FEMS Microbiology Reviews, fuv018, 39, 2015, 670–687

doi: 10.1093/femsre/fuv018
Advance Access Publication Date: 1 May 2015
Review Article

REVIEW ARTICLE

The innate immune response to *Aspergillus fumigatus* at the alveolar surface

Anatte Margalit and Kevin Kavanagh*

Department of Biology, Maynooth University, Co. Kildare, Ireland

*Corresponding author: Department of Biology, Maynooth University, Co Kildare, Ireland. Tel: +00-353-1-7083859; E-mail: kevin.kavanagh@nuim.ie
One sentence summary: The immune response at the alveolar surface to *Aspergillus fumigatus* is described.
Editor: Gerhard Braus

ABSTRACT

Aspergillus fumigatus is an ubiquitous, saprophytic mould that forms and releases airborne conidia which are inhaled by humans on a daily basis. When the immune system is compromised (e.g. immunosuppressive therapy prior to organ transplantation) or there is pre-existing pulmonary malfunction (e.g. asthma, cystic fibrosis, TB lesions), *A. fumigatus* exploits weaknesses in the host defenses which can result in the development of saphrophytic, allergic or invasive aspergillosis. If not effectively eliminated by the innate immune response, conidia germinate and form invasive hyphae which can penetrate pulmonary tissues. The innate immune response to *A. fumigatus* is stage-specific and various components of the host's defenses are recruited to challenge the different cellular forms of the pathogen. In immunocompetent hosts, anatomical barriers (e.g. the mucociliary elevator) and professional phagocytes such as alveolar macrophages (AM) and neutrophils prevent the development of aspergillosis by inhibiting the growth of conidia and hyphae. The recognition of inhaled conidia by AM leads to the intracellular degradation of the spores and the secretion of proinflammatory mediators which recruit neutrophils to assist in fungal clearance. During the later stages of infection, dendritic cells activate a protective *A. fumigatus*-specific adaptive immune response which is driven by Th1 $CD4^+$ T cells.

Keywords: *Aspergillus*; aspergillosis; innate immunity; invasive; immunity; pulmonary

INTRODUCTION

Aspergillus fumigatus is an ubiquitous, saprophytic mould that releases airborne conidia which are inhaled by humans on a daily basis (Latgé 1999). *Aspergillus fumigatus* displays a variety of offensive and defensive virulence factors that enable it to induce a range of diseases in susceptible hosts (Latgé 1999; Daly and Kavanagh 2001; Hohl and Feldmesser 2007; Dagenais and Keller 2009). Three forms of the disease are recognized: saprophytic aspergillosis is characterized by either airway colonization or the development of aspergilloma (fungal ball) in pulmonary tissue. The most common form of allergic aspergillosis is known as allergic bronchopulmonary aspergillosis (ABPA) and it is characterized by the induction of an immune response triggered by the secretion of toxins and allergens from the developing fungus (Fig. 1). Invasive pulmonary aspergillosis is characterized by the proliferation of fungal hyphae within pulmonary tissues and targets severely immunocompromised individuals including organ transplant recipients and chemotherapy patients (Segal and Walsh 2006) (Fig. 2). Although a number of *Aspergillus* species have been associated with invasive aspergillosis (IA), including *A. nidulans* and *A. flavus*, *A. fumigatus* accounts for approximately 90% of these cases (Denning 1998).

For immunocompetent individuals, inhaled conidia are swiftly cleared by cells of the pulmonary immune system (Table 1). Following inhalation, resting conidia become metabolically active and begin to swell (Fig. 3a and b). If not effectively eliminated by the innate immune system, conidia germinate and form invasive hyphae which can penetrate pulmonary tissues (Dagenais and Keller 2009) (Fig. 3c and d). The innate

Received: 2 July 2014; **Accepted:** 17 March 2015

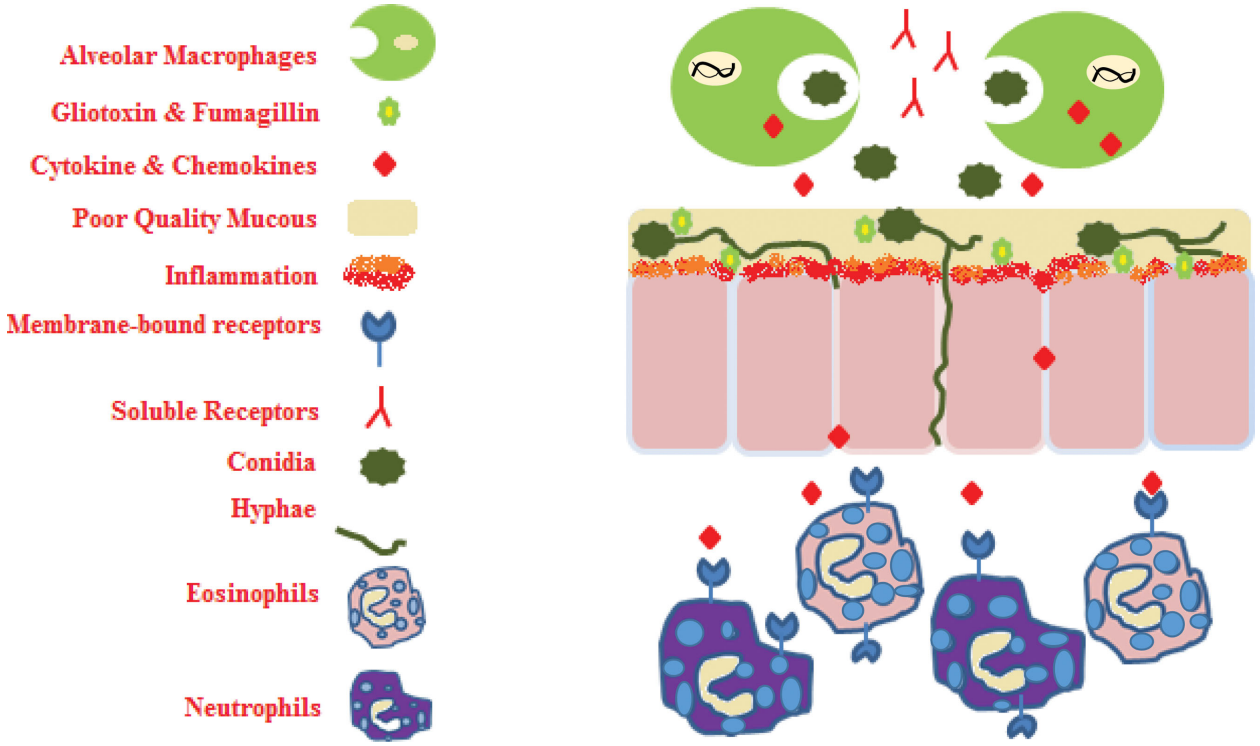

Figure 1. The immunocompromised lung, or the asthmatic and CF lung, provides an environment that is conducive to conidial growth. Poor quality mucous inhibits access to conidia by immunological mediators, thus conidia germinate and penetrate through the alveolar surface. Hyphae produce gliotoxin and fumagillin which deactivate the mucociliary elevator and inhibit neutrophil activity. An overexaggerated inflammatory response to *A. fumigatus* mediated by eosinophiles and neutrophils contributes to tissue necrosis and severe pulmonary damage.

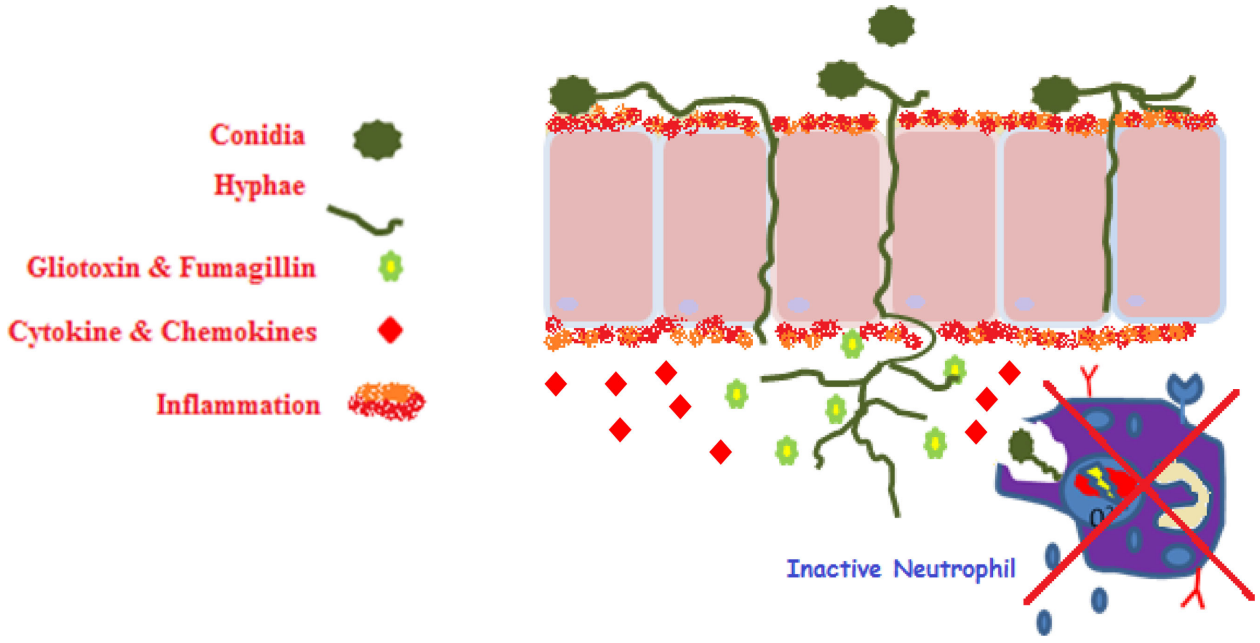

Figure 2. The severely immunocompromised host may experience invasive hyphal growth which can disseminate through the epithelial and endothelial cell membranes due to a defenseless leukocyte-mediated immune response.

immune response against *A. fumigatus* is stage-specific and various components of the host's defenses are activated to challenge the different cellular forms of the pathogen. In immunocompetent hosts, anatomical barriers and alveolar macrophages (AM) ensure that conidia do not proceed past the swelling stage (Fig. 4). In the event of conidial germination, germ tubes are quickly and effectively targeted by neutrophils. In addition, dendritic cells (DCs) activate the adaptive immune response which can rapidly mobilize a T cell-mediated defense against the invading fungus. This review will examine our current

Table 1. Various components of the innate immune response are involved in recognizing, inhibiting the growth of and/or killing different morphological forms of *A. fumigatus*.

Morphology	Resting conidia	Swollen Conidia	Germ tubes	Hyphae
Recognition and opsonization	?	PTX3, SP-A and SP-D, Ficolins, MBL	SP-D	SP-D
Extrcellular recognition	?	Dectin-1, Dectin-2, TLR2, TLR4, DC-SIGN	Dectin-1, Dectin-2, TLR2, TLR4,	Dectin-1, Dectin-2, TLR2, TLR4
Intracellular recognition	–	TLR3, TLR9, Dectin-1 NOD2, NLRP3	–	–
Phagocytosis	–	AM, neutrophils, DCs, monocytes	–	–
Intracellular killing	–	AM, neutrophils, DCs, monocytes	–	–
Endocytosis		ECs		
Extracellular killing	–	Neutrophils	NK cells, eosinophiles	NK cells, neutrophils
Growth inhibition	–	–	Neutrophils, NK cells	Neutrophils, NK cells

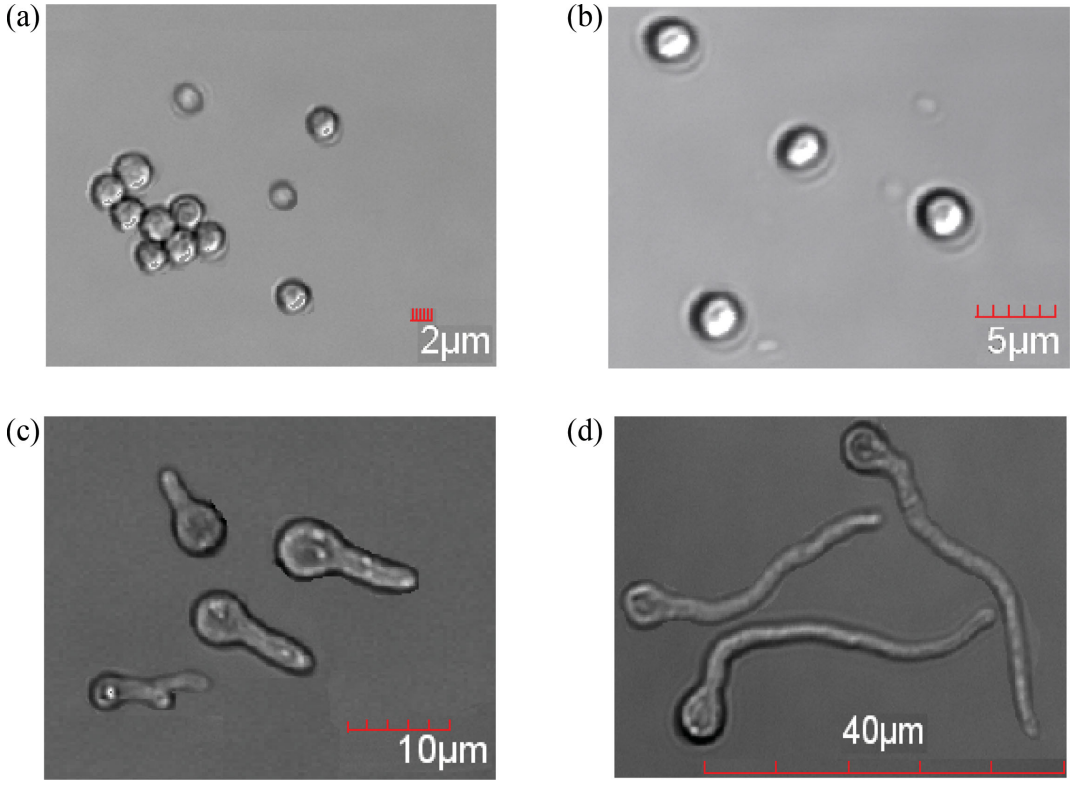

Figure 3. The differing morphological stages of *A. fumigatus* growth; as time proceeds, resting conidia (3a) begin to swell (3b) and germinate (3c), eventually forming hyphae (3d). [*A. fumigatus* conidia (1×10^7 ml) were added to minimal essential medium (Sigma) supplemented with 5% fetal calf serum and incubated at 37°C. A 1 ml aliquot was withdrawn at the times indicated, diluted in ice cold PBS to halt any further development and representative images were captured using an Olympus BX51 Colorview soft imaging system].

understanding of the role of the host immune system in preventing pulmonary colonization by *A. fumigatus* and the development of IA.

INITIAL INTERACTIONS WITH THE HOST

Anatomical barriers to *Aspergillus* infection

The airway epithelium and its secretions, the airway surface liquid, represent the first point of contact for inhaled *A. fumigatus* conidia, and these are involved in the initial immune response to invading microorganisms (Fig. 4). The epithelium of the upper respiratory tract consists of various cell types, including mucous- secreting goblet cells and ciliated cells (Rogers 1994). Inhaled conidia become trapped in mucous, propelled towards the oropharyngeal junction by ciliary beating and either swallowed or exporated (Balloy and Chignard 2009). Developing *A. fumigatus* colonies secrete toxins that inhibit the action of the mucocilary elevator (Amitani *et al.* 1995) and thus may prevent the expulsion of fungal tissue from the lung.

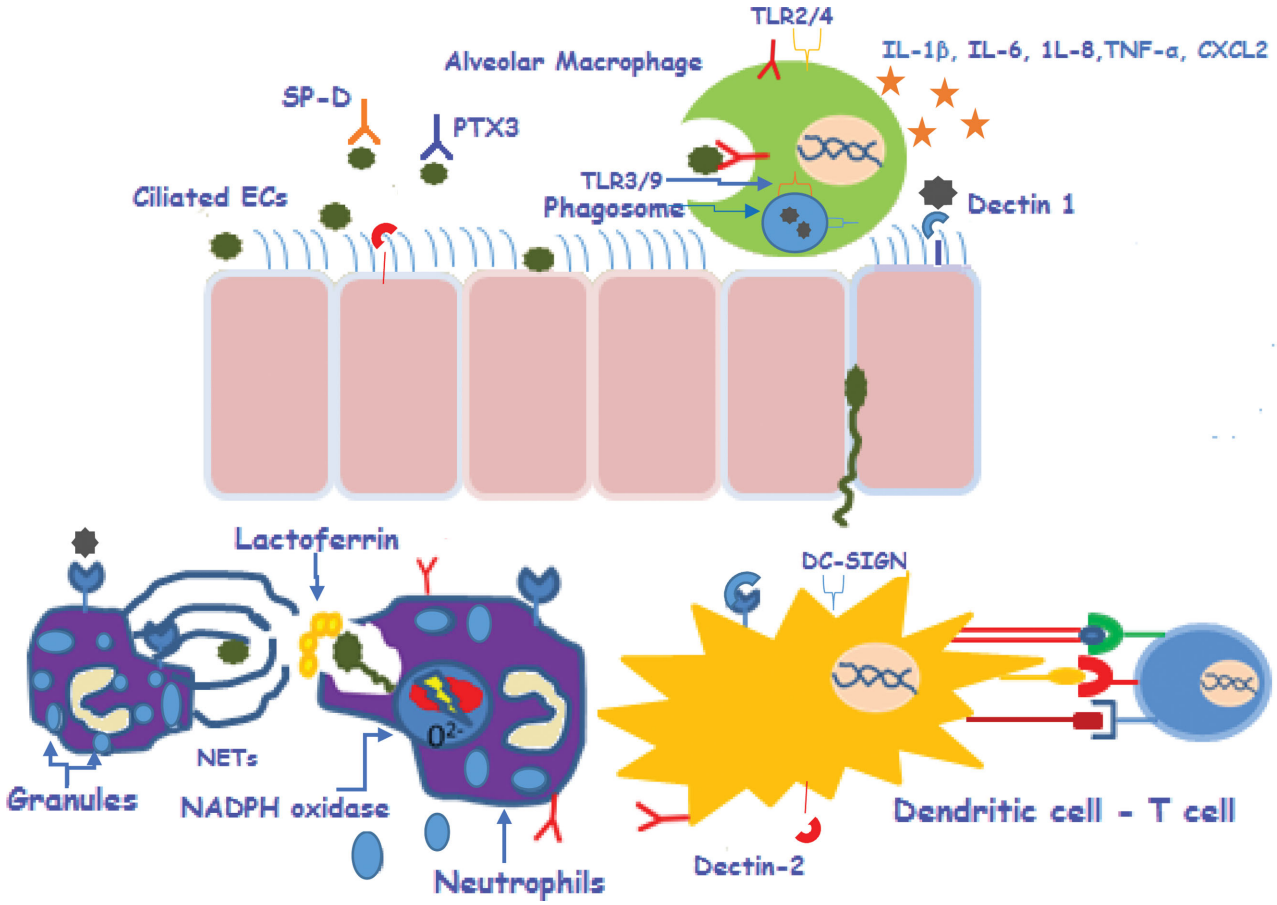

Figure 4. In the immunocompetent lung, conidia are immediately met by a host of soluble recognition receptors including PTX3 and SP-D which bind to and enhance conidial phagocytosis by AM. AM recognition and uptake of conidia is mediated by Dectin-1 and TLRs and leads to the induction of a proinflammatory response. Conidia that have escaped attack by AM, germinate and penetrate through the alveolar surface. AM- and EC-derived proinflammatory mediators recruit neutrophils to the site of infection. Neutrophils employ oxidative-dependent (ROS generation) and oxidative-independent (NET formation, degranulation and lactoferrin production) mechanisms to inactivate germinating conidia and hyphae. At the site of infection, DCs phagocytose and process germinated conidia for subsequent antigen presentation to naïve T cells which in turn activate an adaptive immune response to *A. fumigatus*.

Respiratory epithelial cells (ECs) also secrete a broad range of antimicrobial peptides, some of which possess activity against *A. fumigatus*. This includes lactoferrin, which sequesters iron, and β-defensins (HBD), the synthesis of which increases significantly upon EC exposure to *A. fumigatus* (Alekseeva *et al.* 2009; Balloy and Chignard 2009). An anti-fungal role for the enzyme chitinase has been demonstrated *in vitro* (Chen, Shen and Wu 2009). Chitinase is produced by ECs and macrophages, and degrades chitin a cell wall component of *A. fumigatus*. The secretory leukoprotease inhibitor is produced by ECs and maintains the protease–anti-protease balance within the airways but has also fungicidal activities (Tomee *et al.* 1997; Doumas, Kolokotronis and Stefanopoulos 2005). In cystic fibrosis (CF) patients, poor quality mucus, abnormal airway surface liquid composition and impaired mucociliary clearance are characteristic features which promote fungal colonization in the airways by providing an environment conducive to the growth of *A. fumigatus* (Verkman, Song and Thiagarajah 2003) (Fig. 1).

Role of the airway epithelium in combatting *Aspergillus*

Type II alveolar ECs, tracheal ECs and A549 ECs internalize conidia which are trafficked to late endosomes for processing in acidic cellular compartments (Paris *et al.* 1997; Wasylnka and Moore 2002; Filler and Sheppard 2006). However, in comparison to professional phagocytes (e.g. macrophages), the fungicidal activity of ECs is weaker, and *in vitro* studies have shown that conidia are able to survive and germinate inside the acidic organelles of these host cells (Wasylnka and Moore 2003). Previous studies have demonstrated the ability of dihydroxynaphthalene (DHN) melanin-producing spores to inhibit apoptosis of monocyte-derived macrophages (MDM), and reduce the phagolysosomal acidification of AM, MDM and neutrophils (Thywissen *et al.* 2011; Volling *et al.* 2011). A recent study showed similar effects caused by DHN melanin-producing conidia on alveolar EC; by inhibiting caspase-3-dependent apoptosis and reducing phagolysosomal acidification, these conidia, but not *pksP*, mutants could survive within A549 ECs *in vitro* (Amin *et al.* 2014). Interestingly, DHN melanin-producing conidia are also more efficiently phagocytosed by A549 ECs than non-melanized mutant spores (*pksP*) (Amin *et al.* 2014). Thus, it is suggested that ECs may be exploited by conidia as an immune evasion mechanism, and their ability to persist in ECs allows for their eventual germination into hyphae (Wasylnka and Moore 2003; Amin *et al.* 2014). However, it appears that conidial survival within ECs is dependent on the ability to produce DHN melanin (Amin *et al.* 2014). A possible explanation for this is that the lack of DHN melanin expose β(1,3)-glucan (β-glucan) binding sites for Dectin-1 on the conidial cell

surface, and this allows for the better uptake of *pskP* mutants than melanin-producing conidia by macrophages (Luther *et al.* 2007).

Airway epithelium cells express a range of recognition receptors including C-type lectin receptors (CLRs) and Toll-like receptors (TLRs), with which they can detect *A. fumigatus* and respond through the synthesis of proinflammatory mediators (Balloy *et al.* 2008; Bellanger *et al.* 2009). A Dectin-1-mediated protective response against *A. fumigatus* by bronchiolar ECs has been demonstrated (Sun *et al.* 2012). Interestingly, the generally low expression of Dectin-1 on these cells was significantly upregulated upon TLR2 activation by *A. fumigatus* conidia. Other studies showed that (Gersuk *et al.* 2006; Luther *et al.* 2007) swollen but not resting conidia triggered a proinflammatory response which involved the induction of TNF-α, IL-8, HBD2, HBD9 and the production of reactive oxygen species (ROS) (Sun *et al.* 2012). The ability to distinguish between various morphological forms of conidia is crucial for modulating a correct and proportionate pulmonary inflammatory response. Upon entry into the lung, resting conidia are rapidly ingested by AM, while ECs remain inactive. However, conidia which have escaped capture by AM, begin to swell and can thus be detected by ECs, which, in the activated state can mount a proinflammatory response that involves the synthesis of the neutrophil chemoattractant, IL-8 (Balloy *et al.* 2008).

MOLECULAR RECOGNITION OF *A. FUMIGATUS*

By virtue of their small size (2–3 μm), *A. fumigatus* conidia can bypass mucociliary clearance and penetrate the respiratory tract to reach the bronchoalveolar space where they encounter AM (Dagenais and Keller 2009). AM express soluble and surface pattern recognition receptors (PRRs). These germline receptors, which are also expressed by various other cell types, including neutrophils, DCs and ECs, mediate conidial recognition via pathogen-associated molecular patterns (PAMPs). The *A. fumigatus* cell wall components β-glucan, chitins and mannans are candidate PAMPs for particular PRRs, and their detection triggers a sequence of events that leads to phagocytosis, conidial killing and the production of proinflammatory and anti-inflammatory cytokines and chemokines which when combined lead to effective clearance of the pathogen from the immunocompetent lung (Park and Mehrad 2009; Levitz 2010) (Fig. 4). Several soluble and membrane-bound PRRs are involved in the recognition and killing of *A. fumigatus* (Table 1).

SOLUBLE RECEPTORS TARGETING *ASPERGILLUS*

Pentraxins, complement proteins, ficolins and collectins belong to the humoral arm of the innate immune system (Bottazzi *et al.* 2009). Pentraxin 3 (PTX3) is a soluble recognition receptor that has a non-redundant role in resistance against *A. fumigatus* (Garlanda *et al.* 2002). PTX3 is secreted by a variety of cells including neutrophils, mononuclear and pulmonary ECs in response to conidia and inflammatory signals such as TNF-α and opsonizes conidia for detection by AM (Garlanda *et al.* 2002; Han *et al.* 2005). *In vitro* studies have indicated impaired uptake and killing of conidia by AM in PTX3$^{-/-}$ mice compared to wild-type mice (Garlanda *et al.* 2002).

The critical role of PTX3 in response to *A. fumigatus* infection was demonstrated *in vivo* using otherwise immunocompetent PTX3$^{-/-}$ mice which were rendered highly susceptible to IA upon fungal challenge. Administration of exogenous PTX3 improved phagocytic and fungicidal activities in these animals (Garlanda *et al.* 2002). PTX3 was shown to enhance the recognition of conidia and the phagocytic potential by neutrophils through the Fcγ receptor II *in vitro* (Moalli *et al.* 2010). The role of the FcγRII in *A. fumigatus*-induced PTX3 activity was demonstrated when, upon treatment with exogenous PTX3, FcγR$^{-/-}$ mice displayed increased fungal burden and heightened inflammation in comparison to their wild-type counterparts (Moalli *et al.* 2010). It has been reported that degradation of PTX3 in CF airways may be a contributing factor to inefficient fungal clearance (Hamon *et al.* 2013). Neutrophil elastases, which show persistence in the CF lungs and *A. fumigatus* proteases, were found to be responsible for the degradation of the PTX3 N-terminal domain which functions in conidial recognition (Moalli *et al.* 2010; Hamon *et al.* 2013).

The complement system is an important innate defense mechanism mediated by approximately 30 serum-derived soluble factors and membrane-bound receptors which act in a sequential manner to bring about the death of the pathogen either directly or indirectly (Speth and Rambach 2012). The three pathways of complement activation, the classical, alternative and lectin pathway, converge on a common pathway in which C3 convertase cleaves C3, the products of which can (a) opsonize pathogens for improved phagocytosis, (b) act as chemoattractants for proinflammatory cells such as neutrophils and eosinophiles and (c) produce a pore-forming membrane attack complex resulting in osmotic lysis of the pathogen (Speth *et al.* 2004). Fungal killing is thought to be independent of the latter process, most likely due to the thickness of the fungal cell wall (Kozel 1996). *Aspergillus fumigatus* is known to activate all three complement pathways, although initiation of each pathway appears to be dependent on the morphological form of the fungus (Kozel *et al.* 1989). The alternative pathway is activated by resting conidia and as spores mature, there is progressive involvement of the classical pathway (Kozel 1996). This pattern of complement activation is likely due to the properties of resting conidia, whose immunogenic nature may lead to lack of antibody production (Kozel 1996; Aimanianda *et al.* 2009). Fungal maturation exposes conidia to the immune system enabling antibody development and activation of the classical pathway which is largely driven by the interaction of the complement pattern recognition molecule, C1q with surface-bound IgG and IgM (Kozel 1996; Ricklin *et al.* 2010). C1q can also activate the classical pathway by interacting with long and short pentraxins such as PTX3 and C-reactive protein, respectively (Bottazzi *et al.* 1997; Nauta *et al.* 2003). While C1q deficiency was shown to only marginally reduce phagocytosis of conidia *in vitro*, C1q$^{-/-}$ mice were more susceptible to IA (Garlanda *et al.* 2002; Moalli *et al.* 2010). This susceptibility was reverted upon treatment with exogenous PTX3, demonstrating that PTX3 activity is independent of C1q (Garlanda *et al.* 2002). PTX3 activity is however dependent on C3, and C3$^{-/-}$ mice experienced significantly reduced PTX3-bound conidial uptake by alveolar neutrophils (Moalli *et al.* 2010). Moreover, through the activation of the FcγRII, PTX3-bound conidia were shown *in vitro*, to induce the activation of C3 receptor (CR3), its recruitment to the phagocytic cup and CR3-dependent phagocytosis (Moalli *et al.* 2010). Binding of C3b to C3 convertase catalyzes the formation of a C5 convertase, which cleaves C5 to generate C5a and C5b (Ricklin *et al.* 2010). C5a is a potent chemoattractant for proinflammatory cells such as PMNs. A/Sn and DBA2 mice deficient in the complement component C5 have demonstrated increased susceptibility to IA in comparison to C5-sufficient mice (Svirshchevskaya *et al.* 2009). Compared to C5-sufficent mice, C5$^{-/-}$ murine models of IA showed

decreased neutrophil influx into the lung upon infection with *A. fumigatus*. However PMN influx and survival rates increased upon treatment with complement-sufficient serum, thus indicating an important role for complement during the early stages of *A. fumigatus* infection (Svirshchevskaya *et al.* 2009).

Collectins are soluble CLRs that recognize and bind, in a calcium-dependent manner, carbohydrate moieties such as those found in the fungal cell wall (Turner 2003). The serum protein mannan-binding lectins (MBL) are collectins that activate the complement via the lectin pathway (Ricklin *et al.* 2010). Binding of MBL to its target structure activates MBL-associated serine proteases (MASPs) which generates C4b2a, a C3-convertase, through the cleavage of its substrate C4 and C2 (Møller-Kristensen *et al.* 2007). C4b2a cleaves C3 into C3a and C3b. C3b and its inactive cleavage product, iC3b are potent opsonins and thus enhances conidial phagocytosis by AM and neutrophils (Speth *et al.* 2004). There is evidence to suggest that MBL also initiates the alternative pathway by activating C3 via a C2-bypass mechanism (Dumestre-Pérard *et al.* 2008). Kaur, Gupta and Madan (2007) established a significant role for MBL in mediating innate immunity against IA. Treatment of immunosuppressed BALB/c mice with recombinant human MBL (rhMBL) post-infection with *A. fumigatus* increased survival rates, reduced fungal loads in the lung, enhanced levels of TNF-α, IL-1β and IFN-γ and reduced levels of IL-10 (Kaur, Gupta and Madan 2007). In the same study, *in vitro* analysis of the response by PMNs upon exposure to *A. fumigatus* conidia showed increased deposition of C4b, a product of C4, on PMNs when rhMBL was added to MBL-deficient serum. Enhanced conidial uptake and anti-fungal activity by PMNs also increased in rhMBL-sufficient serum in comparison to MBL-deficient serum (Kaur, Gupta and Madan 2007). Interestingly, MBL deficiency caused by mutations in the *mbl2* gene has been associated with deterioration in individuals with CF (Garred *et al.* 2002; Davies, Turner and Klein 2004).

Ficolins are lectins that, like MBL, form complexes with MASPs and activate complement via the lectin pathway (Endo, Matsushita and Fujita 2011). *In vitro* studies have established a cooperative role for Ficolin-2 (L-ficolin) and PTX3 at the conidial surface of *A. fumigatus* (Ma *et al.* 2009). Interestingly, PTX3 enhanced deposition of Ficolin-2 onto conidial surfaces, while Ficolin-2 enhanced binding of PTX3 to conidia. Furthermore, Ficolin-2 and PTX3 act synergistically by augmenting Ficolin-2-mediated complement deposition on conidial surfaces of *A. fumigatus* (Ma *et al.* 2009).

Aspergillus fumigatus has developed several immune evasion mechanisms to avoid death by complement (Speth and Rambach 2012). The production of pigments regulated by *arp1* and *alb1* appears to be central in conferring conidial protection against complement, and deletion of these genes rendered the fungus more susceptible to C3 deposition and uptake by neutrophils *in vitro* (Tsai *et al.* 1997, 1998). In addition, the ability to *A. fumigatus* conidia to bind soluble complement inhibitors such as factor H, factor H-like protein 1 and factor H-related proteins is thought to be a mechanism employed by the pathogen to down-regulate an active complement system (Behnsen *et al.* 2008).

Pulmonary surfactant protein, SP-A and SP-D are collectins secreted by type II alveolar ECs and Clara cells (Crouch 2000; Balloy and Chignard 2009). *In vitro* studies have shown that binding of SP-A and SP-D to *A. fumigatus* conidia resulted in conidial agglutination, enhanced phagocytic capacity and increased fungicidal effects of AM and neutrophils (Madan *et al.* 1997). Furthermore, SP-A and SP-D were shown to be potent chemotactic agents for neutrophil recruitment (Madan *et al.* 1997). SP-D also binds to hyphae *in vitro* thereby indicating a possible role for this immune molecule during the later stages of fungal infections (Geunes-Boyer *et al.* 2010). In an immunocompromised murine model of IA, the role of SP-D was shown to be significant, since the survival rate of mice challenged with otherwise lethal doses of conidia, followed by treatment with SP-D, was as high as 60% (Madan *et al.* 2001). This is consistent with another study in which THP-1 cells phagocytosed and killed serum-opsonized conidia more efficiently than non-opsonized conidia *in vitro* (Marr *et al.* 2001). However, in a separate study which used murine bronchoalveolar lavage (BAL) fluid as a surfactant to opsonize conidia, no significant conidial aggregation was induced by opsonization (Faro-Trindade *et al.* 2012). Instead, conidial recognition by macrophages and the subsequent proinflammatory response was thought to occur through non-opsonic mechanisms, primarily through Dectin-1-mediated activity. Indeed, it has been demonstrated that non-opsonized conidia can also be taken up by AM (Kan and Bennett 1988; Luther *et al.* 2008). Thus, it would appear that conidial opsonization is a beneficial, yet dispensable process for the recognition and uptake of conidia by AM.

MEMBRANE BOUND PRRs

Recognition of *A. fumigatus* by Dectin-1

Following entry into the lung, conidia undergo maturation by swelling before germinating into hyphae (Fig. 3). Swollen conidia lose the thin hydrophobic RodA protein layer, a surface component of *A. fumigatus* that masks the immunogenic constituents of the cell wall. Loss of RodA thus exposes the β-glucan fractions on the fungal cell wall (Hohl *et al.* 2005; Aimanianda *et al.* 2009).

The type II transmembrane protein, Dectin-1 is a CLR that is highly expressed on macrophages, neutrophils and DCs in both humans and mice and is crucial for mediating a proinflammatory response against *A. fumigatus* (Werner *et al.* 2009). Dectin-1 recognizes β-glucan moieties on swollen and germinating conidia but does not respond to resting conidia, thus allowing macrophages to distinguish between the different morphological forms of *A. fumigatus* (Gersuk *et al.* 2006). *In vitro* studies have shown that swollen conidia are phagocytosed by macrophages with greater efficiency than resting conidia (Luther *et al.* 2007). However, masking the surface of conidia containing β-glucan with β-glucan-specific factor G did not entirely inhibit phagocytosis, thereby indicating that while this surface structure is significant, it is not the only conidial surface component recognized by macrophage recognition receptors (Luther *et al.* 2007). Dectin-1 recognition of swollen conidia can occur at the cell surface or intracellularly (Hohl *et al.* 2005; Steele *et al.* 2005; Faro-Trindade *et al.* 2012). *In vitro,* a Dectin-1-dependent inflammatory response coincided with the recruitment of Dectin-1 to, and its association with, phagolysosomes containing swollen, but not resting, conidia (Faro-Trindade *et al.* 2012). However, it appears, at least *in vitro,* that Dectin-1-mediated cytokine and chemokine production by AM does not require conidial internalization (Steele *et al.* 2005). Dectin-1 signals through Syk kinase, and in part through CARD9, activating NF-κB and inducing the expression of cytokines and chemokines including TNF-α, IL-6, IL-1α, IL-1β, G-CSF, GM-CSF, MIP-1α and MIP-1β (Steele *et al.* 2005; Werner *et al.* 2009; Faro-Trindade *et al.* 2012). IL-10, a key anti-inflammatory cytokine is also induced in a Dectin-1-dependent manner, indicating the immunoregulatory role for Dectin-1 in modulating an inflammatory response (Steele *et al.* 2005). In the context of an early immune

response to A. *fumigatus*, Dectin-1 signaling through AM is critical. A significantly impaired neutrophil influx was observed in the lungs of Dectin-1-deficient mice upon fungal challenge due to depleted production of chemoattractants by unresponsive AM (Werner *et al.* 2009).

Recognition of A. *fumigatus* by Dectin-2

Dectin-2 is a novel CLR that is primarily expressed in DCs and macrophages and has recently been implicated in the host defense against A. *fumigatus* (Saijo and Iwakura 2011; Sun *et al.* 2013). It has been established that the ligands for Dectin-2 are α-mannans, fungal cell wall constituents that are found in the outer layer, thereby masking β-glucan components of the cell (Levitz 2010; Sun *et al.* 2014). As such, it is highly possible that upon inhalation, conidia are more likely to be recognized by Dectin-2 before detection by Dectin-1. Dectin-2 is expressed at high levels by AM in the human lung in response to A. *fumigatus* and was shown to mediate an NF-κB-dependent proinflammatory response in a time-dependent manner against swollen, but not resting conidia (Sun *et al.* 2013, 2014). Upon stimulation with viable conidia, the production of IL-1β, IL-10, 1L-23p19 and TNF-α increased as resting conidia germinated into hyphae *in vitro* (Sun *et al.* 2014). This NF-κB-dependent cytokine production was shown to be mediated by Syk kinase, and silencing Syk expression led to impaired cytokine expression and secretion. Furthermore, silencing Dectin-2 and Syk expression affected the respiratory burst and resulted in significantly reduced conidial killing by THP-1 macrophages, thus further emphasizing the role of this receptor in host defense against A. *fumigatus* (Sun *et al.* 2014).

The role of TLRs 2 and 4 in A. *fumigatus* recognition

TLRs are type I membrane receptors characterized by an extracellular domain consisting of leucine-rich repeats that function in the recognition of PAMPs and an intracellular TIR domain required for downstream signaling (Kawai and Akira 2007). TLR recognition of pathogens triggers downstream signaling cascades that result in the activation of transcription factors such as NF-κB, which mediate expression of pro- and anti-inflammatory cytokines and chemokines (Kawai and Akira 2007). Several studies have implicated the plasma membrane receptors TLR2 and TLR4 as key recognition components for host defense against A. *fumigatus*. However, there are conflicting data on this subject because evidence for and against the role of these TLRs has been reported. Discrepancies between these studies may be due to the origin of cells used and variations in experimental approaches but also because the A. *fumigatus*-associated PAMPs for TLR2 and TLR4 remain undefined (Steele *et al.* 2005).

Hyphae and conidia of A. *fumigatus* were reported to stimulate TLR2-mediated production of cytokines in murine peritoneal macrophages *in vitro*, whereas a TLR4-dependent proinflammatory response was induced by conidia only (Netea *et al.* 2003). Interestingly, TLR4 signaling was lost in response to hyphae, and hyphae but not conidia, stimulated TLR2 production of anti-inflammatory IL-10 thereby indicating that a phenotypic switch from conidia to hyphae may be an immune evasion mechanism of A. *fumigatus* (Netea *et al.* 2003; Chai *et al.* 2011). In contrast, separate studies revealed reduced fungicidal activities in TLR4$^{-/-}$ PMNs to both conidia and hyphae (Bellocchio *et al.* 2004).

An essential role for TLR2 and TLR4 in cytokine production against A. *fumigatus* has been established in a number of *in vitro* studies (Meier *et al.* 2003; Braedel *et al.* 2004; Rubino *et al.* 2012). Using HEK293 cells expressing TLR-encoding plasmids, Meier *et al.* (2003) reported TLR2- and TLR4-dependent cytokine production via the NF-κB pathway but ruled out involvement of all other TLRs, including a synergistic role for TLR2 with TLR1 and TLR6 (Meier *et al.* 2003). In contrast, using HEK293 cells transfected with vectors expressing mouse and human TLR1 and TLR6,Rubino *et al.* (2012) showed that A. *fumigatus* detection by TLR2 involves the formation of a heterodimer with TLR6 or TLR1 in mice and TLR1 but not TLR6 in humans. In response to RodA$^{-/-}$ and wild-type A. *fumigatus* conidia, reduced amounts of IL-6, TNF-α, CXCL2 and IL-12p40 was produced by TLR1$^{-/-}$ murine bone marrow-derived macrophages, while cytokine production was almost abolished in TLR2$^{-/-}$, TLR4$^{-/-}$ and TLR6$^{-/-}$ macrophages but not in TLR3$^{-/-}$ or wild-type macrophages. Although TLR1 and TLR6 are important for contributing to a proinflammatory response, their role in survival is dispensable as 100% survival was observed in TLR1$^{-/-}$ and TLR6$^{-/-}$ C57BL/6 murine models of IA (Rubino *et al.* 2012). In a separate study, Dubourdeau *et al.* (2006) observed that TLR2$^{-/-}$ and TLR4$^{-/-}$ immunocompetent mice were no more susceptible to IA than wild-type mice. Elsewhere, a moderately impaired but otherwise intact inflammatory response in TLR2$^{-/-}$ murine AM upon stimulation with conidia *in vitro* was reported, the authors suggesting that TLR2 is not absolutely necessary for an A. *fumigatus*-induced proinflammatory response (Steele *et al.* 2005).

The role of TLRs 3 and 9 in A. *fumigatus* recognition

Contrary to earlier reports (Meier *et al.* 2003; Bellocchio *et al.* 2004), TLR3 and TLR9 have recently been implicated in the host defense against A. *fumigatus*. TLR3 localizes in endosomal compartments of DCs and ECs primarily, and detects double-stranded RNA which is released from conidia upon entering the endosomal pathway (Beisswenger, Hess and Bals 2012). The role of TLR3 in mediating an adaptive antifungal immune response was demonstrated *in vivo* (Carvalho *et al.* 2012). The migratory capacity of DCs through the lymph nodes of TLR3$^{-/-}$ C57BL/6 mice was reduced, thereby affecting the T-cell priming ability of these cells (Carvalho *et al.* 2012). In line with this, TLR3$^{-/-}$ mice appeared to be unable to activate a CD8^{+} T-cell response to A. *fumigatus* infection (Carvalho *et al.* 2012). TLR3 regulates fungal-induced inflammation by signaling through the adaptor protein, TRIF (Kawai and Akira 2007; de Luca *et al.* 2010). In a murine model of pulmonary aspergillosis, TRIF$^{-/-}$ mice showed a persistent and exacerbated inflammatory response in comparison to MyD88$^{-/-}$ and wild-type mice (de Luca *et al.* 2010). TLR3-expressing ECs provide protection against A. *fumigatus* through the activation of indoleamine 2,3-dioxygenase, a regulator of T cell-mediated proinflammatory responses via the TLR3/TRIF pathway (de Luca *et al.* 2010). Furthermore, EC transfection with conidial RNA induced expression of IFN-β and IP-10 (CXCL10) in a TRIF/RIP1/TBK1-dependent manner (Beisswenger, Hess and Bals 2012). IFN-β and IP-10 are mediators of innate and adaptive immunity, bridging the two arms of the immune system (Le Bon and Tough 2002). Thus, in addition to providing a link between innate and adaptive immunity, TLR3 appears to play a crucial role in modulating a proinflammatory response against A. *fumigatus* in the airways.

TLR9 is a receptor for unmethylated CpG DNA, a component of A. *fumigatus* that becomes exposed during processing in the phagolysososme (Ramirez-Oritz *et al.* 2008). In humans, TLR9 is primarily found on pDCs and B cells, but is also expressed by macrophages, and monocytes in mice (Ramirez-Oritz *et al.* 2008). Upon conidial DNA detection, TLR9 rapidly accumulates at the

spore-containing phagosomes and undergoes proteolytic cleavage, a prerequisite for TLR9 signaling (Kasperkovitz, Cardenas and Vyas 2010). TLR9 recruitment to the phagosome was found to be independent of the conidial germination stage, an indication that the fungal component responsible for inducing this activity is continuously present throughout the varying conidial maturation stages (Kasperkovitz, Cardenas and Vyas 2010). TLR9 recruitment to fungal-containing phagosomes appears to be independent of downstream signaling by other TLRs, since TLR9 activity was unaffected in MyD88$^{-/-}$ and TRIF$^{-/-}$ bone marrow-derived macrophages (Kasperkovitz, Cardenas and Vyas 2010).

TLR9 appears to play an immunoregulatory role during innate defenses against *A. fumigatus* (Ramirez-Oritz *et al.* 2008; Ramaprakash *et al.* 2009) as shown in TLR9$^{-/-}$ neutropenic mice which exhibited reduced inflammatory response 2 days post-fungal challenge and a significantly increased inflammatory response 4 days post-infection as compared to their wild-type counterparts (Ramaprakash *et al.* 2009). Thus, while TLR9 may be required to initiate an inflammatory response, it is also responsible for preventing an excessive response which could result in tissue damage and ultimately facilitate the pathogen (Ramaprakash *et al.* 2009). A correlation between TLR9 and Dectin-1 expression has also been reported; Dectin-1 expression was reduced in TLR9$^{-/-}$ mice when compared to wild-type mice, a finding that may explain the inability of TLR9$^{-/-}$ mice to respond to swollen conidia (Ramaprakash *et al.* 2009).

With the exception of TLR3, all TLRs signal through the universal adaptor, MyD88 (Ramaprakash *et al.* 2009). An important role for TLR signaling through MyD88 in early inflammatory responses to *A. fumigatus* has been reported (Bretz *et al.* 2008). In this study, MyD88$^{-/-}$ immunocompetent murine lungs experienced a delayed fungal clearance and a poorly modulated inflammatory response which was reported to have normalized 3 days post-infection, thus indicating a role for other signaling pathways in mediating inflammatory responses. Indeed, there is strong evidence that *A. fumigatus* induces MyD88-independent inflammatory responses (Marr *et al.* 2003), as appears to be the case in an immunocompetent host (Dubourdeau *et al.* 2006). A significant role for the MAPK (ERK) signaling pathway in early inflammatory responses to *A. fumigatus* was demonstrated and blocking the ERK pathway inhibited TNF-α production in AM in response to conidial swelling (Dubourdeau *et al.* 2006). Furthermore, the previously reported *A. fumigatus*-induced Dectin-1-dependent cytokine and chemokine production (Hohl *et al.* 2005) may provide an explanation for the dispensable role for TLRs and MyD88 reported by others (Marr *et al.* 2003; Dubourdeau *et al.* 2006). Thus, it is evident from these studies that in order to develop a better understanding of the role played by the various TLRs and their respective signaling pathways, further investigations are warranted.

Dectin-1–TLR interactions, DC-SIGN, NOD2 and NLRP3

Dectin-1–TLR2 collaborative responses to fungal stimuli are well established, and it has been reported that TLR2 plays a synergistic role with Dectin-1 to facilitate phagocytosis of *A. fumigatus* conidia (Gantner *et al.* 2003; Luther *et al.* 2007; Dennehy *et al.* 2008; Ferwerda *et al.* 2008; Inoue and Shinohara 2014). *In vitro*, TLR2- and MyD88 deficiency in murine macrophages significantly reduced conidial uptake but not binding of swollen conidia (Luther *et al.* 2007). This indicates that while TLR2-dependent MyD88 signaling promotes Dectin-1-mediated phagocytosis *A. fumigatus*, TLR2 is dispensable for the initial conidial binding process (Luther *et al.* 2007). Elsewhere, it was reported that, Dectin-1 signaling pathways synergize with MyD88 signaling pathways to provide an optimum inflammatory response against swollen, but not resting *A. fumigatus* conidia (Hohl *et al.* 2005).

Dendritic cell-specific (DC-SIGN) ICAM-3-grabbing non-integrin is a CLR that is expressed on DCs and AM (Serrano-Gómez *et al.* 2004; Serrano-Gómez, Leal and Corbí 2005). DC-SIGN may play an important role in the host defense against IA, and *in vitro* studies have established the ability of these cell surface receptors to recognize, bind and mediate the internalization of *A. fumigatus* conidia on both pulmonary DCs and AM (Serrano-Gómez *et al.* 2004).

The intracellular PRR, NOD2 (nucleotide-binding oligomerization domain-2) has recently been implicated in the recognition of *A. fumigatus* (Li *et al.* 2012). *In vitro*, murine macrophages showed increased expression of NOD2 and RIP2 kinase, a signaling component of NODs, in response to conidia. Upon exposure to conidia, upregulation of NF-κB was detected but was downregulated in NOD2-knockout cells, thus demonstrating a possible role for NOD2 in the host defense against *A. fumigatus* (Li *et al.* 2012).

A role for the NLRP3 inflammasome in an *A. fumigatus*-induced inflammatory response was demonstrated *in vitro*, and hyphae but not conidia were shown to induce IL-1β production which was significantly reduced in THP-1 cells carrying silenced NLRP3 and ASC genes (Saïd-Sadier *et al.* 2010). *A. fumigatus*-induced NLRP3 inflammasome activation was found to be dependent on the production of ROS since the neutralization of ROS with antioxidants inhibited both caspase-1 activation and IL-1β secretion (Saïd-Sadier *et al.* 2010). Furthermore, depletion of MyD88 and Syk resulted in decreased IL-1β gene expression, although only Syk signaling seemed to be associated with the activation of the NLRP3 inflammasome (Saïd-Sadier *et al.* 2010). Thus, *A. fumigatus* induces an IL-1β-mediated inflammatory response via the NLRP3 inflammasome, although this may not occur until some hours after conidial inhalation since hyphae but not conidia induced this response (Saïd-Sadier *et al.* 2010).

IL-1β processing by the caspase-8 inflammasome was reported to be dependent on Dectin-1 activation in response to swollen *A. fumigatus* conidia. Since Dectin-1 detects extracellular PAMPs, fungal internalization is not required to activate the caspase-8 inflammasome (Gringhuis *et al.* 2012). This allows for a swift and immediate IL-1β response without the need for conidial internalization, as is the case for NLRs.

CELLULAR RESPONSES TO *A. FUMIGATUS*

AM response to *A. fumigatus*

The principle role of AM is to phagocytose and kill conidia which it does via oxidative mechanisms through the generation of ROS such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and by non-oxidative mechanisms involving phagosomal acidification (Ibrahim-Granet *et al.* 2003; Philippe *et al.* 2003). Upon entry into the lung, AM phagocytose *A. fumigatus* conidia rapidly in an actin-dependent manner (Marr *et al.* 2001; Ibrahim-Granet *et al.* 2003). PI-3-kinase is involved in coordinating the pseudopod extensions that entrap conidia and when treated with wortmannin, an inhibitor of phosphatidylinositol (PI) 3-kinase activity, AM showed reduced conidial uptake (Cox *et al.* 1999; Ibrahim-Granet *et al.* 2003). Members of the src family of tyrosine kinases and myosin motor proteins are also required for the process of phagocytosis (Luther *et al.* 2008). Furthermore, the GTPase dynamin which is responsible for endocytosis was shown

to participate in conidial internalization, and blocking its activity with Dynasore inhibited the uptake of *A. fumigatus* conidia by macrophages (Kasperkovitz, Cardenas and Vyas 2010). Thus, phagocytosis of *A. fumigatus* conidia by AM is dependent upon a series of complex coordinated cellular responses (Ibrahim-Granet *et al.* 2003; Luther *et al.* 2008; Kasperkovitz, Cardenas and Vyas 2010).

Internalized conidia are contained within a phagosome which undergoes maturation by fusing with a lysosome, forming a phagolysosome (Ibrahim-Granet *et al.* 2003). Vacuolar ATPase-mediated acidification of the phagolysosome and activation of hydrolytic enzymes such as cathepsin-D and chitinases contribute to the degradation of the fungal cell wall and blocking ATPase activity was shown to dramatically reduce fungal killing (Ibrahim-Granet *et al.* 2003). Furthermore, phagosomal processing of internalized conidia is a prerequisite for the activation of the intracellular PRRs, Dectin-1 and TLR9 since it is here that the respective ligands become exposed (Kasperkovitz, Cardenas and Vyas 2010; Faro-Trindade *et al.* 2012). Thus, this compartment appears to be central for innate recognition of *A. fumigatus* (Faro-Trindade *et al.* 2012).

Aspergillus fumigatus conidia begin to swell approximately three hours after engulfment (Marr *et al.* 2001; Philippe *et al.* 2003) (Fig. 3b). Coinciding with this event is the generation of ROS, the production of which correlates directly to elevated levels of fungal killing (Philippe *et al.* 2003). ROS production is triggered in response to swollen, but not resting conidia through the activation of nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase, which induces the single electron reduction of oxygen to superoxide anion (O_2^-) (Babior, Kipnes and Curnutte 1973; Forman and Torres 2002; Gersuk *et al.* 2006). The cytosolic proteins, $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and the small GTPase Rac1/Rac2 are recruited to the plasma membrane where they complex with the membrane-bound flavocytochrome subunits, $gp91^{phox}$ and $gp22^{phox}$, forming an active NADPH oxidase (Forman and Torres 2002). The role of NADPH oxidase in AM during *A. fumigatus* infection is a matter for debate. A number of studies have demonstrated an important fungicidal role for the ROS-producing complex during *A. fumigatus* infection (Philippe *et al.* 2003; Grimm *et al.* 2013). Others have suggested that NADPH oxidase is a modulator of the inflammatory response to *A. fumigatus* rather than being directly responsible for fungal killing (Cornish *et al.* 2008). *In vivo* studies by Philippe *et al.* (2003) demonstrated that inhibition of ROS production in NADPH-inactive $p47^{phox-/-}$ mice suppress conidial killing. The critical role of NADPH oxidase in AM-mediated defense against *A. fumigatus* was recently highlighted by Grimm *et al.* (2013). The authors of this study showed that even cyclophosamide-treated (leukopenic) mice were less susceptible to IA than non-leukopenic mice with a $p47^{phox}$-deficiency. Furthermore, in murine models of IA, NADPH $oxidase^{-/-}$ mice with targeted expression of macrophage-restricted NADPH oxidase had increased survival rates and reduced pulmonary inflammation in comparison to globally NADPH $oxidase^{-/-}$ mice. Additionally, in agreement with the findings of Philippe *et al.* (2003), $p47^{phox-/-}$ AM, in contrast to wild-type AM, were unable to prevent growth of phagocytosed conidia *in vitro* (Grimm *et al.* 2013). These studies indicate an important role for NADPH oxidase in conferring protection against *A. fumigates*. However, some studies have suggested a dispensable role for ROS in resistance against this pathogen. For example, Lessing *et al.* (2007) identified AfYap1 as the transcriptional regulator of genes associated with *A. fumigatus* resistance against ROS, and deletion of this gene resulted in increased sensitivity to H_2O_2 but did not affect the virulence of the fungus in *in vitro* and *in vivo* models of IA. Catalase is a scavenger of H_2O_2, and catalase-deficient strains of *A. fumigatus* (Δ*catA*) experienced the same level of killing as wild-type conidia by murine AM *in vivo* thereby indicating that H_2O_2 is not the main ROS responsible for conidial killing (Paris *et al.* 2003). Other studies have suggested a superior role for O_2^- than H_2O_2 in defense against *A. fumigatus* (Lamarre *et al.* 2007). Using $gp91^{phox-/-}$ murine AM, Henriet *et al.* (2011) observed higher growth inhibition of *A. fumigatus* conidia than wild-type cells. Similarly, Cornish *et al.* (2008) observed equal inhibition of conidial germination *in vitro* by AM from $gp91^{phox-/-}$ and C57BL/6 mice. In this study, gene expression analysis of $gp91^{phox-/-}$ and wild-type mice upon exposure to *A. fumigatus* conidia showed no upregulation of transcripts encoding the cytosolic or membrane-bound subunits of the NADPH oxidase complex, or of genes encoding oxidant scavengers. However, genes involved in PMN recruitment including *Cxcl2* and *Ccl3* were significantly upregulated in wild-type mice compared to $gp91^{phox-/-}$ mice upon exposure to conidia. The notable early expression of these genes post-infection suggest the primary role for AM during early *A. fumigatus* infection may be neutrophil recruitment (Cornish *et al.* 2008). Another gene with higher expression in wild-type AM than $gp91^{phox-/-}$ AM was *Socs3*, a negative regulator of the proinflammatory cytokine, IL-6. Indeed, several studies have outlined the role of NADPH oxidase in regulating inflammation. Grimm *et* al. (2013) reported that the lungs of globally NADPH $oxidase^{-/-}$ mice experienced far greater zymosan-induced inflammation than wild-type mice. In a separate study, zymosan-induced pulmonary inflammation was significantly higher in $p47^{phox-/-}$ and $gp91^{phox-/-}$ mice compared with wild-type mice and in comparison to wild-type lungs, resolution of lung inflammation was impaired in NADPH oxidase-defective mice (Segal *et al.* 2010). In this study, $p47^{phox-/-}$ and $gp91^{phox-/-}$ mice showed increased activity of the proinflammatory transcription factor NF-κB, and reduced nuclear translocation of the anti-inflammatory transcription factor Nrf2 compared to wild-type mice. These findings establish a role for NADPH oxidase as a modulator of a proinflammatory response by negatively regulating NF-κB, and activating of Nrf2.

In vivo studies using murine models of IA have shown that *A. fumigatus* infection drives a rapid alternatively activated macrophage-mediated response and depletion of AM resulted in a significantly higher fungal burden than AM-sufficient mice (Bhatia *et al.* 2011). Interestingly, the high number of PMNs recruited to the AM-deficient lungs were unable to control the fungal load on this occasion, thereby indicating the cooperative role of AM and neutrophils that is necessary to eliminate conidia post-inhalation (Bhatia *et al.* 2011). AM orchestrate a robust inflammatory response through the activation of PRRs and the secretion of cytokines and chemokines, amongst which are key mediators of neutrophil recruitment, including TNF-α and CXCR2 ligands: macrophage inflammatory protein-2 (MIP-2)/CXCL2 and keratinocyte-derived chemokine (KC/)CXCL1 (Bhatia *et al.* 2011). This is important because although AM have the capacity to eliminate small inocula of conidia, larger doses of conidia appear to necessitate the involvement of neutrophils (Philippe *et al.* 2003). Conidia that escape macrophage killing begin to germinate, forming germ tubes and hyphae which penetrate through the alveolar surface (Dagenais and Keller 2009). Hyphae are too large to be phagocytosed by AM, and neutrophils are employed to eliminate the invasive fungal form (Bonnett, Cornish and Burritt 2006) (Fig. 4).

The role of TNF-α in *A. fumigatus* infection

Tumor-necrosis factor-α (TNF-α) enhances the host response to the fungus during early and latter stages of infection by augmenting the phagocytic potential of AM and by promoting the capacity of PMNs to generate oxidative burst metabolites in response to hyphae, which ultimately improves PMN-induced hyphal damage (Roilides *et al.* 1998).

TNF-α indirectly mediates neutrophil deployment to the site of infection by inducing expression of adhesion molecules on endothelial cells thereby promoting neutrophil trafficking in the lungs (Vieira *et al.* 2009). Histology of pulmonary tissue from immunocompetent mice that had been treated with anti-TNF-α antibody revealed conidial and hyphal forms just 3 days after challenge with *A. fumigatus* conidia whereas untreated murine lungs contained conidia only (Mehrad, Strieter and Staniford 1999). Consistent with this, a reduced neutrophil influx was observed in TNF-α-neutralized murine lungs (Mehrad, Strieter and Staniford 1999). In a further study investigating the role of CXCR2 ligands in chemokine-mediated immunity against *A. fumigatus* a 90% mortality rate was observed in anti-CXCR2 antibody-treated mice, while histology of lung tissue revealed large quantities of hyphae and a dramatically reduced neutrophil count as compared to untreated mice (Mehrad *et al.* 1999). Similarly, a delayed PMN recruitment to and increased conidial germination in the lungs of CXCR2$^{-/-}$ mice was observed by Bonnett, Cornish and Burritt (2006). However neither mortality nor hyphal formation was reported in this study, perhaps due to the use of BALB/c mice, a less IA-susceptible strain than the C57BL/6 mice used in the study by Mehrad *et al.* (1999) (Bonnett, Cornish and Burritt 2006).

Neutrophil response to *A. fumigatus*

The critical role of neutrophils in innate defense against *A. fumigatus* has been highlighted in several studies which have demonstrated that the outcome of neutrophil depletion in mice is high mortality rates and the establishment of IA (Stephens-Romero, Mednick and Feldmesser 2005; Mircescu *et al.* 2009). In contrast to other studies (Bhatia *et al.* 2011), it has been suggested that AM are dispensable for host defense against *A. fumigatus* (Mircescu *et al.* 2009). *In vivo* studies have revealed that neutrophil recruitment is not entirely dependent upon AM-mediated signaling and support a role for a proinflammatory-mediated response by other immune cells, such as epithelial cells, endothelial cells, natural killer (NK) cells and DCs (Mircescu *et al.* 2009).

Neutrophils employ a range of oxidative and non-oxidative mechanisms that effectively eliminate germinating spores and hyphal forms of *A. fumigatus*, including phagocytosis, NADPH-oxidase-mediated generation of ROS, production of lactoferrin and crucially, the discharge of antimicrobial proteases by degranulation (Feldmesser 2006; Zarember *et al.* 2007). The conidiocidal effect of lactoferrin on *A. fumigatus* has been demonstrated, and a key role for this glycoprotein as a non-oxidative killing mechanism deployed by neutrophils in early defense against conidia has been proposed (Zarember *et al.* 2007).

The necessity of a functional NADPH oxidase in neutrophil-mediated killing of *A. fumigatus* is evident in individuals with chronic granulomatous disease (CGD). CGD is a syndrome characterized by mutations in p47^{phox} which affect the ability of NADPH oxidase to generate ROS, thus leaving patients extremely susceptible to IA (Grimm *et al.* 2011). *In vivo* studies showed NADPH-oxidase-defective (gp91$^{phox-/-}$) mice experienced delayed PMN recruitment and were unable to inhibit conidial germination (Bonnett, Cornish and Burritt 2006). Lung tissue samples obtained from these mice showed extensive hyphal proliferation and tissue invasion which was not observed in the pulmonary tissue of wild-type (C57BL/6 and BALB/C) mice (Bonnett, Cornish and Burritt 2006). Analysis of BAL fluid from wild-type and gp91$^{phox-/-}$ mice following pulmonary exposure to resting conidia revealed that PMN form aggregates around the spores. In contrast to wild-type PMN aggregates, those lacking gp91^{phox} were unable to inhibit conidial germination. However, addition of exogenous H_2O_2 and hypochlorous acid (HClO) to NADPH oxidase-defective cells halted conidial germination *in vitro*, thus indicating the importance of ROS in tackling *A. fumigatus* infection (Bonnett, Cornish and Burritt 2006). In line with previous studies (Paris *et al.* 2003; Lamarre *et al.* 2007), H_2O_2 appeared a less effective ROS, but in this study it contributed to inhibition of conidial germination nonetheless.

The *A. fumigatus* toxins, gliotoxin and fumagillin have demonstrated the ability to inhibit the fungicidal activity of neutrophils by blocking the formation of a functional NADPH oxidase complex (Tsunawaki *et al.* 2004; Fallon, Reeves and Kavanagh 2010) (Fig. 2). The immunosuppressive properties of gliotoxin are well established as evidenced by the ability to induce an immunosuppressive state in mice by injecting the toxin (Sutton, Waring and Mullbacher 1996). Deletion of the *gliP* gene which controls gliotoxin biosynthesis, disabled NADPH oxidase inhibition by mutant strains of *A. fumiagtus* and also reduced fungal virulence in immunosuppressed mice (Sugui *et al.* 2007). *In vivo*, gliotoxin appears to be dispensable for virulence in neutropenic murine models of aspergillosis; however, when exposed to gliP mutants, immunosuppressed non-neutropenic mice were more resistant to *A. fumigatus* than non-neutropenic mice exposed to wild-type spores (Spikes *et al.* 2008). This indicates that neutrophils are a primary target for gliotoxin.

The formation of neutrophil extracellular traps (NETs) was described by Brinkmann *et al.* (2004) as a novel form of neutrophil-mediated antimicrobial defense and has since been implicated in the host defense against *A. fumigatus* (Bruns *et al.* 2010; McCormick *et al.* 2010; Röhm *et al.* 2014). NETs are networks of extracellular fibers composed of decondensed nuclear chromatin that bind histones and antimicrobial granular proteins including neutrophil elastase, myeloperoxidase, cathepsin G, lactoferrin and gelatinase (Brinkmann *et al.* 2004). NET formation (NETosis) is induced by a variety of microbes or proinflammatory mediators such as IL-8 and is particularly important for defense against pathogens that are too large to be phagocytosed, such as *A. fumigatus* hyphae (Brinkmann *et al.* 2004; Urban *et al.* 2006).

Aspergillus fumigatus conidia are a trigger for NET formation (Jaillon *et al.* 2007). However, NET formation depends on conidial morphology and *in vitro*, hyphal forms and *rodA* mutants of resting conidia, but not wild-type resting conidia were able to trigger NETosis (Bruns *et al.* 2010). NETs stimulated by swollen conidia do not appear to inhibit germination although they do inhibit the growth of, but do not kill hyphae, thereby indicating a role for NETs during the latter stages of *A. fumigatus* infection (McCormick *et al.* 2010). Calprotectin, a NET-associated protein chelates zinc ions thereby starving the fungus of an essential nutrient, and evidence suggests that NET-mediated hyphal growth inhibition is at least in part calprotectin dependent (McCormick *et al.* 2010; Bianchi *et al.* 2011). Bianchi *et al.* (2011) observed *in vitro* growth inhibition of *A. nidulans* which was reversed when calprotectin was blocked.

It has been established *in vitro* and *in vivo*, that NET formation is dependent on a functional NADPH oxidase and the generation of ROS (Fuchs *et al.* 2007; Bruns *et al.* 2010; Röhm *et al.* 2014). In a murine model of CGD, the neutrophils of p47$^{phox-/-}$ of mice were unable to form NETs and, in contrast to wild-type mice, were unable to control hyphal burden or stem inflammation in the lungs when exposed to *A. fumigatus* (Röhm *et al.* 2014). In a patient with gp91$^{phox-/-}$ CGD, gene therapy was shown to restore NADPH oxidase and NET activity (Bianchi *et al.* 2009). After gene therapy, gp91^{phox+} neutrophils were shown to kill *A. nidulans* with greater efficiency than gp91$^{phox-/-}$ control neutrophils *in vitro*. Despite inhibition of NET formation by treatment of gp91^{phox+} and gp91$^{phox-/-}$ neutrophils with MNase neutrophil-mediated fungal killing was not prevented and was comparable in gp91^{phox+} and gp91$^{phox-/-}$ neutrophils (Bianchi *et al.* 2009). This indicates that neutrophils also inhibit hyphal growth in a NET-independent manner, such as degranulation. At least with respect to *A. fumigatus*, it appears that NETs have a fungistatic effect rather than a fungicidal effect (Bruns *et al.* 2010; McCormick *et al.* 2010). Indeed, when phagocytosis was inhibited with cytochalasin-D, neutrophil killing of conidia was abrogated, indicating that phagocytic events and not NET formation is the main killer of *A. fumigatus* by neutrophils (Bruns *et al.* 2010).

Neutrophil degranulation involves the discharge of fungicidal hydrolytic enzymes from primary (azurophil) granules into the phagocytic vacuole and it is this non-oxidative mechanism that is primarily responsible for *A. fumigatus* killing (Spitznagel 1990; Segal 2005). While the contents of azurophilic granules mediates direct killing of *A. fumigatus*, NADPH oxidase-derived ROS promotes neutrophil degranulation through the activation of these granular proteases (Reeves *et al.* 2002; Feldmesser 2006). Furthermore, myeloperoxidase (MPO), a component of primary granules, catalyzes the conversion of H_2O_2 to HClO (Roos, van Bruggen and Meischl 2003). Lefkowitz *et al.* (1996) reported a role for MPO as an immunomodulator of macrophage activity against *Candida albicans* by stimulating macrophage-associated respiratory burst and the secretion of TNF-α, a cytokine known to induce neutrophil degranulation and release of MPO (Lefkowitz *et al.* 1996). Thus, NADPH oxidase targets *A. fumigatus* directly through the production of ROS, and indirectly through the formation of NETs and by hydrolytic enzymes released during neutrophil degranulation.

There is no doubt that AM and neutrophils play the major role in the first line of defense against *A. fumigatus*. However, there is mounting evidence that acknowledges the roles played by other innate immune cells in the fight against this fungal pathogen.

Eosinophiles and mast cell response to *A. fumigatus*

The role of eosinophiles in ABPA is well documented and the fungicidal activity of eosinophiles has been reported (Yoon *et al.* 2008; Wark *et al.* 2000; Patterson and Strek 2010) (Fig. 1). More recently, eosinophiles have been implicated specifically in the host defense against *A. fumigatus* (Lilly *et al.* 2014). Eosinophile-deficient but otherwise immunocompetent mice experienced increased hyphal burden and impaired fungal clearance from lungs as compared to wild-type mice (Lilly *et al.* 2014). Moreover, cytokine and chemokine production (IL-1β, IL-6, IL-17A, G-CSF, GM-CSF and CXCL1/KC) was significantly reduced in eosinophile-deficient mice, corresponding with the impaired pulmonary clearance of *A. fumigatus* (Lilly *et al.* 2014). Eosinophiles, like neutrophils, possess granules that contain antimicrobial proteins including eosinophile peroxidase (EPO) and major basic protein (MBP). Upon stimulation, eosinophiles respond by activating a respiratory burst, and discharge their granular contents into the phagosome and extracellular fluid (Henderson and Chi 1985). It has been suggested that decreased levels of EPO and MBP may contribute to the increased fungal burden found in eosinophile$^{-/-}$ lungs (Lilly *et al.* 2014).

The role of mast cells in fungal infection is not well documented (Urb and Sheppard 2012). Mast cell degranulation is classically associated with antigen-specific IgE; however, *in vitro* studies show that *A. fumigatus* hyphae, but not conidia or germ tubes trigger degranulation events in an IgE-independent manner (Urb *et al.* 2009). Since mast cells were not associated directly with fungal killing, their purpose in *A. fumigatus*-induced immune responses appears to be limited to modulating a proinflammatory response (Urb *et al.* 2009).

The NK cell response to *A. fumigatus*

The influence of NK cells on the host defense against *A. fumigatus* has been highlighted by several studies (Morrison *et al.* 2003; Park *et al.* 2009; Bouzani *et al.* 2011; Schmidt *et al.* 2011). While the mechanism of pathogen recognition is unclear, it has been established that NK cells respond to germinating but not resting conidia (Bouzani *et al.* 2011; Schmidt *et al.* 2011). Antifungal activity appears to be multifactorial and mediated by soluble factors, although it is uncertain as to whether the stimulation of NK cells is contact dependent (Bouzani *et al.* 2011; Schmidt *et al.* 2011, 2013). IL-2-producing cells play an important role in promoting NK cell anti-fungal potential, and the fungal killing ability of IL-2 pre-stimulated human NK cells was shown to be significantly greater than non-stimulated cells *in vitro* (Bouzani *et al.* 2011; Schmidt *et al.* 2011).

NK cells induce fungal killing through the release of perforins, and *in vitro*, increased perforin levels correlated with increased killing activity of *A. fumigatus* (Schmidt *et al.* 2011). Others have attributed the fungicidal effect to NK cell-derived IFN-γ (Bouzani *et al.* 2011). The anti-fungal activity of IFN-γ appears to be related to its ability to prevent the growth of germinating conidia into hyphal forms (Park *et al.* 2009; Bouzani *et al.* 2011). The conidial-killing capacity of AM increased when incubated with wild-type NK cells than when incubated alone or with IFN-$\gamma^{-/-}$ NK cells (Park *et al.* 2009). Thus, in addition to exhibiting direct fungicidal effects on hyphae, NK cell-derived IFN-γ augments the killing capacity of AM (Park *et al.* 2009).

NK cell-derived IFN-γ has a significant effect on the pulmonary expression on IFN-inducible chemokines, specifically CXCL9, CXCL10 and CXCL11 (Park *et al.* 2009). These ligands mediate the influx of Th-1 CD4 T cells through their association with their common receptor CXCR3 (Groom and Luster 2011). Interestingly, NK cells also express CXCR3; thus, through the production of CXCR3 ligands NK cells appear to promote a positive feedback cycle during fungal infection (Park *et al.* 2009; Pak-Wittel *et al.* 2013).

In immunocompetent hosts, neutrophils form the first line of defense against hyphal forms of *A. fumigatus*; therefore, the majority of *in vivo* studies carried out to assess the influence of NK cells as a host defense mechanism have been performed in a neutropenic setting (Morrison *et al.* 2003; Park *et al.* 2009). The mortality rate of NK cell-depleted neutropenic mice has shown to be twice that of neutropenic mice models of IA (Morrison *et al.* 2003). Thus, NK cells appear to form an 'extra line' of defense against the invasive form of *A. fumigatus* that appears to be particularly important for the neutropenic host. In fact, it has been proposed that NK cells may potentially

be employed in adoptive immunotherapy procedures in the context of transplantation to reduce the risk of aspergillosis (Schmidt *et al.* 2011).

The role of monocytes in response to A. *fumigatus*

In vitro analysis of the interplay between human monocytes and A. *fumigatus* has identified distinct roles for two monoctye subsets, $CD14^+$ $CD16^+$ and $CD14^+$ $CD16^-$, in defense against the fungus (Serbina *et al.* 2009). Conidial germination and internalization is a prerequisite for monocyte activation and while neither subset were able to kill conidia, $CD14^+$ $CD16^-$ but not $CD14^+$ $CD16^+$ inhibited conidial germination into hyphae, although the latter were found to secrete far more TNF-α upon exposure to the pathogen (Serbina *et al.* 2009). Through *in vitro* coincubation of A. *fumigatus* with human monocytes an induction in the expression of TNF-α, IL-1α, IL-1β, 1L-6, 1L-10, IL-8, CCL7, CCL2 and CCL20 in response to germinating conidia and hyphae but not resting conidia was observed (Loeffler *et al.* 2009). Cortez *et al.* (2006) reported an increase in cytokine and chemokine expression by human monocytes which coincided with an increase in conidial phagocytosis *in vitro*. Murine $Ly6C^{hi}$ monocytes (CCR2-expressing inflammatory monocytes) were shown to mediate an adaptive immune response to A. *fumigatus* through the activation of $CD4^+$ T cells (Hohl *et al.* 2009). Following conidial uptake, $Ly6C^{hi}$ monocytes rapidly convert to $CD11^+$ monocyte-derived DCs (Mo-DCs) and transport conidia to the draining lymph nodes of the lung where they prime A. *fumigatus*-specific $CD4^+$ T cells. Recruitment of $CD11^+$ Mo-DCs to the mediastinal lymph nodes was abrogated in CCR2-depleted mice and the increased fungal burden correlated to a loss of $CD4^+$ T cell-related responses (Hohl *et al.* 2009).

Human monocytes use the lysosomal degradation pathway (autophagy) to eliminate A. *fumigatus* conidia, a process mediated by the recruitment of autophagy protein, LC3 II to the phagosome (Kyrmizi *et al.* 2013). β-glucan-mediated activation of Dectin-1/Syk kinase/ROS signaling is required for recruitment of LC3 II and phagosomal maturation, thus only swollen or germinating, but not resting conidia can trigger this process (Kyrmizi *et al.* 2013). The involvement of NADPH-derived ROS in recruitment of LC3 II to conidia-containing phagosomes renders this pathway defective in the monocytes of patients with CGD (Kyrmizi *et al.* 2013).

In vivo studies using C57BL/6 mice depleted of $CCR2^+$ Mo demonstrated a clear role for $CCR2^+$ monocytes (Mo) and Mo-DCs in resistance to A. *fumigatus*, and CCR2-depleter mice were extremely susceptible to IA compared to $CCR2^+$ Mo-sufficient mice (Espinosa *et al.* 2014). *In vitro*, $CCR2^+$ Mo significantly enhanced the conidiacidal effect of conidia-containing neutrophils and are a potent source of proinflammatory mediators such as TNF, IL-12 and PTX3. In contrast to previous studies which have suggested a fungistatic rather than a fungicidal effect by monocytes (Serbina *et al.* 2009), $CCR2^+$ Mo and Mo-DCs were shown to be involved in direct killing of A. *fumigatus*. Conidial uptake coincided with the differentiation of $CCR2^+$ Mo to Mo-DCs (Espinosa *et al.* 2014). While both cell types were shown to eliminate conidia, Mo-DCs have superior killing abilities which depends in part on NADPH oxidase. Interestingly, while $p47^{phox-/-}$ monocytes had reduced killing capacity compared to $p47^{phox+/+}$ Mo, some killing was maintained in $p47^{phox-/-}$ cells indicating another method of fungicidal activity is employed by monocytes to destroy A. *fumigatus* (Espinosa *et al.* 2014). Thus, it would appear that the distinct contribution of monocytes to defense against A. *fumigatus* is subtype dependent.

The role of platelets in A. *fumigatus* infection

It is not unusual for thrombocytopenia to accompany neutropenia in individuals receiving chemotherapy or organ transplantation (Demetri 2001). *Aspergillus fumigatus* is angioinvasive and hyphal invasion of blood vessels causes characteristic features of IA such as thrombosis and vascular infarction (Bezerra and Filler 2004). A number of *in vitro* studies have identified potential roles for platelets in defense against A. *fumigatus*, particularly against the hyphal form of the fungus (Christin *et al.* 1998; Rødland *et al.* 2010). *In vitro*, it was observed that human platelets surround and adhere to the surface of opsonized conidia and hyphae, but do not engulf fungal spores, perhaps due to the large size of A. *fumigatus* conidia relative to platelets (Christin *et al.* 1998; Perkhofer *et al.* 2008). While studies have reported platelet activation to be contact dependent, Speth *et al.* (2013) observed platelet activation when these cells were exposed to A. *fumigatus*-derived serine proteases and purified gliotoxin *in vitro* and when exposed to the supernatant of gliP mutants, platelets failed to activate.

Activation of platelets is identified by cell surface expression of CD62P antigen and CD63, markers for platelet activation which are released from α-granules and δ-granules, respectively (Christin *et al.* 1998; Perkhofer *et al.* 2008; Rødland *et al.* 2010). Activated platelets were shown to inhibit conidial germination and reduce hyphal elongation, at least in part by damaging hyphal cell wall (Christin *et al.* 1998; Speth *et al.* 2013). Platelets store platelet microbicidal proteins (PMPs) in granules and release these antimicrobial factors upon pathogen-induced activation (Christin *et al.* 1998; Perkhofer *et al.* 2008). One of these PMPs, serotonin (5- hydroxytryptamine; 5-HT) is stored in δ-granules and was shown to have fungistatic effects on several *Aspergillus* species, including A. *fumigatus* (Perkhofer *et al.* 2007).

Furthermore, activated platelets modulate an immune response by recruiting and enhancing the effects of other cells such as PMNs and monocytes (Weyrich and Zimmerman 2004). Rødland *et al.* (2010) observed hyphal-dependent induction of CCL5, CD40L and DKK-1. These soluble factors are released from α-granules and mediate a diverse range of immune responses including chemotaxis and inflammation (Wong and Fish 2003; Elgueta *et al.* 2009; Ueland *et al.* 2009). Additionally, coincubation of hyphal-activated human platelets with THP-1 cells and human adherent monocytes enhanced the expression of IL-8 in monocytes *in vitro* (Rødland *et al.* 2010). Thus, platelets appear to play an important role in mediating proinflammatory responses against A. *fumigatus*, specifically during the later stages of IA when the fungus has germinated and has begun producing mycotoxins such as gliotoxin (Speth *et al.* 2013). While platelet activation may be beneficial in terms of enhancing an immune response against A. *fumigatus*, it may also contribute to unwanted inflammation (Rødland *et al.* 2010).

DCs mediate an adaptive immune response to A. *fumigatus*.

DCs have a number of well-established roles in the host defense against A. *fumigatus*. Immature DCs (iDCs) can phagocytose opsonized or unopsonized conidia and hyphae, which are recognized through a host of PRRs including Dectin-1, DC-SIGN, CR3 and FcγRII (Bozza *et al.* 2002; Serrano-Gómez, Leal and Corbí 2005; Mezger *et al.* 2008). It has been suggested that DC maturation is in fact triggered through the DC-SIGN-mediated binding and internalization of A. *fumigatus* conidia by DCs and that the induction of an iDC-mediated inflammatory response is

primarily due to the activation of Dectin-1 (Serrano-Gómez *et al.* 2004; Mezger *et al.* 2008). Consistent with this, a recent study demonstrated that *in vitro*, β-glucan stimulates DCs to increase the production of pro-inflammatory mediators, specifically IL-12 and IL-8 (Fidan *et al.* 2014). A more recent study claimed that Dectin-2 was the primary recognition receptor for the hyphal form of *A. fumigatus* and activates human pDCs by coupling to Syk indirectly through association with the FcγR chain (Loures *et al.* 2015).

TNF-α, IL-6, IL-12, IL-1α and IL-1β appear to be the main proinflammatory cytokines produced by iDCs upon exposure to *A. fumigatus* conidia and hyphae (Bozza *et al.* 2002; Mezger *et al.* 2008; Morton *et al.* 2011). The differential expression of proinflammatory cytokines appears to be relative to fungal morphology. *In vitro* studies showed that TNF-α was produced in response to conidia and hyphae, an IL-12-response to conidia only, and an IL-4 and IL-10-response to hyphae but not conidia (Bozza *et al.* 2002). Furthermore, time-dependent increase in all measured cytokines and chemokines upon exposure to *A. fumigatus* germ tubes was observed (Mezger *et al.* 2008).

IL-8/CXCL8, a potent chemoattractant for neutrophils, was found to be up-regulated in fungal-infected iDCs, and an increase in IL-8 was observed to coincide with germ tube formation (Gafa *et al.* 2007; Morton *et al.* 2011; Fidan *et al.* 2014). Moreover, neutrophils are known to produce cytokines such as CCL3/MIP-1α and CCL4/MIP-1β, which signal the recruitment of iDCs (Scapini *et al.* 2000). *Aspergillus fumigatus*-stimulated DCs also express a host of inflammatory chemokines involved in T-cell recruitment (Gafa *et al.* 2007). The upregulation of the CCR5 ligands CCL3, CCL4 and CCL5 following coincubation with *A. fumigatus* indicates the involvement of DCs in the recruitment of front-line effector cells such as AM and neutrophils while the expression of CCL20 and CCL19 results in the recruitment of effector memory T cells and naïve T cells expressing CCR6 and CCR7, respectively (Gafa *et al.* 2007; Morton *et al.* 2011). DCs that have internalized conidia express CCR7 and can migrate to secondary lymph nodes to activate naïve T cells (Gafa *et al.* 2006).

The role of plasmacytoid DCs (pDCs) in the host defense against a murine model of IA has recently been highlighted and it appears to be fungistatic rather than fungicidal (Ramirez-Oritz *et al.* 2011). The antifungal activity of pDCs was attributed in part, to the zinc sequestering effect of calprotectin (Ramirez-Oritz *et al.* 2011). Interestingly, a recent study provided evidence that pDCs like neutrophils form extracellular traps when stimulated by *A. fumigatus* hyphae and so-called pETs (pDC extracellular traps) were observed surrounding the hyphae (Loures *et al.* 2015). pDCs are major type I IFN producers and an *in vivo* role for IFNs against aspergillosis was suggested since IFN-$\alpha/\beta R^{-/-}$ mice were more susceptible to aspergillosis than wild-type mice (Ramirez-Ortiz *et al.* 2011). Consistent with this, pDC-depleted mice were significantly more vulnerable to IA than pDC-sufficient mice.

The adaptive immune response to *A. fumigatus*

It is well established that Th1 $CD4^+$ T cells confer protection to the host against the invasive form of *A. fumigatus* (Cenci *et al.* 1998, 1999; Chai *et al.* 2010). In contrast, Th2-mediated responses to IA may be detrimental to the host and (DBA/2) mice with impaired Th2 responses were more resistant to IA than their wild-type counterparts (Cenci *et al.* 1998, 1999). *In vivo* evidence suggests that distinct $CD4^+$ T-cell responses against *A. fumigatus* are influenced by fungal cell morphology (Rivera *et al.* 2005). Immunocompetent (C57BL/6J) mice infected with live conidia produced increased levels of IFN-γ, the signature Th1 cytokine, while mice infected with heat-killed conidia produced far lower amounts of IFN-γ and increased amounts of IL-4, the cytokine associated with differentiation of $CD4^+$ T cells into the Th2 subtype (Rivera *et al.* 2005). Thus, the specific T-cell responses employed to challenge distinct forms of *A. fumigatus* indicate that the adaptive immune system can distinguish between threatening and non-threatening forms of the pathogen and subsequently mount a response appropriate to the invasive potential of the fungus (Rivera *et al.* 2005).

The role for Th17 cells in IA is less clear. Conflicting reports provide evidence for and against a protective role of Th17 cells in murine models of IA (Werner *et al.* 2009; Zelante *et al.* 2007). However in humans, *A. fumigatus* does not appear to induce the same Th17-associated inflammatory response as in mice (Chai *et al.* 2010). *In vitro,* exposure of live *A. fumigatus* conidia to human peripheral blood mononuclear cells induced limited expression (in comparison to *C. albicans*) of IL-17, the signature cytokine of Th17 cells (Chai *et al.* 2010). Furthermore, IL-17 levels in BALs taken from patients at risk of IA and with IA were low and IL-17 concentrations in serum samples taken from patients with IA were lower than controls. One explanation for this is the ability of *A. fumigatus* to inhibit IL-17 release via the tryptophan metabolism pathway, thereby preventing a Th17-mediated inflammatory response against this fungus (Romani *et al.* 2009; Chai *et al.* 2010).

CONCLUSION

Aspergillosis can be a devastating disease and in its most lethal form (IA) can have a mortality rate of over 80% (Latgé 1999; Singh and Paterson 2005). *Aspergillus fumigatus* conidia are inhaled daily, and the immune response is capable of dealing effectively and rapidly with these and thus preventing fungal growth and tissue invasion. This review has outlined the central roles played by some key components of the human innate immune system in protecting the host against *A. fumigatus*. In a stage-specific manner, participants of the innate immune system work in synergy, in a way that is not yet fully elucidated, to ensure an effective clearance of *A. fumigatus* conidia from the respiratory airways of immunocompetent individuals before they have the opportunity to develop. Where there is disruption to the anatomical barriers (e.g. excess mucus in CF patients), reduction in neutrophils (e.g. neutropenia) or the absence of an adequate immune response (e.g. immunosuppression prior to organ transplantation) conidia germination can occur and tissue invasion may commence. Fully understanding the role of the immune response in dealing with *A. fumigatus* conidia may enable us to develop novel strategies to boost the immune response in immunodeficient patients and so assist in limiting fungal infection.

ACKNOWLEDGEMENTS

The authors are grateful for the assistance of Dr. Ilona Dix in capturing the images of conidial germination in Fig. 3.

Conflict of interest. None declared.

REFERENCES

Aimanianda V, Bayry J, Bozza S, *et al.* Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 2009;**460**:1117–21.

Alekseeva L, Huet D, Féménia F, *et al.* Inducible expression of beta defensins by human respiratory epithelial cells exposed to *Aspergillus fumigatus* organisms. *BMC Microbiol* 2009; **9**:33.

Amin S, Thywissen A, Heinekamp T, *et al.* Melanin dependent survival of Apergillus fumigatus conidia in lung epithelial cells. *Int J Med Microbiol* 2014;**304**:626–36.

Amitani R, Taylor G, Elezis EN, *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun* 1995;**63**:3266–71.

Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 1973;**52**:741–4.

Balloy V, Chignard M. The innate immune response to *Aspergillus fumigatus*. *Microbes Infect* 2009;**11**:919–27.

Balloy V, Sallenave JM, Wu Y, *et al.* Aspergillus fumigatus-induced interleukin-8 synthesis by respiratory epithelial cells is controlled by the phosphatidylinositol 3-kinase, p38 MAPK, and ERK1/2 pathways and not by the toll-like receptor-MyD88 pathway. *J Biol Chem* 2008;**283**:30513–21.

Behnsen J, Hartmann A, Schmaler J, *et al.* The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement system. *Infect Immun* 2008;**76**:820–7.

Beisswenger C, Hess C, Bals R. *Aspergillus fumigatus* conidia induce interferon-β signalling in respiratory epithelial cells. *Eur Respir J* 2012;**39**:411–8.

Bellanger AP, Millon L, Khoufache K, *et al.* Aspergillus fumigatus germ tube growth and not conidia ingestion induces expression of inflammatory mediator genes in the human lung epithelial cell line A549. *J Med Microbiol* 2009;**58**:174–9.

Bellocchio S, Moretti S, Perruccio K, *et al.* TLRs govern neutrophil activity in aspergillosis. *J Immunol* 2004;**173**:7406–15.

Bezerra LML, Filler SG. Interactions of *Aspergillus fumigatus* with endothelial cells: internalization, injury, and stimulation of tissue factor activity. *Blood* 2003;**103**:2143–9.

Bhatia S, Fei M, Yarlagadda M, *et al.* Rapid host defense against *Aspergillus fumigatus* involves alveolar macrophages with a predominance of alternatively activated phenotype. *PLoS One* 2011;**6**:e15943.

Bianchi M, Hakkim A, Brinkmann V, *et al.* Restoration of NET formation by gene therapy in CGD controls aspergillosis. *Blood* 2009;**114**:2619–22.

Bianchi M, Niemiec MJ, Siler U, *et al.* Restoration of anti-Aspergillus defense by neutrophil extracellular traps in human chronic granulomatous disease after gene therapy is calprotectin-dependent. *J Allergy Clin Immun* 2011;**127**:1243–52.

Bonnett CR, Cornish EJAG, Burritt JB. Early neutrophil recruitment and aggregation in the murine lung inhibit germination of *Aspergillus fumigatus* Conidia. *Infect Immun* 2006;**74**:6528–39.

Bottazzi B, Doni A, Garlanda C, *et al.* An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol* 2009;**28**:157–83.

Bottazzi B, Vouret-Craviari V, Bastone A, *et al.* Multimer formation and ligand recognition by the long pentraxin PTX3: similarities and differences with the short pentraxins C-reactive protein and serum amyloid p component. *J Biol Chem* 1997;**272**:32817–23.

Bouzani M, Ok M, McCormick A, *et al.* Human NK cells display important antifungal activity against Aspergillus fumigatus, which is directly mediated by IFN-γ release. *J Immunol* 2011;**187**:1369–76.

Bozza S, Gaziano R, Spreca A, *et al.* Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *J Immunol* 2002;**168**:1362–71.

Braedel S, Radsak M, Einsele H, *et al.* *Aspergillus fumigatus* antigens activate innate immune cells via toll-like receptors 2 and 4. *Brit J Haematol* 2004;**125**:392–9.

Bretz C, Gersuk G, Knoblaugh S, *et al.* MyD88 signaling contributes to early pulmonary responses to *Aspergillus fumigatus*. *Infect Immun* 2008;**76**:952–8.

Brinkmann V, Reichard U, Goosmann C, *et al.* Neutrophil extracellular traps kill bacteria. *Science* 2004;**303**:1532–5.

Bruns S, Kniemeyer O, Hasenberg M, *et al.* Production of extracellular traps against Aspergillus fumigatus in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. *PLoS Pathog* 2010;**6**:e1000873.

Carvalho A, De Luca A, Bozza S, *et al.* TLR3 essentially promotes protective class I–restricted memory CD8$^+$ T-cell responses to *Aspergillus fumigatus* in hematopoietic transplanted patients. *Blood* 2012;**119**:967–77.

Cenci E, Mencacci A, DelSero G, *et al.* Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through suppression of protective type I responses. *J Infect Dis* 1999;**180**:1957–68.

Cenci E, Mencacci A, Fè d'Ostiani C, *et al.* Cytokine- and T helper-dependent lung mucosal immunity in mice with invasive pulmonary aspergillosis. *J Infect Dis* 1998;**178**:1750–60.

Chai LY, van de Veerdonk F, Marijnissen RJ, *et al.* Anti-Aspergillus human host defence relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. *Immunology* 2010;**130**:46–54.

Chai LYA, Vonk AG, Kullberg BJ, *et al.* *Aspergillus fumigatus* cell wall components differentially modulate host TLR2 and TLR4 responses. *Microb Infect* 2011;**13**:151–9.

Chen L, Shen Z, Wu J. Expression, puriffication and in vitro antifungal activity of acidic mammalian chitinase against *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton rubrum* strains. *Clin Exp Dermatol* 2009;**34**:55–60.

Christin L, Wysong DR, Meshulam T, *et al.* Human platelets damage *Aspergillus fumigatus* hyphae and may supplement killing by neutrophils. *Infect Immun* 1998;**66**:1181–9.

Cornish EJ, Hurtgen BJ, McInnerney K, *et al.* Reduced nicotinamide adenine dinucleotide phosphate oxidase-independent resistance to *Aspergillus fumigatus* in alveolar macrophages. *J Immunol* 2008;**180**:6854–67.

Cortez KJ, Lyman CA, Kottilil S, *et al.* Functional genomics of innate host defense molecules in normal human monocytes in response to *Aspergillus fumigatus*. *Infect Immun* 2006;**74**:2353–65.

Cox D, Ching-Chun T, Bjekic G, *et al.* A requirement for phosphatidylinositol 3-kinase in pseudopod extension. *J Biol Chem* 1999;**274**:1240–7.

Crouch EC. Surfactant protein-D and pulmonary host defense. *Respir Res* 2000;**1**:93–108.

Dagenais TRT, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009;**22**:447–65.

Daly P, Kavanagh K. Pulmonary aspergillosis: clinical presentation, diagnosis and therapy. *Brit J Biomed Sci* 2001;**58**:197–205.

Davies JC, Turner MW, Klein N. Impaired pulmonary status in cystic fibrosis adults with two mutated MBL-2 alleles. *Eur Respir J* 2004;**24**:798–804.

de Luca A, Bozza S, Zelante T, *et al.* Non-hematopoietic cells contribute to protective tolerance to *Aspergillus fumigatus* via TRIF pathway converging on IDO. *Cell Mol Immunol* 2010;**7**:459–70.

Demetri GD. Targeted approaches for the treatment of thrombocytopenia. *Oncologist* 2001;**6**:15–23.

Dennehy KM, Ferwerda G, Faro-Trindade I, *et al.* Syk kinase is required for collaborative cytokine production induced through Dectin-1 and toll-like receptors. *Eur J Immunol* 2008;**38**:500–6.

Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998;**26**:781–803.

Doumas S, Kolokotronis A, Stefanopoulos P. Anti-inflammatory and antimicrobial roles of secretory leukocyte protease inhibitor. *Infect Immun* 2005;**73**:1271–4.

Dubourdeau M, Athman R, Balloy V, *et al.* *Aspergillus fumigatus* induces innate immune responses in alveolar macrophages through the MAPK pathway independently of TLR2 and TLR4. *J Immunol* 2006;**177**:3994–4001.

Dumestre-Pérard C, Lamy B, Aldebert D, *et al.* Mechanism by the mannan-binding lectin C2 bypass *Aspergillus* conidia activate the complement. *J Immunol* 2008;**181**:7100–5.

Elgueta R, Benson MJ, De Vries VC, *et al.* Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* 2009;**229**:152–72.

Endo Y, Matsushita M, Fujita T. The role of ficolins in the lectin pathway of innate immunity. *Int J Biochem Cell B* 2011;**43**:705–12.

Espinosa V, Jhingran A, Dutta O, *et al.* Inflammatory monocytes orchestrate innate antifungal immunity in the lung. *PLoS Pathog* 2014;**10**:e1003940.

Fallon J, Reeves E, Kavanagh K. Inhibition of neutrophil function following exposure to the *Aspergillus fumigatus* toxin, fumagillin. *J Med Microbiol* 2010;**59**:625–33.

Faro-Trindade I, Willment JA, Kerrigan AM, *et al.* Characterisation of innate fungal recognition in the lung. *PLoS One* 2012;**7**:e35675.

Feldmesser M. Role of neutrophils in invasive aspergillosis. *Infect Immun* 2006;**74**:6514–6.

Ferwerda G, Meyer-Wentrup F, Kullberg BJ, *et al.* Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. *Cell Microbiol* 2008;**10**:2058–66.

Fidan I, Kalkanci A, Yesilyurt E, *et al.* *In vitro* effects of Candida albicans and *Aspergillus fumigatus* on dendritic cells and the role of beta glucan in this effect. *Adv Clin Exp Med* 2014;**23**:17–24.

Filler SG, Sheppard DC. Fungal invasion of normally non-phagocytic host cells. *PLoS One* 2006;**2**:e129.

Forman HJ, Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am J Resp Crit Care* 2002;**166**:4–8.

Fuchs TA, Abed U, Goosmann C, *et al.* Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* 2007;**176**:231–41.

Gafa V, Lande R, Gagliardi MC, *et al.* Human dendritic cells following *Aspergillus fumigatus* infection express the CCR7 receptor and a differential pattern of interleukin-12 (IL-12), IL-23, and IL-27 cytokines, which lead to a Th1 response. *Infect Immun* 2006;**74**:1480–9.

Gafa V, Remoli ME, Giacomini E, *et al.* In vitro infection of human dendritic cells by *Aspergillus fumigatus* conidia triggers the secretion of chemokines for neutrophil and Th1 lymphocyte recruitment. *Microbe Infect* 2007;**9**:971–80.

Gantner BN, Simmons RM, Canavera SJ, *et al.* Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 2003;**197**:1107–17.

Garlanda C, Hirsch E, Bozza S, *et al.* Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* 2002;**420**:182–6.

Garred P, Pressler T, Lanng S, *et al.* Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. *Pediatr Pulm* 2002;**33**:201–7.

Gersuk GM, Underhill DM, Zhu L, *et al.* Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J Immunol* 2006;**176**:3717–24.

Geunes-Boyer S, Heitman J, RaeWright J, *et al.* Surfactant protein D binding to *Aspergillus fumigatus* hyphae is calcineurin-sensitive. *Med Mycol* 2010;**48**:580–8.

Grimm MJ, Vethanayagam RR, Almyroudis NG, *et al.* Role of NADPH oxidase in host defense against aspergillosis. *Med Mycol* 2011;**49**:S144–9.

Grimm MJ, Vethanayagam RR, Almyroudis NG, *et al.* Monocyte- and macrophage-targeted NADPH oxidase mediates antifungal host defense and regulation of acute inflammation in mice. *J Immunol* 2013;**190**:4175–84.

Gringhuis SI, Kaptein TM, Wevers BA, *et al.* Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. *Nat Immunol* 2012;**13**:246–54.

Groom JR, Luster AD. CXCR3 ligands: redundant, collaborative and antagonistic functions. *Immunol Cell Biol* 2011;**89**:207–15.

Hamon Y, Jaillon S, Person C, *et al.* Proteolytic cleavage of the long pentraxin PTX3 in the airways of cystic fibrosis patients. *Innate Immun* 2013;**19**:611–22.

Han B, Mura M, Andrade CF, *et al.* TNFα-induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. *J Immunol* 2005;**175**:8303–11.

Henderson WR, Chi EY. Ultrastructural characterization and morphometric analysis of human eosinophile degranulation. *J Cell Sci* 1985;**73**:33–48.

Henriet SSV, Hermans PWM, Verweij PE, *et al.* Human leukocytes kill *Aspergillus nidulans* by reactive oxygen species-independent mechanisms. *Infect Immun* 2011;**79**:767–73.

Hohl TM, Feldmesser M. *Aspergillus fumigatus*: principles of pathogenesis and host defense. *Eukaryot Cell* 2007;**6**:1953–63.

Hohl TM, Rivera A, Lipuma L, *et al.* Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. *Cell Host Microbe* 2009;**6**:470–81.

Hohl TM, Van Epps HL, Rivera A, *et al.* *Aspergillus fumigatus* triggers inflammatory responses by stage-specific β-glucan display. *PLoS Pathog* 2005;**1**:e30.

Ibrahim-Granet O, Philippe B, Boleti H, *et al.* Phagocytosis and intracellular fate of *Aspergillus fumigatus* conidia in alveolar macrophages. *Infect Immun* 2003;**71**:891–903.

Inoue M, Shinohara ML. Clustering of pattern recognition receptors for fungal detection. *PLoS Pathog* 2014;**10**:e1003873.

Jaillon S, Peri G, Delneste Y, *et al.* The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med* 2007;**204**:793–804.

Kan VL, Bennett JE. Lectin-like attachment sites on murine pulmonary alveolar macrophages bind *Aspergillus fumigatus* conidia. *J Infect Dis* 1988;**158**:407–14.

Kasperkovitz PV, Cardenas ML, Vyas JM. TLR9 is actively recruited to *Aspergillus fumigatus* phagosomes and requires the N-terminal proteolytic cleavage domain for proper intracellular trafficking. *J Immunol* 2010;**185**:7614–22.

Kaur S, Gupta VK, Madan T. Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. *Clin Exp Immunol* 2007;**148**:382–9.

Kawai T, Akira S. TLR signalling. *Semin Immunol* 2007;**19**:24–32.

Kozel TR. Activation of the complement system by pathogenic fungi. *Clin Microbiol Rev* 1996;**9**:34–46.

Kozel TR, Wilson MA, Farrell TP, *et al*. Activation of C3 and binding to *Aspergillus fumigatus* conidia and hyphae. *Infect Immun* 1989;**57**:3412.

Kyrmizi I, Gresnigt MS, Akoumianaki T, *et al*. Corticosteroids block autophagy protein recruitment in *Aspergillus fumigatus* phagosomes via targeting dectin-1/Syk kinase signaling. *J Immunol* 2013;**191**:1287–99.

Lamarre C, Ibrahim-Granet O, Du C, *et al*. Characterization of the SKN7 ortholog of *Aspergillus fumigatus*. *Fungal Genet Biol* 2007;**44**:682–90.

Latgé JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 1999;**12**:310–50.

LeBon A, Tough DF. Links between innate and adaptive immunity via type I interferon. *Curr Opin Immunol* 2002;**14**:432–6.

Lefkowitz SS, Gelderman MP, Lefkowitz DL, *et al*. Phagocytosis and intracellular killing of *Candida albicans* by macrophages exposed to myeloperoxidase. *J Infect Dis* 1996;**173**:1202–7.

Lessing F, Kniemeyer O, Wozniok I, *et al*. The *Aspergillus fumigatus* transcriptional regulator AfYap1 represents the major regulator for defense against reactive oxygen intermediates but is dispensable for pathogenicity in an intranasal mouse infection model. *Eukaryot Cell* 2007;**6**:2290–302.

Levitz SM. Innate recognition of fungal cell walls. *PLoS Pathog* 2010;**6**:e1000758.

Li Z, Tao L, Zhang J, *et al*. Role of NOD2 in regulating the immune response to *Aspergillus fumigatus*. *Inflamm Res* 2012;**61**:643–8.

Lilly LM, Scopel M, Nelson MP, *et al*. Eosinophile deficiency compromises lung defense against *Aspergillus fumigatus*. *Infect Immun* 2014;**82**:1315–25.

Loeffler J, Haddad Z, Bonin M, *et al*. Interaction analyses of human monocytes co-cultured with different forms of *Aspergillus fumigatus*. *J Med Microbiol* 2009;**58**:49–58.

Loures FV, Röhm M, Lee CK, *et al*. Recognition of *Aspergillus fumigatus* hyphae by human plasmacytoid dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. *PLoS Pathog* 2015;**11**:e1004643.

Luther K, Rohde M, Sturm K, *et al*. Characterisation of the phagocytic uptake of *Aspergillus fumigatus* conidia by macrophages. *Microbes Infect* 2008;**10**:175–84.

Luther K, Torosantucci A, Brakhage AA, *et al*. Phagocytosis of *Aspergillus fumigatus* conidia by murine macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. *Cell Microbiol* 2007;**9**:368–81.

Ma YJ, Doni A, Hummelshøi T, *et al*. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem* 2009;**284**:28263–75.

McCormick A, Heesemann L, Wagener J, *et al*. NETs formed by human neutrophils inhibit growth of the pathogenic mold *Aspergillus fumigatus*. *Microbes Infect* 2010;**12**:928–36.

Madan T, Eggleton P, Kishore U, *et al*. Binding of pulmonary surfactant proteins A and D to *Aspergillus fumigatus* conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. *Infect Immun* 1997;**65**:3171–9.

Madan T, Kishore U, Singh M, *et al*. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. *J Clin Invest* 2001;**107**:467–75.

Marr KA, Balajee SA, Hawn TR, *et al*. Differential role of MyD88 in macrophage-mediated responses to opportunistic fungal pathogens. *Infect Immun* 2003;**71**:5280–6.

Marr KA, Koudadoust M, Black M, *et al*. Early events in macrophage killing of *Aspergillus fumigatus* conidia: new flow cytometric viability assay. *Clin Diagn Lab Immun* 2001;**8**:1240–7.

Mehrad B, Strieter RM, Moore TA, *et al*. CXC chemokine receptor-2 ligands are necessary components of neutrophil-mediated host defense in invasive pulmonary aspergillosis. *J Immunol* 1999;**163**:6086–94.

Mehrad B, Strieter RM, Staniford TJ. Role of TNF-α in pulmonary host defense in murine invasive aspergillosis. *J Immunol* 1999;**162**:1633–40.

Meier A, Kirschning CJ, Nikolaus T, *et al*. Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. *Cell Microbiol* 2003;**5**:561–70.

Mezger M, Kneitz S, Wozniok I, *et al*. Proinflammatory response of immature human dendritic cells is mediated by dectin-1 after exposure to *Aspergillus fumigatus* germ tubes. *J Infect Dis* 2008;**197**:924–81.

Mircescu MM, Lipuma L, van Rooijen N, *et al*. Essential role for neutrophils but not alveolar macrophages at early time points following *Aspergillus fumigatus* infection. *J Infect Dis* 2009;**200**:647–56.

Moalli F, Doni A, Deban L, *et al*. Role of complement and Fc{gamma} receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood* 2010;**116**:5170–80.

Møller-Kristensen M, Thiel S, Sjöholm A, *et al*. Cooperation between MASP-1 and MASP-2 in the generation of C3 convertase through the MBL pathway. *Int Immunol* 2007;**19**:141–9.

Morrison BE, Park SJ, Mooney JM, *et al*. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *J Clin Invest* 2003;**112**:1862–70.

Morton CO, Varga JJ, Hornbach A, *et al*. The temporal dynamics of differential gene expression in *Aspergillus fumigatus* interacting with human immature dendritic cells *in vitro*. *PLoS One* 2011;**6**:e16016.

Nauta AJ, Bottazzi B, Mantovani A, *et al*. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol* 2003;**33**:465–73.

Netea MG, Warris A, Van der Meer JWM, *et al*. *Aspergillus fumigatus* evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. *J Infect Dis* 2003;**188**:320–6.

Pak-Wittel MA, Yang L, Sojka DK, *et al*. Interferon-γ mediates chemokine-dependent recruitment of natural killer cells during viral infection. *P Natl Acad Sci USA* 2013;**110**:50–9.

Paris S, Boisvieux-Ulrich E, Crestani B, *et al*. Internalization of *Aspergillus fumigatus* conidia by epithelial and endothelial cells. *Infect Immun* 1997;**65**:1510–4.

Paris S, Wysong D, Debeaupuis JP, *et al*. Catalases of Aspergillus fumigatus. *Infect Immun* 2003;**71**:3551–62.

Park SJ, Hughes MA, Burdick M, *et al*. Early NK cell-derived IFN-{gamma} is essential to host defense in neutropenic invasive aspergillosis. *J Immunol* 2009;**182**:4306–12.

Park SJ, Mehrad B. Innate immunity to *Aspergillus* species. *Clin Microbiol Rev* 2009;**22**:535–51.

Patterson K, Strek ME. Allergic bronchopulmonary aspergillosis. *Proc Am Thorac Soc* 2010;**7**:237–44.

Perkhofer S, Kehrel BE, Dierich MP, *et al*. Human platelets attenuate *Aspergillus* species via granule-dependent mechanisms. *J Infect Dis* 2008;**198**:1243–6.

Perkhofer S, Niederegger H, Blum G, *et al.* Interaction of 5-hydroxytryptamine (serotonin) against Aspergillus spp. in vitro. *Int J Antimicrob Ag* 2007;**29**:424–9.

Philippe B, Ibrahim-Granet O, Prévost MC, *et al.* Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. *Infect Immun* 2003;**71**:3034–42.

Ramaprakash H, Ito T, Standiford TJ, *et al.* Toll-like receptor 9 modulates immune responses to *Aspergillus fumigatus* conidia in immunodeficient and allergic mice. *Infect Immun* 2009;**77**:108–19.

Ramirez-Ortiz ZG, Lee CK, Wang PJ, *et al.* A Nonredundant role for plasmacytoid dendritic cells in host defense against the human fungal pathogen *Aspergillus fumigatus*. *Cell Host Microbe* 2011;**9**:415–24.

Ramirez-Ortiz ZG, Specht CA, Wang JP, *et al.* Toll-like receptor 9-dependent immune activation by unmethylated CpG motifs in *Aspergillus fumigatus* DNA. *Infect Immun* 2008;**76**:2123–9.

Reeves EP, Lu H, Jacobs HL, *et al.* Killing activity of neutrophils is mediated through activation of proteases by K+ flux. *Nature* 2002;**416**:291–6.

Ricklin D, Hajishengallis G, Yang K, *et al.* Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 2010;**11**:785–97.

Rivera A, VanEpps HL, Hohl TM, *et al.* Distinct CD4(+)-T-cell responses to live and heat-inactivated *Aspergillus fumigatus* conidia. *Infect Immun* 2005;**73**:7170–9.

Rødland EK, Ueland T, Pedersen TM, *et al.* Activation of platelets by *Aspergillus fumigatus* and potential role of platelets in the immunopathogenesis of aspergillosis. *Infect Immun* 2010;**78**:1269–75.

Rogers DF. Airway goblet cells: responsive and adaptable front-line defenders. *Eur Respir J* 1994;**7**:1690–706.

Röhm M, Grimm MJ, D'Auria A, *et al.* NADPH oxidase promotes neutrophil extracellular trap formation in pulmonary aspergillosis. *Infect Immun* 2014;**82**:1766–77.

Roilides E, Dimitriadou-Georgiadou A, Sein T, *et al.* Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. *Infect Immun* 1998;**66**:5999–6003.

Romani L, Zelante T, De Luca A, *et al.* Indoleamine 2,3-dioxygenase (IDO) in inflammation and allergy to Aspergillus. *Med Mycol* 2009;**47**:154–61.

Roos D, van Bruggen R, Meischl C. Oxidative killing of microbes by neutrophils. *Microb Infect* 2003;**5**:1307–15.

Rubino I, Coste A, LeRoy D, *et al.* Species-specific recognition of *Aspergillus fumigatus* by toll-like receptor 1 and toll-like receptor 6. *J Infect Dis* 2012;**205**:944–54.

Saijo S, Iwakura Y. Dectin-1 and Dectin-2 in innate immunity against fungi. *Int Immunol* 2011;**23**:467–72.

Saïd-Sadier N, Padilla E, Langsley G, *et al.* *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. *PLoS One* 2010;**5**:e10008.

Scapini P, Lapinet-Vera JA, Gasperini S, *et al.* The neutrophil as a cellular source of chemokines. *Immunol Rev* 2000;**177**:195–203.

Schmidt S, Tramsen L, Hanisch M, *et al.* Human natural killer cells exhibit direct activity against *Aspergillus fumigatus* hyphae, but not against resting conidia. *J Infect Dis* 2011;**203**:430–5.

Schmidt S, Zimmermann SY, Tramsen L, *et al.* Natural killer cells and antifungal host response. *Clin Vaccine Immunol* 2013;**20**:452–8.

Segal AW. How neutrophils kill microbes. *Annu Rev Immunol* 2005;**23**:197–223.

Segal BH, Han W, Bushey JJ, *et al.*, NADPH oxidase limits innate immune responses in the lungs in mice. *PLoS One* 2010;**5**:e9631.

Segal BH, Walsh TJ. Current approaches to diagnosis and treatment of invasive aspergillosis. *Am J Respir Crit Care* 2006;**173**:707–17.

Serbina NV, Cherny M, Shi C, *et al.* Distinct responses of human monocyte subsets to *Aspergillus fumigatus* conidia. *J Immunol* 2009;**183**:2678–87.

Serrano-Gómez D, Domínguez-Soto A, Ancochea J, *et al.* Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of *Aspergillus fumigatus* conidia by dendritic cells and macrophages. *J Immunol* 2004;**173**:5635–43.

Serrano-Gómez D, Leal JA, Corbí AL. DC-SIGN mediates the binding of *Aspergillus fumigatus* and keratinophylic fungi by human dendritic cells. *Immunobiology* 2005;**210**:175–83.

Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. *Clin Microbiol Rev* 2005;**18**:44–69.

Speth C, Hagleitner M, Ott HW, *et al.* *Aspergillus fumigatus* activates thrombocytes by secretion of soluble compounds. *J Infect Dis* 2013;**207**:823–33.

Speth C, Rambach G. Complement attack against *Aspergillus* and corresponding evasion mechanisms. *Interdiscip Perspect Infect Dis* 2012;**2012**:463794.

Speth C, Rambach G, Lass-Flörl C, *et al.* The role of complement in invasive fungal infections. *Mycoses*2004;**47**:93–103.

Spikes S, Xu R, Nguyen CK, *et al.* Gliotoxin production in *Aspergillus fumigatus* contributes to host-specific differences in virulence. *J Infect Dis* 2008;**19**:479–86.

Spitznagel JK. Antibiotic proteins of human neutrophils. *J Clin Invest* 1990;**86**:1381–6.

Steele C, Metz A, Pop SM, *et al.* The beta-glucan receptor Dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog* 2005;**1**:e42.

Stephens-Romero SD, Mednick AJ, Feldmesser M. The pathogenesis of fatal outcome in murine pulmonary aspergillosis dpends on the neutrophil depletion strategy. *Infect Immun* 2005;**73**:114–25.

Sugui JA, Pardo J, Chang YC, *et al.* Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot Cell* 2007;**6**:1562–9.

Sun H, Xu X, Tian X, *et al.* Activation of NF-κB and respiratory burst following *Aspergillus fumigatus* stimulation of macrophages. *Immunobiology* 2014;**219**:25–36.

Sun H, Xu XY, Shao HT, *et al.* Dectin-2 is predominately macrophage restricted and exhibits conspicuous expression during *Aspergillus fumigatus* invasion in human lung. *Cell Immunol* 2013;**284**:60–7.

Sun WK, Lu X, Li X, *et al.* Dectin-1 is inducible and plays a crucial role in Aspergillus-induced innate immune responses in human bronchial epithelial cells. *Eur J Clin Microbiol* 2012;**31**:2755–64.

Sutton P, Waring P, Mullbacher A. Exacerbation of invasive aspergillosis by the immunosuppressive fungal metabolite, gliotoxin. *Immunol Cell Biol* 1996;**74**:318–22.

Svirshchevskaya EV, Shevchenko MA, Huet D, *et al.* Susceptibility of mice to invasive aspergillosis correlates with delayed cell influx into the lungs. *Int J Immunogenet* 2009;**36**:289–99.

Thywissen A, Heinekamp T, Dahse HM, *et al.* Conidial dihydroxynaphthalene melanin of the human pathogenic fungus

Aspergillus fumigatus interferes with the host endocytosis pathway. *Front Microbiol* 2011;**2**:96.

Tomee CJF, Hiemstra PS, Heinzel-Wieland R, *et al.* Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. *J Infect Dis* 1997;**176**:740–7.

Tsai HF, Chang YC, Washburn RG, *et al.* The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. *J Bacteriol* 1998;**180**:3031–8.

Tsai HF, Washburn RG, Chang YC, *et al.* *Aspergillus fumigatus* arp1 modulates conidial pigmentation and complement deposition. *Mol Microbiol* 1997;**26**:175–83.

Tsunawaki S, Yoshida L, Nishida S, *et al.* Fungal metabolite gliotoxin inhibits assembly of the human respiratory burst NADPH oxidase. *Infect Immun* 2004;**72**:3373–82.

Turner MW. The role of mannose-binding lectin in health and disease. *Mol Immunol* 2003;**40**:423–9.

Ueland T, Otterdal K, Lekva T, *et al.* Dickkopf-1 enhances inflammatory interaction between platelets and endothelial cells and shows increased expression in atherosclerosis. *Arterioscl Throm Vas* 2009;**29**:1228–34.

Urb M, Pouliot P, Gravelat FN, *et al.* Aspergillus fumigatus induces immunoglobulin E-independent mast cell degranulation. *J Infect Dis* 2009;**200**:464–72.

Urb M, Sheppard DC. The role of mast cells in the defense against pathogens. *PLoS Pathog* 2012;**8**:e1002619.

Urban CF, Reichard U, Brinkmann V, *et al.* Neutrophil extracellular traps capture and kill *Candida albicans* yeast and hyphal forms. *Cell Microbiol* 2006;**8**:668–76.

Verkman AS, Song Y, Thiagarajah JR. Role of airway surface liquid and submucosal glands in cystic fibrosis lung disease. *Am J Physiol-Cell Ph* 2003;**284**:2–15.

Vieira SM, Lemos HP, Grespan R, *et al.* A crucial role for TNF-α in mediating neutrophil influx induced by endogenously generated or exogenous chemokines, KC/CXCL1 and LIX/CXCL5. *Brit J Pharmacol* 2009;**158**:779–89.

Volling K, Thywissen A, Brakhage AA, *et al.* Phagocytosis of melanised *Aspergillus* conidia by macrophages exerts cytoprotective effects by sustained P13K/Akt signalling. *Cell Microbiol* 2011;**13**:1130–48.

Wark PA, Saltos N, Simpson J, *et al.* Induced sputum eosinophiles and neutrophils and bronchiectasis severity in allergic bronchopulmonary aspergillosis. *Eur Respir J* 2000;**16**:1095–101.

Wasylnka JA, Moore MM. Uptake of *Aspergillus fumigatus* conidia by phagocytic and nonphagocytic cells *in vitro*: quantitation using strains expressing green fluorescent protein. *Infect Immun* 2002;**70**:3156–63.

Wasylnka JA, Moore MM. *Aspergillus fumigatus* conidia survive and germinate in acidic organelles of A549 epithelial cells. *J Cell Sci* 2003;**116**:1579–87.

Werner JL, Metz AE, Horn D, *et al.* Requisite role for the dectin-1 β-glucan receptor in pulmonary defense against *Aspergillus fumigatus*. *J Immunol* 2009;**182**:4938–46.

Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* 2004;**25**:489–95.

Wong MM, Fish EN. Chemokines: attractive mediators of the immune response. *Semin Immunol* 2003;**15**:5–14.

Yoon J, Ponikau JU, Lawrence CB, *et al.* Innate anti-fungal immunity of human eosinophiles mediated by a β2-integrin, CD11b. *J Immunol* 2008;**181**:2907–15.

Zarember KA, Sugui JA, Chang YC, *et al.* Human polymorphnuclear leukocytes inhibit *Aspergillus fumigatus* conidial growth by lactoferrin-mediated iron depletion. *J Immunol* 2007;**178**:6367–73.

Zelante T, De Luca A, Bonifazi P, *et al.* IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol* 2007;**37**:2695–706.