Medical Mycology May 2009, **47**, 227–236

informa
healthcare

Review Article

Fungal strategies for overcoming host innate immune response

LOUIS Y. A. CHAI*†, MIHAI G. NETEA*, ALIEKE G. VONK‡ & BART-JAN KULLBERG*

**Department of Medicine (463), Radboud Nijmegen University Medical Center, Nijmegen and Nijmegen University Center for Infectious Diseases, Nijmegen, The Netherlands, †Department of Medicine, National University Hospital, Singapore, and ‡Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands*

A successful pathogen is one that is able to effectively survive and evade detection by the host innate immune defense. Fungal pathogens have adopted strategies which evade host defense and eventually cause disease in at-risk patients. Shielding of stimulatory surface recognition molecules, shedding of decoy components, induction of anti-inflammatory signals, complement evasion and resilient survival capacity are successful evasion mechanisms employed by fungal pathogens. Understanding these complex pathways of immune evasion can potentially contribute to development of novel therapeutic strategies against fungal infections.

Keywords innate immunity, escape mechanisms, Toll-like receptor

Introduction

Fungal pathogens are invariably ubiquitous, and they often colonize the human host. Most of the time colonization is harmless under normal circumstances and perceived virulence low. In the immunocompetent, opportunistic fungal pathogens are systemically eradicated by the host innate immune defense. However, during immunosuppression induced either by disease or iatrogenically, opportunistic pathogenic fungi to have the capability to proliferate and eventually establish an ensuing disease. The reported mortality and morbidity from invasive fungal infections under such circumstances in the compromised host can be substantial [1,2].

The human innate immune system is the body's first line of defense against the foreign pathogen and is pivotal in determining outcome of such a confrontation. The ability of the pathogen to invade the human host relies on its capacity to evade and circumvent host defense mechanisms. These strategies, innovative yet simple, aid the fungus' survival in the host. As the pathophysiology of fungal infections is slowly unraveled, we begin to understand the mechanisms leading to the presence and persistence of the fungal pathogen in the host. In this review, citing examples from our current understanding on the biology of *Candida*, *Aspergillus*, *Cryptococcus* and other fungal species, we shall attempt to illustrate how fungal pathogens avoid recognition or interfere with host innate systems to enhance their viability during the course of infection.

Attachment to and colonization of body surfaces

The skin and mucous membranes provide an anatomical barrier to fungi such as *Candida spp.* As a prelude to potential invasion, colonization requires a successful means of adherence to epithelial surfaces. To counteract removal by ciliary clearance on mucous membranes, *Candida spp.* expresses attachment factors (so-called adhesins) – surface proteins covalently linked to the β-glucan of the cell wall, to aid adherence to epithelial surfaces. Adhesins, such as the agglutinin-like sequence

Received 25 January 2008; Accepted 16 May 2008
Correspondence: Mihai G. Netea, Department of Medicine (463), Radboud University Nijmegen Medical Center, PO Box 9101, Geert Grootplein 8, 6525 GA Nijmegen, The Netherlands. Tel: +31 24 3618819. E-mail: M.Netea@aig.umcn.nl

DOI: 10.1080/13693780802209082

(ALS) protein and hyphal wall protein-1 (HWP1), participate in biofilm formation by facilitating cell-to-surface and cell-to-cell adherence [3,4]. Clinically, implanted medical devices like central venous catheters provide an entry for microbial penetration through a break in epithelial surface. The formation of an overlying biofilm may shield the embedded *Candida spp.* from therapeutic levels of antifungal medications and provide a safe harbour for genetic variation to arise. This facilitates the emergence of resistance and forms a nidus for persistence or re-infection unless the implant is fully removed [5,6].

Immune recognition and surveillance of fungal pathogens

The activation of the host defense depends on appropriate detection of the invading pathogen. The mechanism responsible for the recognition is regulated by host pattern-recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs) expressed by microbes, but not by the host. This has been an area of intense research in recent years and the interested reader is encouraged to refer to comprehensive reviews on this topic [7–9]. Upon recognition of microbial ligands by PRRs, the innate defense system is activated. Firstly, a direct anti-fungal response resulting in either a phagocytic process or secretion of microbicidal compounds, and secondly, a facilitative role through the production of proinflammatory mediators such as cytokines and chemokines is initiated (Fig. 1). Finally, antigen uptake and presentation for the triggering of the adaptive immune system is induced [10].

The role of recognition of fungal pathogens has first been ascribed to a major class of PRRs, the Toll-like receptors (TLRs). TLRs are expressed on various immune and non-immune cell types. Within the TLR family, TLR2 and TLR4 have been highlighted as being involved in recognition of *Candida* and *Aspergillus*. Fungal wall components – zymosan, phospholipomannan and glucuronoxylomannan (GXM) have been identified as ligands (or specific PAMPs) for TLR2, while glucoronoxylomannan and O-linked mannan are ligands for TLR4 [11,12]. Nonetheless, there have been differences in reports on the relative importance and role of either receptors in mediating recognition of yeast and hyphal form of fungal pathogens. The decreased resistance to invasive candidiasis in TLR2 $^{-/-}$ mice in a single study [13] was not supported by

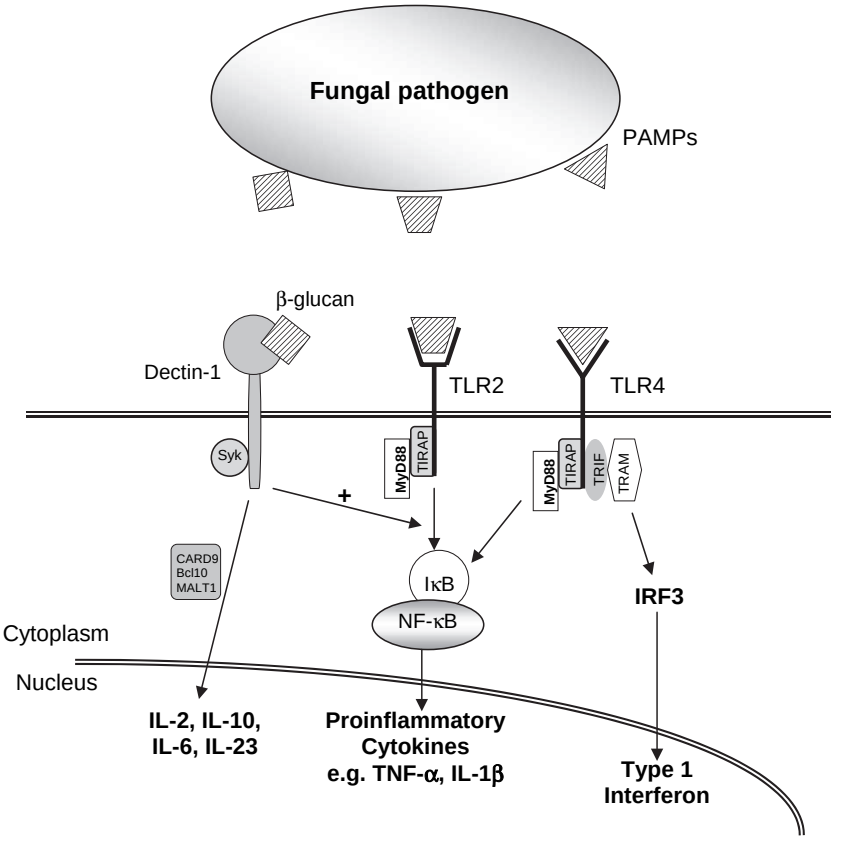

Fig. 1 Pattern recognition of fungal pathogen and representative signaling pathways.

Specific pathogen-associated molecular patterns (PAMPs) on the surface of fungal cell wall engage their respective pattern-recognition receptor (PRR) like TLR2, TLR4 and dectin-1 receptor on immune cells. Complex signaling pathways are activated involving adaptors and protien kinases (see below) resulting in the release of transicription factor to the nucleus to regulate expression of inflammatory cytokines
Adaptors: myeloid differentiation primary response gene 88 (MyD88), Tol/interleukin-1 receptor (TIR) adaptor protein (TIRAP), TIR-containing adaptor-inducing interferon β (TRIF), TRIF-related adaptor molecule (TRAM), caspase recruitment domain 9 (CARD9)-B-cell chronic lymphocytic leukaemia/lymphoma 10 (BCl10)-mucosal-associated lymphoid tissue translocation gene 1 (MALT1) complex
Protein Kinase: spleen tyrosine kinase (Syk)
IRF, Interferon regulatory factor
NF-κB, nuclear factor-kappa B
IκB, inhibitor or NF-κB

other studies that reported increased resistance to infection and a reduced fungal burden in TLR2$^{-/-}$ mice [14,15]. These latter studies attributed the observation to TLR2 as inducing the host immunity towards an 'anti-inflammatory'-type response. Likewise, studies with TLR4$^{-/-}$ mice had either demonstrated increased [14,16] or similar susceptibility [17] to *C. albicans* infections as compared to wild-type animals. Most of these differences are likely attributable to the differences in the cell lines employed or variation in methodologies in the respective studies. In addition TLR6 and TLR9 are also reported to have supplementary roles in *Candida* recognition [14,18]. Recognition of *A. fumigatus* conidia and hyphae have been attributed to both TLR2 and TLR4 although the relative importance of the role of each receptor differ depending on the morphologic stadium of the fungus [14, 19–21].

A second class of PRRs involved in the recognition of fungi is the C-type lectin receptors (CLR). Recently, the CLR dectin-1, has been demonstrated to mediate specific recognition of β-glucans found in the cell walls of *Candida* and *Aspergillus spp.* [22–24]. Dectin-1 and TLR2 may collaborate to augment proinflammatory cytokine production. Phagocytosis of (blasto)conidia can also be mediated by either dectin-1 or TLR2 [25,26]. Though dectin-1-deficient mice have shown increased susceptibility to disseminated candidemia [27], the actual importance of this receptor has been debated in another study that did not validate its anti-*Candida* effects using independently-developed dectin-1$^{-/-}$ mice model [28]. Other CLRs, like the macrophage mannose receptor and DC-SIGN have also been implicated by recognizing N-linked branched mannan structures of fungi [11,29,30].

The main cells in the host innate immune system which are responsible for immune surveillance against fungal pathogens are the circulating neutrophils and monocytes as well as tissue macrophages. Dendritic cells are involved in antigen-presentation and aid initiation of host adaptive immunity [10]. The major PRRs involved in fungal recognition: TLR2, TLR4 and dectin-1 are found in all the above-mentioned cell populations [9]. The relative importance of these respective professional phagocytes in contributing to the body's innate armour depends on the invading pathogen and its morphotype. Against *Candida*, neutrophils form an important frontline of the host defense. Organs of granulocytopenic mice yielded increased fungal burden following *in vivo* challenge with *C. albicans* [31,32]. *C. albicans* yeasts exposed to blood components including monocytes but devoid of neutrophils still retained the capacity to germinate [33]. Nonetheless macrophages and monocytes also play important roles especially in the absence of adequate neutrophil function [34,35]. Against *A. fumigatus*, it is believed that macrophages and monocytes form the first line of defence against inhaled conidia, while neutrophils target hyphal growth [10,36]. Targeted non-phagocytic oxidative and non-oxidative modes of hyphal damage by neutrophils have been demonstrated for *C. albicans* and *A. fumigatus* [37–39].

Clinically, occurrence of invasive fungal infection is usually associated with a quantitative or qualitative deficiency in host immunity, especially with opportunistic pathogens like *Aspergillus* and *Cryptococcus*. Nonetheless, acquisition of disease is also determined by pathogen virulence and its ability to effectively evade host immune defense. The following are strategies employed by fungal pathogens to evade host defense mechanisms (Fig. 2):

1. Shielding of stimulatory PAMPs,
2. Modulation of inflammatory signals,
3. Shedding of decoy components,
4. Persistence in intracellular environments,
5. Complement evasion.

Stealth mechanisms: shielding proinflammatory PAMPs

One important escape mechanism of the fungal pathogen is to shield its proinflammatory PAMPs from recognition by PRRs (Fig. 2a). Dectin-1 has been proposed as a pivotal PRR for recognition of fungal pathogens, by sensing β-glucans, a common conserved component in the fungal cell wall [40,41]. *C. albicans* is a polymorphic fungi which is able to switch its phenotype between yeast and filamentous forms pending environmental pressures. After budding, the bud scars of *Candida* yeast wall expose the otherwise concealed β-glucan predisposing to dectin-1 recognition. It has been demonstrated that during hyphal growth, *C. albicans* β-glucans are obscured from recognition by dectin-1 by outer wall components [24,42]. Dectin-1 also promotes fungicidal activity of human neutrophils [43], which are the important effector cells against hyphal morphologies. We postulate that β-glucan shielding in hyphal growth, and the subsequent inability of dectin-1 to detect the fungus, protects these larger morphologies from being internalized. In dendritic cells, hyphal forms also preferentially induced a T-helper cell type 2 (Th2) rather than a protective T-helper cell type 1 (Th1) immune response [44,45]. Hence, immune cells respond differently to yeast and hyphal forms.

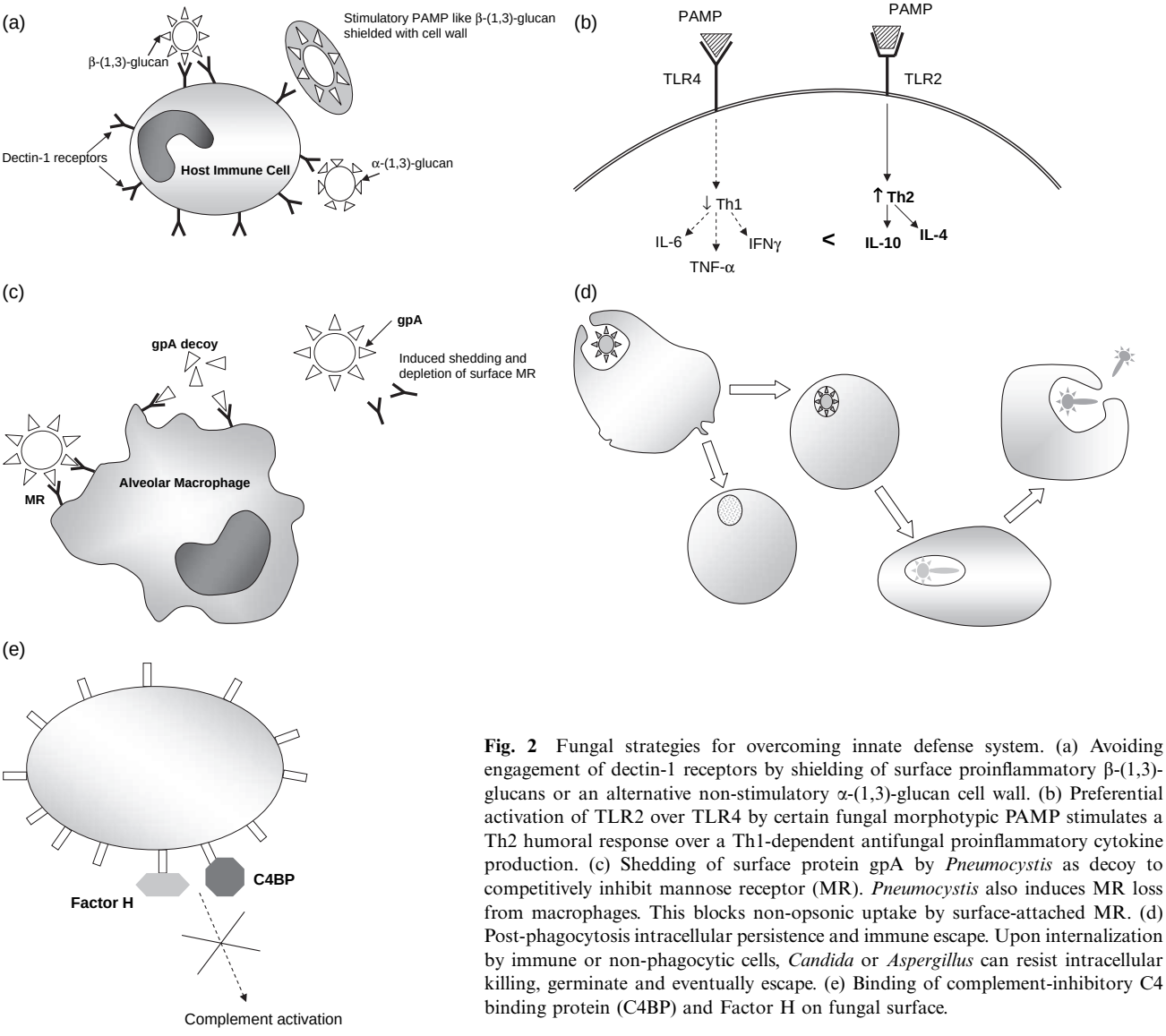

Fig. 2 Fungal strategies for overcoming innate defense system. (a) Avoiding engagement of dectin-1 receptors by shielding of surface proinflammatory β-(1,3)-glucans or an alternative non-stimulatory α-(1,3)-glucan cell wall. (b) Preferential activation of TLR2 over TLR4 by certain fungal morphotypic PAMP stimulates a Th2 humoral response over a Th1-dependent antifungal proinflammatory cytokine production. (c) Shedding of surface protein gpA by *Pneumocystis* as decoy to competitively inhibit mannose receptor (MR). *Pneumocystis* also induces MR loss from macrophages. This blocks non-opsonic uptake by surface-attached MR. (d) Post-phagocytosis intracellular persistence and immune escape. Upon internalization by immune or non-phagocytic cells, *Candida* or *Aspergillus* can resist intracellular killing, germinate and eventually escape. (e) Binding of complement-inhibitory C4 binding protein (C4BP) and Factor H on fungal surface.

Similar to *Candida* in which surface mannans shield β-glucan from recognition by dectin-1, in *Histoplasma capsulatum* the α-(1,3)-glucan present in its outer layer cell wall contributes to pathogenesis by shielding its immunostimulatory β-glucans. Proinflammatory tumor necrosis factor-α (TNF-α) production by phagocytic cells was demonstrated to be elevated in *H. Capsulatum* strains which lack α-(1,3)-glucan in contrast to strains which display in-situ α-(1,3)-glucan. Conversely, the TNF-α level became suppressed with depletion of host dectin-1 receptor for β-glucan [46]. Likewise, as *Paracoccidioides brasiliensis* transforms into its pathogenic yeast form, a change in its cell wall glucan polymer linkage occurs from β-(1,3) to α-(1,3)-glucan [47]. Shielding of β-glucan exposure is one possible mechanism of immune evasion, as α-(1,3)-glucan is not recognized and does not stimulate any known PRR. *Cryptococcus neoformans* masks its surface PAMPs through the production of an extracellular capsule of glucuronoxylomannan (GXM) which results in down-regulation of proinflammatory TNF-α and interleukin-1beta (IL-1β) production [48].

Another means of avoiding immune recognition is illustrated by *Coccidioides posadasii*, a fungal respiratory pathogen, that is recognized by the host by its immunodominant spherule outer wall glycoprotein (SOWgp). During endospore differentiation, the fungus secretes a metalloproteinase (Mep1) which digests SOWgp [49]. The resultant down-regulation of SOWgp during endospore formation enables the fungus to

evade phagocytosis and killing at its vulnerable endospore stage of development [50].

One way of masking its signature from the host immune system is the ability of the fungus to exist in different morphotypes and to reversibly switch from one form to another during the course of infection [10,51]. These polymorphic states are associated with phenotypic switching and results in differential recognition by PRRs. This capability has likely evolved to optimize the survival of fungi in different environments.

As exemplified earlier in experiments in which *C. albicans* filament, unlike its yeast-form, is able to escape immune recognition by dectin-1 [24], differential recognition of *C. albicans* blastoconidia and hyphae via TLR-mediated cytokine production has also been demonstrated. Whereas *C. albicans* blastoconidia activate both TLR2 and TLR4 in peripheral blood mononuclear cells and peritoneal macrophages, the hyphal form, which is responsible for tissue-invasive disease, fails to be recognized by TLR4, the receptor which activates interferon-gamma (IFN-γ) production, a critical cytokine in determining a successful outcome against candidiasis [52]. Phenotypic switching during germination, as such, may be an important escape mechanism for the organism. Likewise, TLR4-mediated signals are lost during *A. fumigatus* germination: whereas *A. fumigatus* conidia induce signals from TLR2 and TLR4; during tissue invasion, the conidia germinate into hyphae with loss of TLR4 stimulation, leading to a less pronounced stimulation of proinflammatory cytokines [21]. TLR4-mediated proinflammatory effects have been demonstrated to be important in the protection against invasive aspergillosis [14].

Modulation of inflammatory signals

The activation of TLR2 versus TLR4 differs in terms of nett pro- and anti-inflammatory cytokine effect, with the induction of proinflammatory cytokines that is weaker after TLR2 than TLR4 engagement [53] (Fig. 2b). Activation of dendritic cells using TLR4 agonists specifically induced Th1-inducing cytokine responses, while TLR2 stimulation induced a more pronounced anti-inflammatory Th2 response [54].

The balance between Th1/Th2 responses is perceived as critical in determining the outcome to infection [55–57] as each effector arm evokes a distinct immune response. On the one hand, the Th1 pathway produces proinflammatory cytokines like IFN-γ that induce cell-mediated immune responses needed for phagocyte activation and cytotoxicity – important against intracellular and fungal pathogens. The Th2 pathway, on the other hand, represented by cytokines such as IL-4, IL-5 and IL-10, stimulates a humoral response and in turn inhibits the Th1-dependent cellular mechanisms [58]. During anti-fungal responses, Th2 cytokines can inhibit monocyte anti-hyphal activity and oxidative burst [57]. Moreover, it has been demonstrated that *C. albicans* can evade the host defense through TLR2-derived signals: TLR2-deleted macrophages have been found to have enhanced anti-candidal capabilities [59] and *in-vivo*, $TLR2^{-/-}$ mice are relatively more resistant to disseminated *C. albicans* infection [14,15]. Hence the tissue-invasive hyphal forms of *C. albicans* and *A. fumigatus*, by means of evading TLR4 in favor of a predominant TLR2 activation, are able to tilt the balance towards a Th2 response.

Similar immunosuppressive potential has also been exhibited by *C. neoformans*. The capsule of *C. neoformans*, which is its main virulence factor, consists mainly of the polysaccharide glucuronoxylomannan which has been shown to be a potent inducer of the anti-inflammatory cytokine IL-10 in human monocytes and a pro-Th2 cytokine profiling [60,61]. Interestingly, the melanin pigment, commonly produced in pathogenic fungal species, has been linked to fungal virulence and its immunomodulatory effect has been studied in *C. neoformans*. Melanized strains of *C. neoformans* induce higher pulmonary IL-4 levels polarizing the host towards a Th2 immune response [62].

Likewise, *Blastomyces dermatitidis* which causes a progressive pulmonary and systemic mycosis, has the capability to induce 'relative immunosuppression' by limiting the production of proinflammatory cytokine TNF-α [63]. It does so through a *Blastomyces* surface adhesin and established virulence factor BAD1 which binds to complement receptor 3 on macrophages, whose physiological role is to regulate excess TNF-α production during clearance of apoptotic cells [64]. The resultant ligation suppresses TNF-α release which would have been detrimental to the survival of *B. dermatitidis*.

Shedding of decoy components

Other strategies of evasion have been highlighted for *Pneumocystis*, the cause of the opportunistic *Pneumocystis jiroveci (formerly mentioned carinii)* pneumonia (PCP) mainly in AIDS patients. Glycoprotein A (gpA) complex is the principal protein antigen on the surface of *Pneumocystis,* which is heavily glycosylated with mannose, glucose and galactose-containing carbohydrate moieties [65]. These structures are recognized by mannose receptors (MR) on alveolar macrophages (AMs). By prematurely shedding its gpA as a decoy, *Pneumocystis* seeks to competitively block MR on AMs

and impair their phagocytic function [66]. In addition, *Pneumocystis* has been shown to induce the depletion of MR from the surface of AMs and consequently block the non-opsonic uptake by surface-expressed mannose receptor [67] (Fig. 2c).

Escape from phagocytic response

To escape from immune surveillance and phagocytosis, fungi may resort to hiding in sanctuary sites within the host (Fig. 2d). These niches can be host cells that are functionally non-phagocytic by designation, such as the host epithelial or endothelial cells, in which the pathogen is protected from the hostile external environment.

It has been demonstrated that *A. fumigatus* conidia can bind and become internalized by human epithelial cell lines [68]. Although the invasion index may be lower than professional phagocytes, the internalized conidia within epithelial cells remained viable for relatively longer periods compared to conidia within macrophages [69]. Following inhalation, *Aspergillus* conidia which become internalized by airway epithelial cells can limit the induced levels of proinflammatory cytokines IL-6 and IL-8 [70]. *A. fumigatus* conidia endocytosed by type II pneumocytes can hide within late endosomes. The conidia eventually germinate with the potential to disseminate [71].

C. albicans can induce its own uptake via endocytosis by endothelial cells through N-cadherin, a surface protein [72]. *C. neoformans* can induce its own endocytosis by microvascular endothelial cells and subsequently cross the blood-brain barrier to cause meningitis [73]. These findings provide evidence that fungal pathogens may use their internalization by non-phagocytic cells to hide from detection by professional phagocytes, and when the opportunity presents, disseminate from its reservoir within the host.

Persistence despite adversity

In confronting macrophages, some fungal pathogens have also developed the capability to resist phagocytosis as exemplified by *C. neoformans*, which can undergo phenotypic switching to a mucoid colony variant that produces a larger capsular polysaccharide GXM (with altered biophysical and biochemical properties) thus reducing the phagocytic efficacy of alveolar macrophages [74].

For pathogenic strains which fail to evade host recognition, phagocytosis by immune cells does not mean inevitable demise and the end of its life cycle as the fungus may still be able to persist, despite the adverse environment of the phagolysosome. Some strains of *C. albicans* can resist intracellular killing, develop hyphal forms and eventually escape from the macrophage [75]. Against the oxygen free radicals and reactive nitrogen molecules such as nitric oxide (NO) produced by monocytes and macrophages upon confrontation, *C. albicans* possesses a highly specific, readily inducible anti-NO defense mechanism involving NO-scavenging flavohemoglobin genes which converts NO to less toxic molecules [76]. Detailed morphologic studies have demonstrated that despite phagocytosis by macrophages, with rapid recruitment of lysosomes to fuse with the phagosome, *Candida* yeast may still form germ tubes, propagate and escape from the host cell [77]. Phagocytosis induces *C. albicans* within the macrophage to adopt a self-preserving state: a slower growth rate, alternative carbon utilization and an oxidative stress response as for a 'hibernation mode' for continued survival in the harsh environment until it re-declares its presence with the eventual demise of the macrophage [78]. Phagocytic activity alone may be inadequate to contain the pathogen [79] and should be augmented by Th1-type cytokines like IFN-γ for effective microbial killing capacity.

Likewise, *C. neoformans* may be viewed as practicing a form of intracellular parasitism [80]. It has been observed that the yeast can survive and replicate within phagocytic cells during chronic infection [81,82]. In addition to resisting intracellular killing, it is thought that *C. neoformans* upon being phagocytosed, induces disordered lysosomal trafficking and extensive host cell cytoplasmic vacuolation with eventual host cell disruption [80]. *H. capsulatum*, another facultative intracellular fungus, is known to be able to persist in the host for long periods after initial infection and re-activate when immunity wanes by surviving within macrophages [83,84]. After phagocytosis, *Histoplasma* is able to inhibit phagolysosome fusion and actively modulate phagosomal pH to optimize its survival within phagosomes [85,86]. In addition, *Histoplasma* can also inhibit the release of toxic superoxide radicals detrimental to its survival [87].

Evasion of complement system

The complement system represents another arm of the host innate immunity. This consists of intricate cascades involving serum proteins via three biochemical pathways – the classical, alternative and lectin activating pathways – resulting in antimicrobial activity via opsonophagocytosis and recruitment of inflammatory cells. The lectin pathway is thought to play a minor anti-fungal role. *Aspergillus* and *Candida* are known to

trigger complement activation through C3 deposition on fungal surface facilitating opsonization and production of chemoattractant C5a, which recruits leukocytes to the site of infection [88–90]. Pigmentation on *A. fumigatus* conidial surface has been shown to affect virulence by limiting C3 complement deposition [91] and neutrophil activation. Physiologically, the complement system is kept in check against excessive activation by regulatory proteins, such as Factor H (alternative pathway), Factor H-like protein 1 (FHL-1) and C4 binding protein (C4BP) for the classical pathway. In attempting immune evasion, *C. albicans*, and more recently, *A. fumigatus* have shown ability to bind Factor H, FHL-1 and C4BP on their surface to down-regulate the complement cascade [92–95] (Fig. 2e). Moreover the thick fungal cell wall is largely resistant to direct lysis by the terminal membrane-attack complex of the complement system [96].

Conclusion

The defense mechanisms in the immunocompetent host are generally effective against fungal pathogens. However, it is not rare that the integrity of the innate immune system is breached: iatrogenically with medical procedures (e.g., central catheters, major surgery) or immunosuppressive therapy (e.g., chemotherapy/ transplant regimens leading to neutropenia and immune depletion). Despite the varied capabilities possessed by the fungal pathogen to evade host detection, it remains to be highlighted that a normal host defense is generally effective against most fungal infections and a permissive immune suppressive state has first to exist before the host becomes susceptible to opportunistic pathogens. In endemic mycoses however, immune suppression may not be a pre-requisite and the pathogen's capability to evade host immune detection may yet determine eventual disease acquisition. In the face of significant morbidity and mortality rates, treatment options for invasive fungal infections remain generally limited by the range of anti-microbial medications which act against the few established drug targets known to date. In recent years, increased knowledge on the mechanisms of fungal recognition by the host immune system has become available. In addition to understanding how the host defense is activated, it also becomes clear that fungal pathogens do possess strategies to evade recognition and inhibit anti-fungal defenses, as presented in this review. As more is understood of the pathogenesis of fungal diseases from the perspectives of immunology, infectious diseases and microbiology, it is hoped that this knowledge will help direct the development of future therapeutics and minimize risk of disease in the ever-increasing population of immunocompromised patients seen in medical practice today.

Acknowledgements

The authors are grateful to Mdm Khoo Ai Leng for the production of the illustrations.

L.C. was supported by the Health Manpower Development Plan (HMDP) Fellowship, Ministry of Health, Singapore and the International Fellowship, Agency for Science, Technology and Research (A*STAR), Singapore.

M.G.N. was supported by a Vidi grant of the Netherlands Organization for Scientific Research.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Post MJ, Lass-Floerl C, Gastl G, Nachbaur D. Invasive fungal infections in allogeneic and autologous stem cell transplant recipients: a single-center study of 166 transplanted patients. *Transpl Infect Dis* 2007; **9**: 189–195.
2 Silveira FP, Husain S. Fungal infections in solid organ transplantation. *Med Mycol* 2007; **45**: 305–320.
3 Nobile CJ, Andes DR, Nett JE, *et al*. Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation in vitro and in vivo. *PLoS Pathog* 2006; **2**: 63.
4 Nobile CJ, Nett JE, Andes DR, Mitchell AP. Function of *Candida albicans* adhesin Hwp1 in biofilm formation. *Eukaryot Cell* 2006; **5**: 1604–1610.
5 Douglas LJ. *Candida* biofilms and their role in infection. *Trends Microbiol* 2003; **11**: 30–36.
6 Nobile CJ, Mitchell AP. Microbial biofilms: e pluribus unum. *Curr Biol* 2007; **17**: R349–53.
7 Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; **124**: 783–801.
8 Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* 2003; **3**: 169–176.
9 Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. *Nat Rev Microbiol* 2008; **6**: 67–78.
10 Romani L. Immunity to fungal infections. *Nat Rev Immunol* 2004; **4**: 1–23.
11 Netea MG, Gow NA, Munro CA, *et al*. Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest* 2006; **116**: 1642–1650.
12 Tada H, Nemoto E, Shimauchi H, *et al*. *Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. *Microbiol Immunol* 2002; **46**: 503–512.
13 Villamon E, Gozalbo D, Roig P, *et al*. Toll-like receptor-2 is essential in murine defenses against *Candida albicans* infections. *Microbes Infect* 2004; **6**: 1–7.

14 Bellocchio S, Montagnoli C, Bozza S, *et al*. The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens *in vivo*. *J Immunol* 2004; **172**: 3059–3069.
15 Netea MG, Sutmuller R, Hermann C, *et al*. Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. *J Immunol* 2004; **172**: 3712–3718.
16 Netea MG, Van Der Graaf CA, Vonk AG, *et al*. The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. *J Infect Dis* 2002; **185**: 1483–1489.
17 Murciano C, Villamon E, Gozalbo D, *et al*. Toll-like receptor 4 defective mice carrying point or null mutations do not show increased susceptibility to *Candida albicans* in a model of hematogenously disseminated infection. *Med Mycol* 2006; **44**: 149–157.
18 Netea MG, van de Veerdonk F, Verschueren I, van der Meer JW, Kullberg BJ. Role of TLR1 and TLR6 in the host defense against disseminated candidiasis. *FEMS Immunol Med Microbiol* 2008; **52**: 118–123.
19 Mambula SS, Sau K, Henneke P, Golenbock DT, Levitz SM. Toll-like receptor (TLR) signaling in response to *Aspergillus fumigatus*. *J Biol Chem* 2002; **277**: 39320–39326.
20 Wang JE, Warris A, Ellingsen EA, *et al*. Involvement of CD14 and toll-like receptors in activation of human monocytes by *Aspergillus fumigatus* hyphae. *Infect Immun* 2001; **69**: 2402–2406.
21 Netea MG, Warris A, Van der Meer JW, *et al*. *Aspergillus fumigatus* evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. *J Infect Dis* 2003; **188**: 320–326.
22 Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. *Nature* 2001; **413**: 36–37.
23 Brown GD, Herre J, Williams DL, *et al*. Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* 2003; **197**: 1119–1124.
24 Gantner BN, Simmons RM, Underhill DM. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *Embo J* 2005; **24**: 1277–1286.
25 Dennehy KM, Brown GD. The role of the beta-glucan receptor Dectin-1 in control of fungal infection. *J Leukoc Biol* 2007; **82**: 253–258.
26 Luther K, Torosantucci A, Brakhage AA, Heesemann J, Ebel F. 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**: 368–381.
27 Taylor PR, Tsoni SV, Willment JA, *et al*. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat Immunol* 2007; **8**: 31–38.
28 Saijo S, Fujikado N, Furuta T, *et al*. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nat Immunol* 2007; **8**: 39–46.
29 Porcaro I, Vidal M, Jouvert S, Stahl PD, Giaimis J. Mannose receptor contribution to *Candida albicans* phagocytosis by murine E-clone J774 macrophages. *J Leukoc Biol* 2003; **74**: 206–215.
30 Cambi A, Gijzen K, de Vries JM, *et al*. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur J Immunol* 2003; **33**: 532–538.
31 van't Wout JW, Linde I, Leijh PC, van Furth R. Contribution of granulocytes and monocytes to resistance against experimental disseminated *Candida albicans* infection. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 736–741.
32 Kullberg BJ, van't Wout JW, Poell RJ, van Furth R. Combined effect of fluconazole and recombinant human interleukin-1 on systemic candidiasis in neutropenic mice. *Antimicrob Agents Chemother* 1992; **36**: 1225–1229.
33 Fradin C, De Groot P, MacCallum D, *et al*. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol Microbiol* 2005; **56**: 397–415.
34 Ashman RB, Papadimitriou JM. Production and function of cytokines in natural and acquired immunity to *Candida albicans* infection. *Microbiol Rev* 1995; **59**: 646–672.
35 Jensen J, Warner T, Balish E. The role of phagocytic cells in resistance to disseminated candidiasis in granulocytopenic mice. *J Infect Dis* 1994; **170**: 900–905.
36 Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*. Observations on these two lines of defense *in vivo* and *in vitro* with human and mouse phagocytes. *J Clin Invest* 1982; **69**: 617–631.
37 Diamond RD, Krzesicki R, Jao W. Damage to pseudohyphal forms of *Candida albicans* by neutrophils in the absence of serum *in vitro*. *J Clin Invest* 1978; **61**: 349–359.
38 Diamond RD, Clark RA. Damage to *Aspergillus fumigatus* and *Rhizopus oryzae* hyphae by oxidative and nonoxidative microbicidal products of human neutrophils in vitro. *Infect Immun* 1982; **38**: 487–495.
39 Christin L, Wysong DR, Meshulam T, Wang S, Diamond RD. Mechanisms and target sites of damage in killing of *Candida albicans* hyphae by human polymorphonuclear neutrophils. *J Infect Dis* 1997; **176**: 1567–1578.
40 Latge JP, Mouyna I, Tekaia F, *et al*. Specific molecular features in the organization and biosynthesis of the cell wall of *Aspergillus fumigatus*. *Med Mycol* 2005; **43**: S15–22.
41 Gow NA, Netea MG, Munro CA, *et al*. Immune recognition of *Candida albicans* beta-glucan by dectin-1. *J Infect Dis* 2007; **196**: 1565–1571.
42 Heinsbroek SE, Brown GD, Gordon S. Dectin-1 escape by fungal dimorphism. *Trends Immunol* 2005; **26**: 352–354.
43 Kennedy AD, Willment JA, Dorward DW, *et al*. Dectin-1 promotes fungicidal activity of human neutrophils. *Eur J Immunol* 2007; **37**: 467–478.
44 Romani L, Bistoni F, Puccetti P. Fungi, dendritic cells and receptors: a host perspective of fungal virulence. *Trends Microbiol* 2002; **10**: 508–514.
45 d'Ostiani CF, Del Sero G, Bacci A, *et al*. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity *in vitro* and *in vivo*. *J Exp Med* 2000; **191**: 1661–1674.
46 Rappleye CA, Eissenberg LG, Goldman WE. *Histoplasma capsulatum* alpha-(1,3)-glucan blocks innate immune recognition by the beta-glucan receptor. *Proc Natl Acad Sci USA* 2007; **104**: 1366–1370.
47 Borges-Walmsley MI, Chen D, Shu X, Walmsley AR. The pathobiology of *Paracoccidioides brasiliensis*. *Trends Microbiol* 2002; **10**: 80–87.
48 Vecchiarelli A, Retini C, Pietrella D, *et al*. Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1 beta secretion from human monocytes. *Infect Immun* 1995; **63**: 2919–2923.
49 Hung CY, Seshan KR, Yu JJ, *et al*. A metalloproteinase of *Coccidioides posadasii* contributes to evasion of host detection. *Infect Immun* 2005; **73**: 6689–6703.
50 Hung CY, Yu JJ, Seshan KR, Reichard U, Cole GT. A parasitic phase-specific adhesin of *Coccidioides immitis* contributes to the

virulence of this respiratory fungal pathogen. *Infect Immun* 2002; **70**: 3443–3456.
51 Hogan LH, Klein BS, Levitz SM. Virulence factors of medically important fungi. *Clin Microbiol Rev* 1996; **9**: 469–488.
52 van der Graaf CA, Netea MG, Verschueren I, van der Meer JW, Kullberg BJ. Differential cytokine production and Toll-like receptor signaling pathways by *Candida albicans* blastoconidia and hyphae. *Infect Immun* 2005; **73**: 7458–7464.
53 Hirschfeld M, Weis JJ, Toshchakov V, *et al*. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect Immun* 2001; **69**: 1477–1482.
54 Re F, Strominger JL. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* 2001; **276**: 37692–37699.
55 Netea MG, Van der Meer JW, Sutmuller RP, Adema GJ, Kullberg BJ. From the Th1/Th2 paradigm towards a Toll-like receptor/T-helper bias. *Antimicrob Agents Chemother* 2005; **49**: 3991–3996.
56 Romani L. Immunity to *Candida albicans*: Th1, Th2 cells and beyond. *Curr Opin Microbiol* 1999; **2**: 363–367.
57 Stevens DA. Th1/Th2 in aspergillosis. *Med Mycol* 2006; **44**: 229–235.
58 Jankovic D, Liu Z, Gause WC. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. *Trends Immunol* 2001; **22**: 450–457.
59 Blasi E, Mucci A, Neglia R, *et al*. Biological importance of the two Toll-like receptors, TLR2 and TLR4, in macrophage response to infection with *Candida albicans*. *FEMS Immunol Med Microbiol* 2005; **44**: 69–79.
60 Vecchiarelli A, Retini C, Monari C, *et al*. Purified capsular polysaccharide of *Cryptococcus neoformans* induces interleukin-10 secretion by human monocytes. *Infect Immun* 1996; **64**: 2846–2849.
61 Chiapello LS, Baronetti JL, Aoki MP, *et al*. Immunosuppression, interleukin-10 synthesis and apoptosis are induced in rats inoculated with *Cryptococcus neoformans* glucuronoxylomannan. *Immunology* 2004; **113**: 392–400.
62 Mednick AJ, Nosanchuk JD, Casadevall A. Melanization of *Cryptococcus neoformans* affects lung inflammatory responses during cryptococcal infection. *Infect Immun* 2005; **73**: 2012–2019.
63 Finkel-Jimenez B, Wuthrich M, Brandhorst T, Klein BS. The WI-1 adhesin blocks phagocyte TNF-alpha production, imparting pathogenicity on *Blastomyces dermatitidis*. *J Immunol* 2001; **166**: 2665–2673.
64 Brandhorst TT, Wuthrich M, Finkel-Jimenez B, Warner T, Klein BS. Exploiting type 3 complement receptor for TNF-alpha suppression, immune evasion, and progressive pulmonary fungal infection. *J Immunol* 2004; **173**: 7444–7453.
65 Stringer JR, Keely SP. Genetics of surface antigen expression in *Pneumocystis carinii*. *Infect Immun* 2001; **69**: 627–639.
66 Pop SM, Kolls JK, Steele C. *Pneumocystis*: immune recognition and evasion. *Int J Biochem Cell Biol* 2006; **38**: 17–22.
67 Fraser IP, Takahashi K, Koziel H, *et al*. *Pneumocystis carinii* enhances soluble mannose receptor production by macrophages. *Microbes Infect* 2000; **2**: 1305–1310.
68 Paris S, Boisvieux-Ulrich E, Crestani B, *et al*. Internalization of *Aspergillus fumigatus* conidia by epithelial and endothelial cells. *Infect Immun* 1997; **65**: 1510–1514.
69 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**: 3156–3163.
70 Zhang Z, Liu R, Noordhoek JA, Kauffman HF. Interaction of airway epithelial cells (A549) with spores and mycelium of *Aspergillus fumigatus*. *J Infect* 2005; **51**: 375–382.
71 Wasylnka JA, Moore MM. *Aspergillus fumigatus* conidia survive and germinate in acidic organelles of A549 epithelial cells. *J Cell Sci* 2003; **116**: 1579–1587.
72 Filler SG, Sheppard DC. Fungal invasion of normally non-phagocytic host cells. *PLoS Pathog* 2006; **2**: 129.
73 Chang YC, Stins MF, McCaffery MJ, *et al*. Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. *Infect Immun* 2004; **72**: 4985–4995.
74 Fries BC, Taborda CP, Serfass E, Casadevall A. Phenotypic switching of *Cryptococcus neoformans* occurs *in vivo* and influences the outcome of infection. *J Clin Invest* 2001; **108**: 1639–1648.
75 Tavanti A, Campa D, Bertozzi A, *et al*. *Candida albicans* isolates with different genomic backgrounds display a differential response to macrophage infection. *Microbes Infect* 2006; **8**: 791–800.
76 Ullmann BD, Myers H, Chiranand W, *et al*. Inducible defense mechanism against nitric oxide in *Candida albicans*. *Eukaryot Cell* 2004; **3**: 715–723.
77 Kaposzta R, Marodi L, Hollinshead M, Gordon S, da Silva RP. Rapid recruitment of late endosomes and lysosomes in mouse macrophages ingesting *Candida albicans*. *J Cell Sci* 1999; **112**: 3237–3248.
78 Lorenz MC, Bender JA, Fink GR. Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot Cell* 2004; **3**: 1076–1087.
79 Richardson MD, Smith H. Resistance of virulent and attenuated strains of *Candida albicans* to intracellular killing by human and mouse phagocytes. *J Infect Dis* 1981; **144**: 557–564.
80 Feldmesser M, Tucker S, Casadevall A. Intracellular parasitism of macrophages by *Cryptococcus neoformans*. *Trends Microbiol* 2001; **9**: 273–278.
81 Goldman DL, Lee SC, Mednick AJ, Montella L, Casadevall A. Persistent *Cryptococcus neoformans* pulmonary infection in the rat is associated with intracellular parasitism, decreased inducible nitric oxide synthase expression, and altered antibody responsiveness to cryptococcal polysaccharide. *Infect Immun* 2000; **68**: 832–838.
82 Lee SC, Kress Y, Zhao ML, Dickson DW, Casadevall A. *Cryptococcus neoformans* survive and replicate in human microglia. *Lab Invest* 1995; **73**: 871–879.
83 Woods JP, Heinecke EL, Luecke JW, *et al*. Pathogenesis of *Histoplasma capsulatum*. *Semin Respir Infect* 2001; **16**: 91–101.
84 Porta A, Maresca B. Host response and *Histoplasma capsulatum*/macrophage molecular interactions. *Med Mycol* 2000; **38**: 399–406.
85 Strasser JE, Newman SL, Ciraolo GM, *et al*. Regulation of the macrophage vacuolar ATPase and phagosome-lysosome fusion by *Histoplasma capsulatum*. *J Immunol* 1999; **162**: 6148–6154.
86 Eissenberg LG, Goldman WE, Schlesinger PH. *Histoplasma capsulatum* modulates the acidification of phagolysosomes. *J Exp Med* 1993; **177**: 1605–1611.
87 Eissenberg LG, Goldman WE. *Histoplasma capsulatum* fails to trigger release of superoxide from macrophages. *Infect Immun* 1987; **55**: 29–34.
88 Speth C, Rambach G, Lass-Florl C, Dierich MP, Wurzner R. The role of complement in invasive fungal infections. *Mycoses* 2004; **47**: 93–103.
89 Kozel TR, Weinhold LC, Lupan DM. Distinct characteristics of initiation of the classical and alternative complement pathways by *Candida albicans*. *Infect Immun* 1996; **64**: 3360–3368.

90 Hector RF, Yee E, Collins MS. Use of DBA/2N mice in models of systemic candidiasis and pulmonary and systemic aspergillosis. *Infect Immun* 1990; **58**: 1476–1478.
91 Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ. The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. *J Bacteriol* 1998; **180**: 3031–3038.
92 Meri T, Hartmann A, Lenk D, *et al*. The yeast *Candida albicans* binds complement regulators factor H and FHL-1. *Infect Immun* 2002; **70**: 5185–5192.
93 Meri T, Blom AM, Hartmann A, *et al*. The hyphal and yeast forms of *Candida albicans* bind the complement regulator C4b-binding protein. *Infect Immun* 2004; **72**: 6633–6641.
94 Vogl G, Lesiak I, Jensen DB, *et al*. Immune evasion by acquisition of complement inhibitors: the mould *Aspergillus* binds both factor H and C4b binding protein. *Mol Immunol* 2008; **45**: 1485–1493.
95 Behnsen J, Hartmann A, Schmaler J, *et al*. The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement system. *Infect Immun* 2008; 76: 820–827.
96 Kozel TR. Activation of the complement system by pathogenic fungi. *Clin Microbiol Rev* 1996; **9**: 34–46.

This paper was first published online on iFirst on 24 July 2008.