whose generation and efficacy are regulated by multiple mechanisms, presumably with the aim of delivering neutrophils to the site of inflammation whilst minimising bystander tissue damage en route. Interestingly, a hierarchy regulates neutrophil recruitment and elimination in ways permitting careful control of neutrophil function and numbers. Factors that directly cause neutrophil recruitment, such as chemokines, tend to be poor inducers of neutrophil survival; conversely, cytokines that delay neutrophil apoptosis tend to show little efficacy in assays of chemotaxis (though may prime responses to chemotactic factors). Overarching proinflammatory mediators and pathways orchestrate the production of recruitment and survival factors, and on cessation of the inflammatory insult these may be withdrawn to favour resolution.

1.4.1. Chemokines

Chemokines have major roles in driving the selective recruitment of leukocytes. The first chemokines to be identified and characterised in the late 1980s and early 1990s included monocyte-chemoattractant protein-1 (MCP-1) (Yoshimura et al., 1989), RANTES (Schall et al., 1988) (named in honour of its near-mystical powers of cell recruitment (Cohen, 1996)), and interleukin-8 (IL-8) (Matsushima et al., 1988; Yoshimura et al., 1987, Yoshimura et al., 1987); these chemokines recruiting principally monocytes, lymphocytes, and neutrophils respectively. Now renamed according to structural considerations as part of a rational nomenclature as CCL2, CCL5 and CXCL8 respectively (Sabroe et al., 2002), these chemokines provided hope that the mechanisms regulating selective cell recruitment could be identified and therapeutically targeted. Inevitably, passing time has revealed a complex network of chemokines showing redundancy and overlapping spectra of activity, which has been complicated by important differences between experimental species (e.g. mice) and men. In man, neutrophils principally express two chemokine receptor types, CXCR1 and CXCR2 (Chuntharapai and Kim, 1995). The best-known

neutrophil-recruiting chemokine, CXCL8, acts upon both of these receptors, but there appears to be some division of responsibilities between CXCR1 and CXCR2. The latter receptor is relatively promiscuous, binding a range of CXC chemokines containing an ELR amino acid motif, including CXCL8, CXCL1 (MGSA), CXCL7 (NAP-2), CXCL5 (ENA-78), and CXCL6 (GCP-2). Activation of CXCR2 appears to be crucial for neutrophil recruitment (Chuntharapai and Kim, 1995; Pease and Sabroe, 2002; Del Rio et al., 2001), and small molecule inhibitors of CXCR2 are showing substantial promise as effective therapies to inhibit neutrophil recruitment where neutrophilic inflammation is destructive (e.g., potentially, in asthma, chronic obstructive pulmonary disease, and the acute respiratory distress syndrome) (Pease and Sabroe, 2002; White et al., 1998; Jones et al., 1997; Podolin et al., 2002). Although CXCR1 and CXCR2 are both high-affinity receptors for CXCL8, it appears that activation of CXCR1 is linked more with neutrophil activation and respiratory burst generation rather than recruitment (Chuntharapai and Kim, 1995; Pease and Sabroe, 2002). CXCR1 is relatively fastidious, binding CXCL8 and CXCL6 only. CXCR1 and CXCR2 have high homology, yet their expression is independently regulated by proinflammatory stimuli, with CXCR2 showing rapid downregulation on activation of neutrophils by a variety of chemokines, cytokines, and pathogen-derived molecules, whilst CXCR1 expression is relatively preserved (Doroshenko et al., 2002; Sabroe et al., 2005, Sabroe et al., 1997; Ali et al., 1999; Richardson et al., 2003; Tomhave et al., 1994). Such divisions of roles between these two receptors are unlikely to be absolute, and it is conceivable that the properties of these receptors are arranged such that CXCR2 function is responsible for initial cell recruitment, and after its downregulation, signalling via CXCR1 takes over to regulate activation and, potentially, further tissue positioning (Ludwig et al., 1997). There is limited evidence that activated neutrophils may express other chemokine receptors, such as CCR1 (Bonecchi et al., 1999), which may contribute to regulation of their recruitment, tissue positioning, or activation, but in general it is thought CCR1 has little role in human neutrophil function (Hall et al., 2001). This is in contrast to results obtained from studies in mice, where it is clear that CC chemokines, acting on receptors including CCR1, may have important roles in neutrophil recruitment (Gao et al., 1997). As a further complication, a mouse homologue of CXCR1 has only just been identified (Fu et al., 2005), making dissection of the individual roles of these receptors difficult. In general, it is probably true that CC chemokines have a very minor or no role in the direct induction of neutrophil recruitment in humans, and thus caution is required when extrapolating from data generated in the mouse. Indirect roles for CC chemokines, through the recruitment of monocytes and T cells and their subsequent induction of neutrophil recruitment, are, however, likely.

Neutrophil-recruiting chemokines are themselves generated from an extremely wide range of tissues. There are few cell types that cannot make CXCL8, and at sites of inflammation, epithelial cells, endothelial cells, fibroblasts, and infiltrating leukocytes will all represent potentially important sources of chemokines. Heat-killed opsonised *Candida albicans* and *Saccharomyces cerevisiae* as well as zymosan are

able to induce significant production of CXCL8 from human neutrophil cultures *in vitro* (Hachicha et al., 1998). Production of the CC chemokine, CCL3, following challenge in the same experiments, was however only minimally increased after exposure to *C. albicans* and was not increased compared to control following exposure to *S. cerevisiae* and zymosan. These results contrasted with findings for most of the bacterial pathogens tested, which induced potent induction of both chemokines. Theses findings held true even after priming of neutrophils with TNFα after which *S. cerevisiae* and zymosan still failed to stimulate CCL3, and in fact induced lower levels of the chemokine after neutrophil priming than did control cells. The importance of CXCL8 to neutrophil recruitment in yeast infections has been confirmed in genetically modified BALB/c mice which lack the CXCR2 orthologue (Balish et al., 1999). These $CXCR2^{-/-}$ mice demonstrated increased susceptibility to invasive and gastric candidiasis. Furthermore, neutrophil recruitment was retarded compared to wild-type mice both in tissues and in peritoneal exudate following challenge with heat-killed *Candida albicans*.

The role of CC chemokines in neutrophil recruitment, as noted above, remains challenging to dissect. Most studies are based in mice, which have a different repertoire of neutrophil chemokine receptors compared to man, moreover, CC chemokine-mediated monocyte recruitment may indirectly result in downstream amplification of inflammation and subsequent tissue neutrophilia. The lack of CC chemokine production in response to infectious challenge has been confirmed for many other yeasts. Whether this is a host-specific tuning of the immune response, or an evolved response of the pathogen to try to minimise phagocyte recruitment, is not known. *Cryptococcal neoformans* infection of murine lungs, for example, fails to induce significant mRNA or protein for a variety of CC chemokines including CCL2, CCL3, and CCL5 (Kawakami et al., 1999). Although for many yeasts production of CCL3 is minimal and seems unnecessary for neutrophil recruitment, CCL3 production has been shown to be an important determinant of neutrophil recruitment into the peritoneal cavity of mice challenged with the yeast form of the dimorphic fungus *Histoplasma capsulatum* (Medeiros et al., 2004). In this case, CCL3 production was induced by live yeast but not β-glucan from the cell wall, and production was regulated by leukotrienes (Medeiros et al., 2004), and in part mediated neutrophil recruitment indirectly via recruitment of mononuclear cells that released second chemotactic signals.

Some yeasts are capable of inhibiting chemokine-induced migration of neutrophils. A constituent of the capsule of *Cryptococcus neoformans*, mannoprotein (MP)-4, inhibits migration of neutrophils towards CXCL8 (as well as to other chemotactic factors such as fMLP and platelet activating factor) possibly through a mechanism involving chemoattractant cross-desensitisation and premature neutrophil activation (Coenjaerts et al., 2001). Thus, although cryptococcal capsule polysaccharides, such as glucuronoxylomannan, may be potent inducers of CXCL8, cryptococcal meningitis is associated with a paucity of recruited neutrophils to the cerebrospinal fluid (Lipovsky et al., 1998). Therefore *Cryptococcus neoformans* is well equipped to inhibit neutrophil recruitment, both by preventing responses

to CXCL8, and by inducing only low levels of many CC chemokines that might amplify inflammatory responses.

CXC chemokines are also important in responses to filamentous fungi. Invasive aspergillosis is associated with neutropenia or neutrophil dysfunction. Following exposure to *Aspergillus fumigatus*, the murine CXCR2 agonists KC and MIP-2 are critical to prevention of invasive aspergillosis in the lung (Kawakami et al., 1999). In this model, blocking antibodies against CXCR2 induced invasive aspergillosis in immunocompetent mice. Similarly, transient overexpression of KC improved fungal clearance in a model of murine invasive aspergillosis (Mehrad et al., 2002). Roles for CC chemokines in neutrophil recruitment and host defence against *Aspergillus fumigatus* (Gao et al., 1997) have also been observed. Mice lacking CCR1, which binds CCL3 and CCL5, also demonstrate increased mortality suggesting that for this infection (Gao et al., 1997), though again CCL3 effects may in part be mediated through actions on monocyte recruitment, cells that in themselves also have potent phagocytic, antimicrobial properties (Mehrad et al., 2000).

1.4.2. Other chemotactic factors

A variety of other molecules of various classes also serve to regulate neutrophil recruitment, including lipid mediators such as platelet activating factor (PAF) and leukotriene B_4 (LTB_4). The activated complement fragment, C5a, is also a potent stimulus of neutrophil recruitment, potentially acting sequentially with chemokines (Hopken et al., 1996; Ivey et al., 1995). In contrast to chemokines, C5a is an extremely potent inducer of respiratory burst. Chemoattractants such as chemokines, C5a, and bacterial peptides acting on the fMLPR, exhibit a hierarchy of function, whereby, for example, C5a signalling will desensitise responses to chemokines, but chemokines cannot desensitise responses to C5a (Sabroe et al., 1997). The upshot of this complicated interaction between chemoattractants is probably that multiple chemoattractant molecules are responsible for tissue positioning of neutrophils, acting in a sequential manner, and these mechanisms may also allow neutrophils to continue to migrate along chemotactic gradients of molecules that may vary over many orders of magnitude of concentration (Foxman et al., 1997). Signalling via the C5a receptor is important for clearance of some bacterial infections (Hopken et al., 1996), and likewise has a role in fungal infections since during infection with *Cryptococcus neoformans* C5a enhances killing of yeast (Lovchik and Lipscomb, 1993). This effect is influenced by the site of inoculation, with a role for C5a demonstrated in a murine model of intravenous infection in which C5 deficient mice had decreased recruitment of neutrophils into pulmonary vessels and decreased clearance of yeast as compared to wild-type mice. In keeping with lower concentrations of complement in the alveolar space, however, C5 deficient mice demonstrated no defect in early recruitment or killing in response to intratracheal fungal challenge (Lovchik and Lipscomb, 1993). *Cryptoccocus neoformans* has developed adaptations to prevent this host response: the capsule polysaccharide glucuronoxylomannan (GXM) downregulates neutrophil expression of the C5a

receptor (C5aR/CD88) (Monari et al., 2002). In contrast, unencapsulated strains induce upregulation of C5aR expression, emphasising the importance of the cryptococcal capsule as a virulence determinant that inhibits neutrophil recruitment. Filamentous fungi also appear capable of inducing complement-mediated neutrophil chemotaxis, since, following germination, *Aspergillus fumigatus* conidia and *Rhizopus oryzae* spores activate chemotactic complement components (Waldorf and Diamond, 1985). In addition, extracts of the dimorphic fungus *Coccidioides immitis* demonstrate chemotactic activity for neutrophils in the presence of serum but not heat-inactivated serum, suggesting a complement-mediated effect (Galgiani et al., 1978).

Bacterial peptides are initiated by a formlyated methionine residue, and small peptides with this motif, such as fMLP, are potent neutrophil recruiting and activating factors. *Candida albicans* also produces a chemotactic factor that is capable of inducing neutrophil recruitment via the formyl peptide receptor (FPR) (Edens et al., 1999). A variety of *Candida* spp., including C. *tropicalis, C. parapsilosis* and *C. glabrata*, produce these factors but *Saccharomyces cerevisiae* does not (Geiger et al., 2004). The chemotactic factor is approximately 1kDa in size and further analysis suggests it is a low molecular mass polypeptide, like fMLP. Since this factor is produced by the white phase but not the opaque phase (mating competent) phenotype it has been suggested that *C. albicans* downregulates production of this factor during mating to prevent neutrophil recruitment during this critical stage of the life-cycle.

2. OVERARCHING REGULATORY MECHANISMS

It is evident that cytokines such as TNFα and IL-1β have major roles in the orchestration of neutrophil recruitment, through the production of cytokines such as CXCL8 from other leukocytes and tissue cells. We have shown that activation of tissue cells by monocytes exposed to TLR agonists results in marked CXCL8 generation, dependent upon TLR-induced IL-1β secretion from the monocyte that drives CXCL8 production from tissue cells (Morris et al., 2005). TNFα/lymphotoxin-α double knock-out mice have increased susceptibility to *Candida albicans* infection in a model of intra-peritoneal challenge (Netea et al., 1999). While these factors are well recognised to contribute to activation of neutrophils, these mice also demonstrated significant impairment in neutrophil recruitment, and phagocytosis of yeast, although killing of ingested yeast was not altered (Netea et al., 1999). Production of TNFα in response to fungal infection is regulated at multiple levels, as illustrated by studies of mice deficient in Fas, which produce more TNFα and show increased neutrophil recruitment in fungal infection, resulting in greater fungal clearance and host survival (Netea et al., 1999).

IL-17A is increasingly recognised as playing an important role in neutrophil granulopoiesis and recruitment. It is produced by T-lymphocytes and therefore interconnects lymphoid and myeloid lineages in host defence. T-lymphocytes

are important in host defence against a variety of fungi, in particular yeast and dimorphic fungi. Accordingly, mice lacking the IL-17 receptor demonstrated increased fungal loads and decreased neutrophil recruitment to target organs during systemic challenge with *Candida albicans* (Huang et al., 2004).

2.1. Neutrophil Adherence

Neutrophil recruitment from the microvasculature proceeds according to the classical three-stage model, whereby selectin-mediated interactions between the neutrophil and endothelium allow the neutrophil to roll along the vessel wall. Upon encountering chemoattractants such as chemokines, probably displayed bound to glycosaminoglycans (GAGs) on the vessel wall, upregulation of integrin-mediated adhesion results in tight bonding of the leukocyte to the vessel wall, followed by transmigration (diapedesis) between endothelial cells and into tissue (Luster, 1998). Effective migration relies upon L-selectin-mediated rolling of neutrophils, but subsequently L-selectin is shed from the neutrophil, a process that is probably important in allowing effective transmigration of the vessel wall. Engagement of selectins may also activate specific signalling pathways contributing to the regulation of leukocyte recruitment (Simon et al., 1999). Activation of pattern recognition receptors and chemoattractant receptors results in L-selectin shedding, and inappropriate L-selectin shedding could impede effective leukocyte recruitment. Many components of fungi activate pattern recognition receptors such as TLRs, which will result in L-selectin shedding (Sabroe et al., 2002, 2003), and this has been proposed as a mechanism that might explain reduced leukocyte recruitment in disseminated cryptococcal infection. *Cryptococcus neoformans* capsule polysaccharides glucuronoxylomannan (GXM) and galactoxylomannan as well as mannoprotein induce shedding of L-selectin from neutrophils (Dong and Murphy, 1996). GXM also induces shedding of the TNF receptor, TNFR p75–80 (Dong and Murphy, 1996), and interferes with E-selectin-mediated binding of neutrophils to endothelial cells (Ellerbroek et al., 2004), in a CD14/TLR4-dependent fashion. In keeping with these *in vitro* results, individuals with systemic cryptococcal infection and detectable serum levels of cryptococcal polysaccharide have decreased levels of L-selectin in peripheral blood neutrophils and increased levels of soluble L-selectin in serum (Jackson et al., 2005). GXM also binds to a principal neutrophil integrin, CD18, and may inhibit the interaction of β2 integrins with their ligand ICAM-1 (Dong and Murphy, 1997). Leukocyte adhesion deficiency results from deficiencies in CD11b/CD18, giving rise to recurrent infections by bacteria and fungi, and in severe forms are associated with a very abbreviated lifespan.

2.2. Neutrophil Activation

Recruited neutrophils that encounter pathogens respond by induction of antimicrobial systems and the production of further chemokines, in particular CXCL8, to amplify the innate immune response. The processes of transmigration of the

vessel wall and local tissues under the influence of chemotactic factors, and the exposure to cytokines such as GM-CSF and TNFα and lipid mediators such as PAF, results in enhanced (primed) responses to microbial factors and other neutrophil activators, seen for example in marked increases in ROS production in primed cells (Cadwallader et al., 2002; Condliffe et al., 1996; Fuhler et al., 2004; Kitchen et al., 1996). Extensive evidence links cytokines known to prime neutrophil function with enhanced killing of a variety of fungal species. In particular, G-CSF and IFNγ cause enhanced killing of fungal components both in vitro and in vivo (Gil-Lamaignere et al., 2005; Liles et al., 1997; Roilides et al., 1995). Interestingly, although increased killing can reflect increased respiratory burst, this is not always a direct relationship, and a marked discordance between effects of TNFα and IFNγ on pathogen killing and respiratory burst has been reported (Diamond et al., 1991). The detection of pathogens is potentially mediated by molecules including CD11b/CD18, CD14, the fMLPR, and TLRs, in cooperation with Fc receptors that interact with opsonised particles. Signalling via these pathways triggers phagocytosis and delivery to the phagosome of toxic antimicrobial molecules, including proteases and reactive ions such as reactive oxygen species. Neutrophil activation, as characterised by alterations in adhesion molecule expression, regulation of chemotaxis, production of ROS and the potential to degranulate, can be triggered by a variety of factors that signal inflammation as well as direct encounter with pathogens. Molecules such as PAF and C5a serve roles both as endogenous chemoattractants and neutrophil activators, and generation of such factors at sites of inflammation is likely to be an important mechanism amplifying antimicrobial responses.

2.2.1. Toll-like receptors (TLRs)

The biology and roles of TLRs is covered in chapter 11. In brief, TLRs enable responses to a broad range of pathogen-associated molecules, ranging from lipoproteins (TLR2) to LPS (TLR4) to foreign DNA (TLR9) and viral RNA (TLRs 3, 7, and 8) (Sabroe et al., 2003; Akira and Takeda, 2004). Signalling via TLRs activates multiple pathways of host defence, regulating macrophage phagocytosis, cytokine production, cell survival, and ROS production. In the neutrophil, TLR activation has been shown to influence many aspects of neutrophil function, including the ability to (a) prolong cell survival both directly and indirectly via monocyte activation (Sabroe et al., 2003); (b) regulate neutrophil recruitment directly through effects on chemokine receptor expression and indirectly through effects on CXCL8 generation by neutrophils, monocytes, and tissue cells (Morris et al., 2005; Sabroe et al., 2002, 2003); (c) modulate neutrophil expression of adhesion molecules (Sabroe et al., 2002, 2003); and (d) prime neutrophils to induce ROS production (Sabroe et al., 2002, 2003). Neutrophils express the majority of TLRs (except TLR3), and their function can be primed by GM-CSF (Sabroe et al., 2002; Hayashi et al., 2003; Kurt-Jones et al., 2002). There is particularly strong evidence for important roles for TLRs 2 & 4 in neutrophil function. The relative contribution of other TLRs to neutrophil antimicrobial responses will no doubt become clearer over time. TLRs contribute to antifungal host defence (Romani,

2004; Roeder et al., 2004). TLR2 recognises a cell-wall glycolipid in *Candida albicans* phospholipomannan (Jouault et al., 2003). β-glucans, found in yeast cell wall preparations such as zymosan, and which exist as carbohydrate polymers in the cell walls of fungi such as *Saccharomyces cerevisiae, Candida albicans* and *Paracoccidioides brasiliensis* (Brown et al., 2003), cause activation of leukocytes that is dependent, at least in monocytic cells, on collaborative signalling of TLR2 and dectin-1. In the neutrophil, zymosan signalling also involves the CD11b/CD18 integrin (which can also act as a signalling molecule and a partner to other signalling molecules such as TLR4), and autocrine generation of platelet-activating factor (PAF), a potent chemoattractant lipid and leukocyte activator (Au et al., 1994). CD14, a non-signalling protein that is required for efficient TLR4-mediated responses to LPS, also appears to be involved in the TLR-mediated recognition of fungal components (Mambula et al., 2002; Wang et al., 2001). In contrast the *Cryptococcus neoformans* capsular polysaccharide GXM appears to activate TLR4/CD14 but not TLR2 (Shoham et al., 2001). *Aspergillus* species activate macrophages via both TLR2 and TLR4 (Meier et al, 2003) although the complexity of hyphal and conidial cell walls has meant that the polysaccharide and protein PAMPs responsible are still being evaluated (Roeder et al., 2004). TLR9, which recognises CpG DNA, is also activated by fungi, including *Candida albicans* and *Aspergillus fumigatus* (Bellocchio et al., 2004, Bellocchio et al., 2004). Thus, neutrophils respond to zymosan in a complex fashion that is likely to involve TLR signalling, and it is highly probable that neutrophil activation by fungal components, acting via a range of TLRs including TLR2, 4, and 9, will contribute to the induction of a neutrophil anti-fungal response. The pattern of TLR activated determines the specific responses of neutrophils to fungal challenge. For example, in responding to *Aspergillus fumigatus*, TLR2 may govern fungal killing via release of extracellular gelatinases such as MMP-9, and production of proinflammatory cytokines, while TLR4 may govern both the fungicidal effects mediated by reactive oxygen species generated in myeloperoxidase-positive granules, and also production of anti-inflammatory cytokines such as IL-10 (Bellocchio et al., 2004).

TLRs are highly expressed by other immune cells such as monocytes, which appear likely to play an important role in the orchestration of neutrophilic inflammation by the indirect regulation of neutrophil recruitment, activation, and survival (Morris et al., 2005; Sabroe et al., 2004). In a model of disseminated candidiasis in C3H/HeJ mice, which lack functional TLR4, decreased production of KC and MIP-2 (Netea et al., 2002) was observed compared to wild type mice, that was associated with decreased neutrophil recruitment and decreased fungal clearance. In the same study it was shown that TNFα and IL-1β production by macrophages was TLR2-dependent, demonstrating roles for both TLRs in the production of mediators influencing neutrophilic inflammation. In genetically modified mice optimal neutrophil recruitment in response to *Aspergillus fumigatus* required both TLR2 and TLR4 activation (Meier et al, 2003). Regardless of the specific TLRs involved, fungicidal pathways to both yeast and filamentous fungi

appear to be dependent on signalling via MyD88, the signalling adapter that is a major component of TLR signalling, and an essential component of IL-1R signalling (Bellocchio et al., 2004).

2.2.2. Phagocytosis

One of the important consequences of neutrophil activation is to ensure effector functions such as phagocytosis of fungi (Figure 2). In general, yeasts are phagocytosed well, with similar rates of internalization reported for a variety of *Candida* spp. (Lyman and Walsh, 1994). *Candida krusei*, an important medical pathogen appears, however, to be significantly less efficiently phagocytosed than is *Candida albicans* (Richardson and Donaldson, 1994). *Cryptococcus neoformans* is less well phagocytosed than other yeasts and, as is also the case for many bacteria, the presence of a polysaccharide capsule is likely to be a significant adaptation to prevent phagocytosis (Lyman and Walsh, 1994). Of interest, *Trichosporon beigelii*, which is phylogenetically related to *Cryptococcus neoformans*, is also less well phagocytosed even though it lacks a capsule, so other surface antigens may also modify internalization (Lyman and Walsh, 1994). Another fungal adaptation to inhibit phagocytosis by neutrophils is the glycoprotein extracellular fibrillar matrix on the spherules of *Coccioides immitis* (Frey and Drutz, 1986).

Opsonization enhances fungal internalization. Although various monoclonal antibodies against *Candida* spp. enhance phagocytosis of yeast *in vitro* (Wellington et al., 2003) their effect *in vivo* is less marked (Casadevall, 1995), but nonetheless potentially important (Valerius et al., 1997; van Spriel et al., 1999, 2001). Opsonization is particularly important for *Cryptococcus neoformans* in view of its capsule, but also mediates non-significant alterations in phagocytosis of unencapsulated *Cryptococcus neoformans* or *Candida albicans* in some studies (Monari et al., 1999). Opsonized *Cryptococcus neoformans* is taken up predominantly via the FcγRI (CD16) and FcγRIII (CD64) receptors, at least in HIV-infected individuals

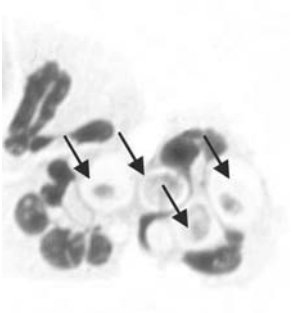

Figure 2. Neutrophils engulfing fungal particles. This photomicrograph shows neutrophils that have taken up particles of zymosan (derived from yeast cell walls) into phagosomes (arrowed) for destruction. The ability of the neutrophil to engulf multiple foreign particles in an attempt to neutralise infection is shown in the cell on the right (See Color Section.)

in whom a monoclonal antibody against this organism enhances phagocytosis and killing of yeast (Monari et al., 1999).

Other factors implicated in the opsonization of fungi include complement components and surfactant proteins. Complement components generated by the alternative pathway enhance phagocytosis of *Aspergillus fumigatus* conidia (Sturtevant and Latge, 1992). Receptors involved in recognising opsonized fungi can cooperate in order to induce specific effector functions. For example, CR3 (Mac-1, CD11b/CD18), which recognises *Candida albicans* opsonized with C3bi, does not appear to further enhance levels of FcR-mediated yeast internalization when yeast are optimally opsonized (van Spriel et al., 2001). However activation of both FcRs and CR3 during phagocytosis can modify some effector functions, such as antibody-dependent cellular cytotoxicity, though respiratory burst and degranulation are not effected by simultaneous engagement of CR3 and FcRs (van Spriel et al., 2001). Surfactant protein D can opsonize fungi, in which context it enhances phagocytosis of *Aspergillus fumigatus* (as does surfactant protein A), but not phagocytosis of *Candida albicans* (Tacken et al., 2004; Madan et al., 1997).

A complete overview of all the downstream signalling pathways activated within the neutrophil by encounter with fungi is beyond the scope of this work. Evidence to date suggests that signalling pathways activated by TLRs are similar in neutrophils and monocytes, this latter cell type being better characterised with respect to TLR signalling. Certainly engagement of TLRs, activation of integrins, production of autocrine activators such as PAF, and engagement of a phagocytosis programme will activate a broad range of signalling pathways within the neutrophil, including PI-3 kinases, MAP kinases, generation of free NF-κB, etc., as reviewed in many excellent articles including those cited here (Akira and Takeda, 2004; Kawai and Akira, 2005). Small G proteins such as Rac and Cdc42 are involved in MAPK activation following *Candida albicans* phagocytosis, and contribute to effective internalisation and microbicidal responses (Zhong et al., 2003).

2.2.3. Killing

Reactive oxygen and nitrogen species and the proteases activated in association with their generation represent major mechanisms of microbial killing by neutrophils (Christin et al., 1997; Fierro et al.). Antimicrobial proteins such as defensins also contribute to the ability of the neutrophil to kill fungi (Schneider et al., 2005), and neutrophils can release complement proteins that contribute to the formation of a membrane attack complex on the surface of yeasts (Lukasser-Vogl et al., 2000). Proteins present in azurophil granules, including defensins and also cathepsin G and BPI, can work together to inhibit the grown of yeasts such as *Histoplasma capsulatum* (Newman et al.), 2000). Fungistasis is a property of other proteins that can be derived from neutrophils, such as lactoferrin (Palma et al., 1992). Both the myeloperoxidase (MPO) and NADPH-oxidase systems generate reactive ions that contribute to defence against fungal infections (Aratani et al., 2002). Relative resistance to neutrophil hydrogen peroxide is a significant virulence factor with respect to pathogenic dimorphic fungi (Schaffner et al., 1986).

Deficiencies in the NADPH oxidase system are a feature of chronic granulomatous disease (CGD), and result in a markedly enhanced susceptibility to *Aspergillus* spp. This X-linked condition usually manifests itself in early childhood. Individuals are susceptible to most pathogens, including less virulent species such as *Staphylococcus epidermidis*. The defect occurs in the respiratory burst pathway, such that sufferers are unable to produce hydrogen peroxide. Clinically, the individual develops chronic severe forms of pneumonia, abscesses, osteomyeltis and lymphadenitis. Diagnosis may involve a combination of enzyme assays and phagocytosis function tests including quantitative nitroblue tetrazolium (NBT), chemiluminence and quantitative intracellular killing curve. Mouse models of CGD, such as the gp91 phox knock-out mice, demonstrate that deficiency of the NADPH oxidase system results in enhanced susceptibility to pulmonary disease after intratracheal challenge and greater degrees of lung inflammation (Morgenstern et al., 1997). Glucose – 6 – phosphate dehydrogenase deficiency is an X- linked inherited disorder resulting in the absence of this enzyme's activity. This results in inadequate production of NADPH, which is needed for the respiratory burst. The clinical picture is very similar to CGD. Genetic myeloperoxidase deficiencies, with varying consequences for susceptibility to microbial infection (including *Candida spp*.), have also been described.

Unsurprisingly, there is also evidence fungi may have attempted to evolve strategies that limit their killing by neutrophils (Du et al., 2005; Levitz and Diamond, 1985; Murayama et al., 1996; Smail et al., 1992). The basis of the respiratory burst generated in response to fungi involves a response to common cell wall constituents such as mannans and zymosan and can be inhibited by mannose (Danley and Hilger, 1981). However the dimorphic fungi may have evolved mechanisms to downregulate this host response. The spherule form of *Coccidioides immitis* is relatively resistant to hydrogen peroxide (Galgiani, 1986). In addition, mature *Coccidioides immitis* spherules appear to be able to inhibit hydrogen peroxide and hypochlorous acid generation, despite being capable of inducing superoxide generation (Galgiani, 1995). *Histoplasma capsulatum* binding to neutrophils is enhanced by complement-mediated opsonization and occurs via CD18, but despite effective phagocytosis under these conditions superoxide generation is minimal (Schnur and Newman, 1990). This is despite multiple lines of experimental evidence suggesting a respiratory burst occurs in neutrophils and phagolysosomal fusion occurs under these conditions, suggesting that this dimorphic fungus may have developed mechanisms to trap intracellular superoxide to subvert oxidative host defence in neutrophils (Schnur and Newman, 1990; Kurita et al., 1991).

2.2.4. Resolution of inflammation and regulation of neutrophil lifespan

Neutrophils are the most short-lived of all cell types, with a half-life in the circulation of a few hours. In culture, neutrophils die rapidly, with a half-life of around 12 hours (there being some variations in death rates between laboratories) (Sabroe et al., 2004), and thereafter die rapidly by apoptosis (Figure 3). Apoptosis can result from activation of either an 'intrinsic' or an extrinsic pathway, the former

Figure 3. Removal of aptoptotic neutrophils. In this smear, leukocytes were isolated from the joint of a patient with rheumatoid arthritis. The two cells shown here are monocytes, one of which has engulfed an apoptotic neutrophil (arrowed), for removal in an injury-limiting fashion that is associated with downregulation of proinflammatory macrophage function (See Color Section.)

mediated via ligation of death receptors such as Fas and TRAIL and the latter via stress-induced changes in mitochondrial permeability and activation of both caspase-dependent and independent pathways. This intrinsic pathway is regulated by both pro- and anti-apoptotic members of the Bcl-2 family. These complex processes are reviewed in a number of excellent articles to which the reader's attention is directed (Simon, 2003; Adams and Cory, 1998; Ashkenazi and Dixit, 1998; Thornberry and Lazebnik, 1998; Green and Reed, 1998).

The key role of apoptosis in resolution of neutrophilic inflammation was first appreciated by Savill and Haslett (Savill et al., 1989), allowing safe clearance of these potentially dangerous cells from tissues. Ideally, in the context of infection, neutrophils will remain viable until invading pathogens are killed, but thereafter apoptosis would proceed promptly to abrogate inflammation and avoid tissue damage (Haslett, 1997). With resolution of inflammation, apoptotic neutrophils are cleared by phagocytes, particularly macrophages (Savill and Fadok, 2000). In a further layer of anti-inflammatory regulation, macrophage ingestion of apoptotic neutrophils results in induction of an anti-inflammatory cytokine profile in these cells. In contrast, neutrophils that die by necrosis rather than apoptosis are proinflammatory, both through release of cell contents into the local milieu, and through induction of proinflammatory cytokines from monocytes and macrophages (Fadok et al., 2001). In the majority of circumstances, as illustrated by the extraordinary manner in which a bacterial or fungal pneumonia resolves to leave normal or near-normal lung architecture, the process of removal of neutrophils by apoptotic cell death is an exceptionally efficient resolution mechanism (Haslett, 1997).

Activated neutrophils show a marked relative prolongation of their lifespan (Lee et al., 1993; Colotta et al., 1992). The ability to directly prolong lifespan is largely restricted to a group of proinflammatory cytokines such as TNFα and GM-CSF: it is interesting that chemokines such as CXCL8 are relatively poor stimulators of enhanced survival, implying compartmentalised regulation of neutrophil recruitment, activation, and survival. Growth factor-mediated delay of apoptosis thus acts in concert with effects on bone marrow to increase the numbers of functionally competent neutrophils present at a site of infection. Pathogens themselves can cause enhanced survival by direct engagement of TLRs, but this

survival response appears to be relatively small compared to that induced by pathogen activation of TLRs on bystander monocytes, which results in the release of potent survival factors from these cells (Sabroe et al., 2002). It is plausible that the direct survival response to TLR agonists is relatively weak in order to prevent a single contact with a pathogen resulting in the generation of an activated neutrophil with a very long lifespan, whose production of antimicrobial factors could outlive the pathogen. The regulation of neutrophil lifespan through TLR signalling of other cell types such as monocytes provides an attractive external control mechanism, allowing withdrawal of survival factors and resolution of inflammation once the infective insult has been cleared.

Some pathogens have evolved strategies to manipulate neutrophil apoptosis to their advantage. For example, the Gram-negative bacterium, *Pseudomonas aeruginosa*, secretes a toxin that accelerates neutrophil apoptosis *in vitro* (Usher et al., 2002) and may favour bacterial persistence at inflammatory sites (Allen et al., 2005). There is also some evidence that the interaction between fungi and neutrophils may change rates of apoptosis to the advantage of the pathogen (Medeiros et al., 2004).

3. EXCESSIVE ACTIVATION AND DISEASE

Although defective neutrophil function can result in disseminated infection, inappropriate or overwhelming neutrophil activation can also be a major cause of disease. Destructive neutrophilic inflammation is thought to contribute to a range of pathologies, from asthma to rheumatoid arthritis to vasculitis. In the context of fungal disease, the role of the neutrophil is beneficial to the host, but progession of sepsis to syndromes such as the acute respiratory distress syndrome (ARDS) is associated with an extremely high mortality. ARDS is a neutrophil-driven acute inflammatory disease, in which neutrophil-mediated capillary damage results in a dramatic non-cardiogenic pulmonary oedema and severe respiratory failure. In this context, it is relevant that anecdotally, engraftment of bone marrow and reappearance of neutrophils in the peripheral circulation can, in the context of pulmonary infection, be associated with a transient worsening of oxygenation and a deterioration in respiratory status. Clearly, neutralisation of neutrophil function in this setting would not be desirable, but such scenarios demonstrate the requirement for careful regulation of neutrophil function by the host in health and disease.

4. CONCLUSION

The neutrophil is central to our defence against fungal infection and disease. Its function is regulated at all levels from production to recruitment to activation and survival, providing a robust defence mechanism that is relatively rarely implicated in unwanted disease. Therapeutic manipulation of neutrophilic inflammation, for example by the administration of G-CSF to encourage bone marrow production and prime neutrophil function, is already an established strategy in the treatment of fungal disease.

REFERENCES

Adams, J.M. and Cory, S. (1998) The Bcl-2 protein family: arbiters of cell survival. Science *281*, 1322.

Akira, S. and Takeda, K. (2004) Toll-like receptor signalling. Nat. Rev. Immunol. *4*, 499.

Ali, H., Richardson, R.M., Haribabu, B. and Snyderman, R. (1999) Chemoattractant receptor cross-desensitization. J. Biol. Chem. *274*, 6027.

Allen, L., Dockrell, D.H., Pattery, T. Lee, D.G., Cornelis, P. Hellewell, P.G. and Whyte, M.K. (2005) Pyocyanin production by Pseudomonas aeruginosa induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. J. Immunol. *174*, 364–3.

Aratani, Y., Kura, F., Watanabe, H. Akagawa, H., Takano, Y., Suzuki, K., Dinauer, M.C., Maeda, N. and Koyama, H. (2002) Relative contributions of myeloperoxidase and NADPH-oxidase to the early host defense against pulmonary infections with Candida albicans and Aspergillus fumigatus. Med. Mycol. *40*, 557.

Ashkenazi, A. and Dixit, V.M. (1998) Death receptors: signaling and modulation. Science *281*, 1305.

Au, B.-A., Williams, T.J. and Collins, P.D. (1994) Zymosan-induced IL-8 release from human neutrophils involves activation via CD11b/CD18 receptor and endogenous platelet-activating factor as an autocrine modulator. J. Immunol. *152*, 5411.

Balish, E., Wagner, R.D., Vazquez-Torres, A., Jones-Carson, J., Pierson, C. and Warner, T. (1999) Mucosal and systemic candidiasis in IL-8Rh$^{-/-}$ BALB/c mice. J. Leukoc. Biol. *66*, 144.

Bellocchio, S., Moretti, S., Perruccio, K., Fallarino, F., Bozza, S., Montagnoli, C., Mosci, P., Lipford, G.B., Pitzurra, L. and Romani, L. (2004) TLRs govern neutrophil activity in aspergillosis. J. Immunol. *173*, 7406.

Bellocchio, S., Montagnoli, C., Bozza, S., Gaziano, R., Rossi, G., Mambula, S.S., Vecchi, A., Mantovani, A., Levitz, S.M. and Romani, L. (2004) The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo. J. Immunol. *172*, 3059.

Bonecchi, R., Polentarutti, N., Luini, W., Borsatti, A., Bernasconi, S., Locati, M., Power, C., Proudfoot, A., Wells, T.N.C., Mackay, C., Mantovani, A. and Sozzani, S. (1999) Up-regulation of CCR1 and CCR3 and induction of chemotaxis to CC chemokines by IFN-γ in human neutrophils. J. Immunol. *162*, 474.

Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S. and Gordon, S. (2003) Dectin-1 mediates the biological effects of beta-glucans. J. Exp. Med. *197*, 1119.

Burdon, P.C., Martin, C. and Rankin, S.M. (2005) The CXC chemokine MIP-2 stimulates neutrophil mobilization from the rat bone marrow in a CD49d-dependent manner. Blood *105*, 2543.

Cadwallader, K.A., Condliffe, A.M., McGregor, A., Walker, T.R., White, J.F., Stephens, L.R. and Chilvers, E.R. (2002) Regulation of phosphatidylinositol 3-kinase activity and phosphatidylinositol 3,4,5-trisphosphate accumulation by neutrophil priming agents. J. Immunol. *169*, 3336.

Casadevall, A. (1995) Antibody immunity and invasive fungal infections. Infect. Immun. *63*, 4211.

Christin, L., Wysong, D.R., Meshulam, T., Wang, S. and Diamond, R.D. (1997) Mechanisms and target sites of damage in killing of Candida albicans hyphae by human polymorphonuclear neutrophils. J. Infect. Dis. *176*, 1567.

Chuntharapai, A. and Kim, K.J. (1995) Regulation of the expression of IL-8 receptor A/B by IL-8: possible functions of each receptor. J. Immunol. *155*, 2587.

Coenjaerts, F.E., Walenkamp, A.M., Mwinzi, P.N., Scharringa, J., Dekker, H.A., van Strijp, J.A., Cherniak, R. and Hoepelman, A.I. (2001) Potent inhibition of neutrophil migration by cryptococcal mannoprotein-4-induced desensitization. J. Immunol. *167*, 3988.

Cohen, P. (1996) Hooked on HIV. New Scientist *152*, 24.

Colotta, F., Re, F., Polentarutti, N., Sozzani, S. and Mantovani, A. (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood *80*, 2012.

Condliffe, A.M., Chilvers, E.R., Haslett, C. and Dransfield, I. (1996) Priming differentially regulates neutrophil adhesion molecule expression/function. Immunology *89*, 105.

Danley, D.L. and Hilger, A.E. (1981) Stimulation of oxidative metabolism in murine polymorphonuclear leukocytes by unopsonized fungal cells: evidence for a mannose-specific mechanism. J. Immunol. *127*, 551.

Del Rio, L., Bennouna, S., Salinas, J. and Denkers, E.Y. (2001) CXCR2 deficiency confers impaired neutrophil recruitment and increased susceptibility during Toxoplasma gondii infection. J. Immunol. *167*, 6503.

Diamond, R.D., Lyman, C.A. and Wysong, D.R. (1991) Disparate effects of interferon-gamma and tumor necrosis factor-alpha on early neutrophil respiratory burst and fungicidal responses to Candida albicans hyphae in vitro. J. Clin. Invest. *87*, 711.

Dong, Z.M. and Murphy, J.W. (1996) Cryptococcal polysaccharides induce L-selectin shedding and tumor necrosis factor receptor loss from the surface of human neutrophils. J. Clin. Invest. *97*, 689.

Dong, Z.M. and Murphy, J.W. (1997) Cryptococcal polysaccharides bind to CD18 on human neutrophils. Infect. Immun. *65*, 557.

Doroshenko, T., Chaly, Y., Savitskiy, V., Maslakova, O., Portyanko, A., Gorudko, I. and Voitenok, N.N. (2002) Phagocytosing neutrophils down-regulate the expression of chemokine receptors CXCR1 and CXCR2. Blood *100*, 2668.

Du, C., Calderone, R., Richert, J. and Li, D. (2005) Deletion of the SSK1 response regulator gene in Candida albicans contributes to enhanced killing by human polymorphonuclear neutrophils. Infect. Immun. *73*, 865.

Edens, H.A., Parkos, C.A., Liang, T.W., Jesaitis, A.J., Cutler, J.E. and Miettinen, H.M. (1999) Non-serum-dependent chemotactic factors produced by Candida albicans stimulate chemotaxis by binding to the formyl peptide receptor on neutrophils and to an unknown receptor on macrophages. Infect Immun. *67*, 1063.

Ellerbroek, P.M., Ulfman, L.H., Hoepelman, A.I. and Coenjaerts, F.E. (2004) Cryptococcal glucuronoxylomannan interferes with neutrophil rolling on the endothelium. Cell Microbiol. *6*, 581.

Fadok, V.A., Bratton, D.L. and Henson, P.M. (2001) Phagocyte receptors for apoptotic cells: recognition, uptake, and consequences. J. Clin. Invest. *108*, 957.

Fierro, I.M., Barja-Fidalgo, C., Cunha, F.Q. and Ferreira, S.H. (1996) The involvement of nitric oxide in the anti-Candida albicans activity of rat neutrophils. Immunology *89*, 295.

Foxman, E.F., Campbell, J.J. and Butcher, E.C. (1997) Multistep navigation and the combinatorial control of leukocyte chemotaxis. J. Cell Biol. *139*, 1349.

Frey, C.L. and Drutz, D.J. (1986) Influence of fungal surface components on the interaction of Coccidioides immitis with polymorphonuclear neutrophils. J. Infect. Dis. *153*, 933.

Fu, W., Zhang, Y., Zhang, J. and Chen, W.F. (2005) Cloning and characterization of mouse homolog of the CXC chemokine receptor CXCR1. Cytokine *31*, 9.

Fuhler, G.M., Cadwallader, K.A., Knol, G.J., Chilvers, E.R., Drayer, A.L. and Vellenga, E. (2004) Disturbed granulocyte macrophage-colony stimulating factor priming of phosphatidylinositol 3,4,5-trisphosphate accumulation and Rac activation in fMLP-stimulated neutrophils from patients with myelodysplasia. Journal of Leukocyte Biology *76*, 254.

Galgiani, J.N., Isenberg, R.A. and Stevens, D.A. (1978) Chemotaxigenic activity of extracts from the mycelial and spherule phases of Coccidioides immitis for human polymorphonuclear leukocytes. Infect Immun. *21*, 862.

Galgiani, J.N. (1986) Inhibition of different phases of Coccidioides immitis by human neutrophils or hydrogen peroxide. J. Infect. Dis. *153*, 217.

Galgiani, J.N. (1995) Differences in oxidant release by human polymorphonuclear leukocytes produced by stimulation with different phases of Coccidioides immitis. J. Infect. Dis. *172*, 199.

Gao, J.L., Wynn, T.A., Chang, Y., Lee, E.J., Broxmeyer, H.E., Cooper, S. Tiffany, H.L., Westphal, H., Kwon-Chung, J. and Murphy, P.M. (1997) Impaired host defense, hematopoiesis, granulomatous inflammation and type 1-type 2 cytokine balance in mice lacking CC chemokine receptor 1. J. Exp. Med. *185*, 1959.

Geiger, J., Wessels, D. Lockhart, S.R. and Soll, D.R. (2004) Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white-opaque switching in Candida albicans. Infect Immun. *72*, 667.

Gil-Lamaignere, C., Simitsopoulou, M., Roilides, E., Maloukou, A., Winn, R.M. and Walsh, T.J. (2005) Interferon- gamma and granulocyte-macrophage colony-stimulating factor augment the activity of polymorphonuclear leukocytes against medically important zygomycetes. J. Infect. Dis. *191*, 1180.

Green, D.R. and Reed, J.C. (1998) Mitochondria and apoptosis. Science *281*, 1309.

Hachicha, M., Rathanaswami, P., Naccache, P.H. and McColl, S.R. (1998) Regulation of chemokine gene expression in human peripheral blood neutrophils phagocytosing microbial pathogens. J. Immunol. *160*, 449.

Hall, L.R., Diaconu, E., Patel, R. and Pearlman, E. (2001) Cxc chemokine receptor 2 but not c-c chemokine receptor 1 expression is essential for neutrophil recruitment to the cornea in helminth-mediated keratitis (river blindness). J. Immunol. *166*, 4035.

Haslett, C. (1997) Granulocyte apoptosis and inflammatory disease. Br. Med. Bull. *53*, 669.

Hayashi, F., Means, T.K. and Luster, A.D. (2003) Toll-like receptors stimulate human neutrophil function. Blood *102*, 2660.

Hopken, U.E., Lu, B., Gerard, N.P. and Gerard, C. (1996) The C5a chemoattractant receptor mediates mucosal defence to infection. Nature *383*, 86.

Huang, W., Na, L., Fidel, P.L. and Schwarzenberger, P. (2004) Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J. Infect. Dis. *190*, 624.

Ivey, C.L., Williams F.M., Collins P.D., Jose P.J. and Williams, T.J. (1995) Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit: evidence for a role for C5a and interleukin-8. J. Clin. Invest. *95*, 2720.

Jackson, L.A., Drevets, D.A., Dong, Z.M., Greenfield, R.A. and Murphy, J.W. (2005) Levels of L-selectin (CD62L) on human leukocytes in disseminated cryptococcosis with and without associated HIV-1 infection. J. Infect. Dis. *191*, 1361.

Jones, S.A., Dewald, B., Clark-Lewis, I. and Baggiolini, M. (1997) Chemokine antagonists that discriminate between interleukin-8 receptors. Selective blockers of CXCR2. J. Biol. Chem. *272*, 16166.

Jouault, T., Ibata-Ombetta, S., Takeuchi, O., Trinel, P.A., Sacchetti, P., Lefebvre, P., Akira, S. and Poulain, D. (2003) Candida albicans phospholipomannan is sensed through toll-like receptors. J. Infect. Dis. *188*, 165.

Kawai, T. and Akira, S. (2005) Toll-like receptor downstream signaling. Arthritis Res. Ther. *7*, 12.

Kawakami, K., Shibuya, K., Qureshi, M.H., Zhang, T., Koguchi, Y., Tohyama, M., Xie, Q., Naoe, S. and Saito A. (1999) Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent Cryptococcus neoformans: effects of interleukin-12. FEMS Immunol. Med. Microbiol *25*, 391.

Kitchen, E., Rossi, A.G., Condliffe, A.M., Haslett, C. and Chilvers, E.R. (1996) Demonstration of reversible priming of human neutrophils using platelet-activating factor. Blood *88*, 4330.

Kurita, N., Terao, K., Brummer, E., Ito, E., Nishimura, K. and Miyaji, M. (1991) Resistance of Histoplasma capsulatum to killing by human neutrophils. Evasion of oxidative burst and lysosomal-fusion products. Mycopathologia *115*, 207.

Kurt-Jones, E.A., Mandell, L., Whitney, C., Padgett, A., Gosselin, K., Newburger, P.E. and Finberg, R.W. (2002) Role of Toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin 8 responses in neutrophils. Blood *100*, 1860.

Lee, A., Whyte, M.K. and Haslett, C. (1993) Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. J. Leukoc. Biol. *54*, 283.

Levitz, S.M. and Diamond, R.D. (1985) Mechanisms of resistance of Aspergillus fumigatus Conidia to killing by neutrophils in vitro. J. Infect. Dis. *152*, 33.

Liles, W.C., Huang, J.E., van Burik, J.A., Bowden, R.A. and Dale, D.C. (1997) Granulocyte colony-stimulating factor administered in vivo augments neutrophil-mediated activity against opportunistic fungal pathogens. J. Infect. Dis. *175*, 1012.

Lipovsky, M.M., Gekker, G., Hu, S., Ehrlich L.C., Hoepelman, A.I. and Peterson P.K. (1998) Cryptococcal glucuronoxylomannan induces interleukin (IL)-8 production by human microglia but inhibits neutrophil migration toward IL-8. J. Infect. Dis. *177*, 260.

Lovchik, J.A. and Lipscomb, M.F. (1993) Role for C5 and neutrophils in the pulmonary intravascular clearance of circulating Cryptococcus neoformans. Am. J. Respir. Cell Mol. Biol. *9*, 617.

Ludwig, A., Petersen, F., Zahn, S., Götze, O., Schröder, J.-M., Flad, H.-D. and Brandt, E. (1997) The CXC-chemokine neutrophil activating peptide-2 induces two distinct optima of neutrophil chemotaxis by differential interaction with interleukin-8 receptors CXCR-1 and CXCR-2. Blood *90*, 4588.

Lukasser-Vogl, E., Gruber, A., Lass-Florl, C., Eder, A., Hogasen, A.K., Morgan, B.P., Dierich, M.P. and Wurzner, R. (2000) Membrane attack complex formation on yeast as trigger of selective release of terminal complement proteins from human polymorphonuclear leukocytes. FEMS Immunol. Med. Microbiol. *28*, 15.

Luster, A.D. (1998) Chemokines - chemotactic cytokines that mediate inflammation. N. Engl. J. Med. *338*, 436.

Lyman, C.A. and Walsh, T.J. (1994) Phagocytosis of medically important yeasts by polymorphonuclear leukocytes. Infect. Immun. *62*, 1489.

Madan, T., Eggleton, P., Kishore, U., Strong, P., Aggrawal, S.S., Sarma, P.U. and Reid, K.B. (1997) Binding of pulmonary surfactant proteins A and D to Aspergillus fumigatus conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. Infect. Immun. *65*, 3171.

Mambula, S.S., Sau, K., Henneke, P., Golenbock, D.T. and Levitz, S.M. (2002) Toll-like receptor (TLR) signaling in response to Aspergillus fumigatus. J. Biol. Chem. *277*, 39320.

Martin, C., Burdon, P.C., Bridger, G., Gutierrez-Ramos, J.C., Williams, T.J. and Rankin, S.M. (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. Immunity *19*, 583.

Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H.F., Leonard, E.J. and Oppenheim, J.J. (1988) Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J. Exp. Med. *167*, 1883.

Medeiros, A.I., Malheiro, A., Jose, P.J., Conroy, D.M., Williams, T.J. and Faccioli, L.H. (2004) Differential release of MIP-1alpha and eotaxin during infection of mice by Histoplasma capsulatum or inoculation of beta-glucan. Inflamm. Res. *53*, 351.

Mehrad, B., Moore, T.A. and Standiford, T.J. (2000) Macrophage inflammatory protein-1 alpha is a critical mediator of host defense against invasive pulmonary aspergillosis in neutropenic hosts. J. Immunol. *165*, 962.

Mehrad, B., Wiekowski, M., Morrison, B.E., Chen, S.C., Coronel, E.C., Manfra, D.J. and Lira, S.A. (2002) Transient lung-specific expression of the chemokine KC improves outcome in invasive aspergillosis. Am. J. Respir. Crit. Care Med. *166*, 1263.

Meier, A., Kirschning, C.J., Nikolaus, T., Wagner, H., Heesemann, J. and Ebel, F. (2003) Toll-like receptor (TLR) 2 and TLR4 are essential for Aspergillus-induced activation of murine macrophages. Cell Microbiol. *5*, 561.

Monari, C., Casadevall, A., Pietrella, D., Bistoni, F. and Vecchiarelli, A. (1999) Neutrophils from patients with advanced human immunodeficiency virus infection have impaired complement receptor function and preserved Fcgamma receptor function. J. Infect. Dis. *180*, 1542.

Monari, C., Casadevall, A., Retini, C., Baldelli, F., Bistoni, F. and Vecchiarelli, A. (1999) Antibody to capsular polysaccharide enhances the function of neutrophils from patients with AIDS against Cryptococcus neoformans. AIDS *13*, 653.

Monari, C., Kozel, T.R., Bistoni, F. and Vecchiarelli, A. (2002) Modulation of C5aR expression on human neutrophils by encapsulated and acapsular Cryptococcus neoformans. Infect Immun. *70*, 3363.

Morgenstern, D.E., Gifford, M.A., Li, L.L., Doerschuk, C.M. and Dinauer, M.C. (1997) Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defense and inflammatory response to Aspergillus fumigatus. J. Exp. Med. *185*, 207.

Morris, G.E., Whyte, M.K.B., Martin, G.F., Jose, P.J., Dower, S.K. and Sabroe, I. (2005) Agonists of Toll-like receptors 2 and 4 activate airway smooth muscle via mononuclear leukocytes. Am. J. Respir. Crit. Care Med. *171*, 814.

Murayama, T., Amitani, R., Ikegami, Y., Nawada, R., Lee, W.J. and Kuze, F. (1996) Suppressive effects of Aspergillus fumigatus culture filtrates on human alveolar macrophages and polymorphonuclear leucocytes. Eur. Respir. J. *9*, 293.

Netea, M.G., van Tits, L.J., Curfs, J.H., Amiot, F.J., Meis, F. van der Meer, J.W. and Kullberg, B.J. (1999) Increased susceptibility of TNF-alpha lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of Candida albicans. J. Immunol. *163*, 1498.

Netea, M.G., van Der Meer, J.W., Meis, J.F. and Kullberg, B.J. (1999) Fas-FasL interactions modulate host defense against systemic Candida albicans infection. J. Infect. Dis. *180*, 1648.

Netea, M.G., Van Der Graaf, C.A., Vonk, A.G., Verschueren, I., Van Der Meer, J.W. and Kullberg, B.J. (2002) The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. J. Infect. Dis. *185*, 1483.

Newman, S.L., Gootee, L., Gabay, J.E. and Selsted, M.E. (2000) Identification of constituents of human neutrophil azurophil granules that mediate fungistasis against Histoplasma capsulatum. Infect. Immun. *68*, 5668.

Palma, C., Serbousek, D., Torosantucci, A., Cassone, A. and Djeu, J.Y. (1992) Identification of a mannoprotein fraction from Candida albicans that enhances human polymorphonuclear leukocyte (PMNL) functions and stimulates lactoferrin in PMNL inhibition of candidal growth. J. Infect. Dis. *166*, 1103.

Pease, J.E. and Sabroe, I. (2002) The role of interleukin 8 and its receptors in inflammatory lung disease. Am. J. Respir. Med. *1*, 19.

Podolin, P.L., Bolognese, B.J., Foley, J.J., Schmidt, D.B., Buckley, P.T., Widdowson, K.L., Jin, Q., White, J.R., Lee, J.M., Goodman, R.B., Hagen, T.R., Kajikawa, O., Marshall, L.A., Hay, D.W.P. and Sarau, H.M. (2002) A potent and selective nonpeptide antagonist of CXCR2 inhibits acute and chronic models of arthritis in the rabbit. J. Immunol. *169*, 6435.

Richardson, M.D. and Donaldson, F. (1994) Interaction of Candida krusei with human neutrophils in vitro. J. Med. Microbiol. *41*, 384.

Richardson, R.M., Marjoram, R.J., Barak, L.S. and Snyderman, R. (2003) Role of the cytoplasmic tails of CXCR1 and CXCR2 in mediating leukocyte migration, activation, and regulation. J. Immunol. *170*, 2904.

Roeder, A., Kirschning, C.J., Rupec, R.A., Schaller, M. and Korting, H.C. (2004) Toll-like receptors and innate antifungal responses. Trends Microbiol. *12*, 44.

Roilides, E., Holmes, A. Blake, C. Pizzo, P.A. and Walsh. T.J. (1995) Effects of granulocyte colony-stimulating factor and interferon-gamma on antifungal activity of human polymorphonuclear neutrophils against pseudohyphae of different medically important Candida species. J. Leukoc. Biol. *57*, 651.

Romani, L. (2004) Immunity to fungal infections. Nat. Rev. Immunol. *4*, 1.

Sabroe, I., Williams, T.J., Hébert, C.A. and Collins, P.D. (1997) Chemoattractant cross-desensitization of the human neutrophil IL-8 receptor involves receptor internalization and differential receptor subtype regulation. J. Immunol. *158*, 1361.

Sabroe, I., Jones, E.C., Usher, L.R., Whyte, M.K.B. and Dower, S.K. (2002) Toll-Like Receptor (TLR)2 and TLR4 in Human Peripheral Blood Granulocytes: A Critical Role for Monocytes in Leukocyte Lipopolysaccharide Responses. J. Immunol. *168*, 4701.

Sabroe, I., Lloyd, C.M., Whyte, M.K., Dower, S.K., Williams, T.J. and Pease, J.E. (2002) Chemokines, innate and adaptive immunity, and respiratory disease. Eur. Respir. J. *19*, 350.

Sabroe, I., Prince, L.R., Jones, E.C., Horsburgh, M.J., Foster, S.J., Vogel, S.N., Dower, S.K. and Whyte, M.K.B. (2003) Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. J. Immunol. *170*, 5268.

Sabroe, I., Read, R.C., Whyte, M.K., B. Dockrell, D.H., Vogel, S.N. and Dower, S.K. (2003) Toll-like receptors in health and disease: complex questions remain. J. Immunol. *171*, 1630.

Sabroe, I., Prince, L.R., Dower, S.K., Walmsley, S.R., Chilvers, E.R. and Whyte, M.K.B. (2004) What can we learn from highly purified neutrophils? Biochem. Soc. Trans. *32*, 468.

Sabroe, I., Jones, E.C., Whyte, M.K.B. and Dower, S.K. (2005) Regulation of human neutrophil chemokine receptor expression and function by activators of Toll-like Receptors (TLR)2 and TLR4. Immunology *115*, 90.

Savill, J. and Fadok, V. (2000) Corpse clearance defines the meaning of cell death. Nature *407*, 784.

Savill, J.S., Wyllie, A.H., Henson, J.E., Walport, M.J., Henson, P.M. and Haslett, C. (1989) Macrophage phagocytosis of aging neutrophils in inflammation. J. Clin. Invest. *83*.

Schaffner, A., Davis, C.E., Schaffner, T., Markert, M., Douglas, H. and Braude, A.I. (1986) In vitro susceptibility of fungi to killing by neutrophil granulocytes discriminates between primary pathogenicity and opportunism. J. Clin. Invest. *78*, 511.

Schall, T.J., Jongstra, J., Dyer, B.J., Jorgensen, J., Clayberger, C., Davis, M.M. and Krensky, A.M. (1988) A human T cell-specific molecule is a member of a new gene family. J. Immunol. *141*, 1018.

Schneider, J.J., Unholzer, A., Schaller, M., Schafer-Korting, M. and Korting, H.C. (2005) Human defensins. J. Mol. Med.

Schnur, R.A. and Newman, S.L. (1990) The respiratory burst response to Histoplasma capsulatum by human neutrophils. Evidence for intracellular trapping of superoxide anion. J. Immunol. *144*, 4765.

Shoham, S., Huang, C., Chen, J.M., Golenbock, D.T. and Levitz, S.M. (2001) Toll-like receptor 4 mediates intracellular signaling without TNF-alpha release in response to Cryptococcus neoformans polysaccharide capsule. J. Immunol. *166*, 4620.

Simon, S.I., Cherapanov, V., Nadra, I., Waddell, T.K., Seo, S.M., Wang, Q., Doerschuk, C.M. and Downey, G.P. (1999) Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and colocalization with CD18. J. Immunol. *163*, 2891.

Simon, H.U. (2003) Neutrophil apoptosis pathways and their modifications in inflammation. Immunol. Rev. *193*, 101.

Smail, E.H., Cronstein, B.N., Meshulam, T., Esposito, A.L., Ruggeri, R.W. and Diamond, R.D. (1992) In vitro, Candida albicans releases the immune modulator adenosine and a second, high-molecular weight agent that blocks neutrophil killing. J. Immunol. *148*, 3588.

Sturtevant, J. and Latge, J.P. (1992) Participation of complement in the phagocytosis of the conidia of Aspergillus fumigatus by human polymorphonuclear cells. J. Infect. Dis. *166*, 580.

Tacken, P.J., Hartshorn, K.L., White, M.R., van Kooten, C., van de Winkel, J.G., Reid, K.B. and Batenburg, J.J. (2004). Effective targeting of pathogens to neutrophils via chimeric surfactant protein D/anti-CD89 protein. J. Immunol. *172*, 4934.

Thornberry, N.A. and Lazebnik, Y. (1998) Caspases: enemies within. Science *281*, 1312.

Tomhave, E.D., Richardson, R.M., Didsbury, J.R., Menard, L., Snyderman, R. and Ali, H. (1994) Cross-desensitization of receptors for peptide chemoattractants. J. Immunol. *153*, 3267.

Usher, L.R., Lawson, R.A., Geary, I., Taylor, C.J., Bingle, C.D., Taylor, G.W. and Whyte, M.K. (2002) Induction of neutrophil apoptosis by the Pseudomonas aeruginosa exotoxin pyocyanin: a potential mechanism of persistent infection. J. Immunol. *168*, 1861.

Valerius, T., Stockmeyer, B., van Spriel, A.B., Graziano, R.F., van den Herik-Oudijk, I.E., Repp, R., Deo, Y.M., Lund, J., Kalden, J.R., Gramatzki, M. and van de Winkel, J.G. (1997) FcalphaRI (CD89) as a novel trigger molecule for bispecific antibody therapy. Blood *90*, 4485.

van Spriel, A.B., van den Herik-Oudijk, I.E., van Sorge, N.M., Vile, H.A., van Strijp, J.A. and van de Winkel, J.G. (1999) Effective phagocytosis and killing of Candida albicans via targeting FcgammaRI (CD64) or FcalphaRI (CD89) on neutrophils. J. Infect. Dis. *179*, 661.

van Spriel, A.B., van den Herik-Oudijk, I.E. and van de Winkel, J.G. (2001) Neutrophil Fc gamma RI as target for immunotherapy of invasive candidiasis. J. Immunol. *166*, 7019.

van Spriel, A.B., Leusen, J.H., van Egmond, M., Dijkman, H.B., Assmann, K.J., Mayadas, T.N. and van de Winkel, J.G. (2001) Mac-1 (CD11b/CD18) is essential for Fc receptor-mediated neutrophil cytotoxicity and immunologic synapse formation. Blood *97*, 2478.

Waldorf, A.R. and Diamond, R.D. (1985) Neutrophil chemotactic responses induced by fresh and swollen Rhizopus oryzae spores and Aspergillus fumigatus conidia. Infect Immun. *48*, 458.

Wang, J.E., Warris, A., Ellingsen, E.A., Jorgensen, P.F., Flo, T.H., Espevik, T., Solberg, R., Verweij, P.E. and Aasen, A.O. (2001) Involvement of CD14 and toll-like receptors in activation of human monocytes by Aspergillus fumigatus hyphae. Infect. Immun. *69*, 2402.

Wellington, M., Bliss, J.M. and Haidaris, C.G. (2003) Enhanced phagocytosis of Candida species mediated by opsonization with a recombinant human antibody single-chain variable fragment. Infect. Immun. *71*, 7228.

White, J.R., Lee, J.M., Young, P.R., Hertzberg, R.P., Jurewicz, A.J., Chaikin, M.A., Widdowson, K., Foley, J.J., Martin, L.D., Griswold, D.E. and Sarau H.M. (1998) Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. J. Biol. Chem. *273*, 10095.

Yoshimura, T., Matsushima, K., Oppenheim, J.J. and Leonard, E.J. (1987) Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). J. Immunol. *139*, 788.

Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E.A., Appella, E., Oppenheim, J.J. and Leonard, E.J. (1987) 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. *84*, 9233.

Yoshimura, T., Yuhki, N., Moore, S.K., Appella, E., Lerman, M.I. and Leonard, E.J. (1989) Human monocyte chemoattractant protein-1 (MCP-1). Full length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEBS Lett. *244*, 487.

Zhong, B., Jiang, K., Gilvary, D.L., Epling-Burnette, P.K., Ritchey, C., Liu, J., Jackson, R.J., Hong-Geller, E. and Wei, S. (2003) Human neutrophils utilize a Rac/Cdc42-dependent MAPK pathway to direct intracellular granule mobilization toward ingested microbial pathogens. Blood *101*, 3240.