A Sweet Response to a Sour Situation: The Role of Soluble Pattern Recognition Receptors in the Innate Immune Response to Invasive *Aspergillus fumigatus* Infections

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Introduction

*Aspergillus* spp. infect around 11,000,000 patients, resulting in about 600,000 deaths per year, but these numbers are on the rise due to the emergence of antifungal-resistant strains and a lack of sensitive diagnostic tests [1].

It is increasingly acknowledged that soluble pattern recognition receptors (PRRs), such as the complement component C1q, the collectins (MBL, SP, and CL-11), PTX3, and the ficolins (ficolin-1, 2, 3 and A), are important within anti-*Aspergillus* immunity [2]. Moreover, studies have highlighted that they may be used as a possible alternative to current antifungal drugs or used in combination to increase efficacy [3].

Binding of pathogen-associated molecular patterns (PAMPs) on the pathogen surface by soluble PRRs often results in opsonisation. This enhances interactions with membrane-associated PRRs on phagocytes, such as the important β-glucan receptor Dectin-1, Toll-like receptors (TLRs), complement receptors (CR1), and Fc receptors; ultimately augmenting phagocytosis, which is essential in controlling the infection [2].

Alternatively, opsonins can promote fungal damage directly or further promote opsonisation by C3b deposition via activation of the conserved complement system [4]. There are three main arms of the complement system, which are the classical, alternative, and lectin pathways. C1q primarily activates the classical antibody-mediated pathway, whereas MBL, CL-11, and the ficolins are known to activate the lectin complement pathway via activation of the mannos-binding lectin-associated serine proteases (MASPs). However, SP-A and SP-D are not involved in complement activation, and the role of CL-11 in *Aspergillus* immunity is yet to be explored. Furthermore, PTX3 can interact with complement activators and inhibitory components to modulate all three pathways [5]. The role of each of these PRRs in anti-*Aspergillus* immunity will be discussed further.

PTX3 Plays a Non-redundant Role in *Aspergillus fumigatus* Immunity

PTX3 is a globally expressed acute-phase protein that is synthesised locally at inflammatory sites by several cell types, particularly mononuclear phagocytes, dendritic cells (DCs), epithelial, and endothelial cells. Furthermore, PTX3 is stored within neutrophil granules containing
lactoferrin and once secreted, associates with neutrophil extracellular traps (NETs), acting as a focal point for antimicrobial effector molecules [6].

PTX3 primarily functions as an opsonin in *A. fumigatus* immune responses, whereby it binds to galactomannan residues of dormant spores, facilitating recognition and phagocytosis [7]. PTX3 can also interact with numerous important opsonins, complement proteins, and membrane-associated PRRs to enhance antifungal immunity, including MBL, ficolin-2, C1q, Factor H, and Dectin-1, and more recently has been shown to exert its antifungal effects through TLR4/MD-2 mediated signalling [8,9]. Moreover, PTX3 can modulate all three complement pathways [5]. Current evidence indicates that PTX3 activates complement on the *Aspergillus* conidial surface and interacts with FcγRIIa, which mediates activation of the complement receptor CR3, leading to recognition and internalization of conidia [10].

There have been several human studies reporting single nucleotide polymorphisms (SNPs) in the PTX3 gene that are associated with susceptibility to *A. fumigatus* infections in haematopoietic stem cell and whole organ transplant patients [11,12].

In support of these findings, studies utilising PTX3 knockout mice have indicated a non-redundant role within immunity to *A. fumigatus* pulmonary infection [7]. Furthermore, PTX3 has been demonstrated to be protective against invasive aspergillosis (IA) in mice receiving allogeneic bone marrow transplants, in chronic granulomatous disease mice (p47phox−/−), and corticosteroid-treated rats [13].

**Mannose-Binding Lectin (MBL) Is Essential for Defence Against *A. fumigatus***

MBL is one of the best characterised lectins involved in innate antifungal immunity. It is found predominantly within the serum, but, during inflammation, loss of vascular integrity can result in leakage of MBL into alveola where it can interact with *A. fumigatus* (Fig 1) [14]. Binding here is primarily achieved via selective and calcium-dependent binding to the carbohydrate moieties D-mannose, L-fucose, and N-acetylglucosamine (GlcNAc) in the *A. fumigatus* cell wall.

Neth et al. [15] were the first to show demonstrable binding of *A. fumigatus* by the MBL carbohydrate recognition domain (CRD). It wasn’t until much later that MBL was described to be protective against *Aspergillus* infection via the activation of the lectin-complement pathway on *A. fumigatus* conidia.

It has since been well established in humans that natural MBL deficiencies, or MBL deficiencies due to genetic polymorphisms, are significantly correlated with increased susceptibility to acute IA and chronic necrotizing pulmonary aspergillosis (CPA), respectively [16,17].

This importance has been well documented in murine models by Kaur et al., [18] in which they comprehensively demonstrated that MBL-deficiency was linked to significantly reduced phagocytosis, diminished complement activation, impaired cytokine responses, and greater mortality in a murine model of IA.

Furthermore, studies utilising serum obtained from transgenic animals have indicated that only MBL-C, and not MBL-A, can recognise *A. fumigatus* and is essential for complement activation [19].

Conversely, a more recent study indicated that loss of MBL in a systemic model of aspergillosis resulted in a resistant phenotype and may play a deleterious role [20], suggesting an importance within pulmonary infection rather than disseminated disease.
Surfactant Protein-D Is an Important Initiator of the Fungal Immune Response to *A. fumigatus*

The roles of SP-A and SP-D in *Aspergillus* defence have been extensively studied, with SP-D exhibiting particular importance. SP-D is found in alveolar lung lining and primarily binds β-1,6-glucan in the *A. fumigatus* cell wall. Interestingly, SP-D can also bind *A. fumigatus* hyphae in a calcineurin-sensitive manner, hinting at an additional role in the later stages of infection [21].

Recognition by SP-D has been observed to augment the immune response to *Aspergillus* in vitro and in vivo. In particular, SP-D is essential in vivo, whereby it has been observed that administration of SP-D can protect immunosuppressed mice against an otherwise fatal dose of
Aspergillus, and SP-D–deficient mice are highly susceptible to IA [22,23]. Conversely, SP-A–deficient mice become more resistant to invasive infection, indicating SP-A may even facilitate pathology [23]. However, it appears that surfactant proteins may play a greater role within allergic broncho-pulmonary aspergillosis (ABPA) rather than IA. Human studies have indicated a polymorphism in the collagen region of SP-A (SP-A2) that is correlated with increased risk of ABPA and increased allergic responses, but no SNPs have so far been shown to enhance susceptibility to IA [24].

**Ficolins: The Emergence of a Novel Participant in the Host Fungal Response**

We and others have recently implicated ficolins within fungal host–microbe interactions. L-ficolin and H-ficolin, in addition to rodent ficolin-A, bind avidly to *A. fumigatus* via a range of carbohydrate moieties, including GlcNAc, N-acetylgalactosamine, D-mannose, and L-fucose [19,25–28]. Furthermore, ficolin-A also recognises the resting, swollen, and germinating morphotypes of *A. fumigatus*, in addition to the less pathogenic species: *A. flavus*, *A. terreus*, and *A. niger* [19].

Following binding to *A. fumigatus*, both L- and H-ficolin activate the lectin-complement pathway on *A. fumigatus* conidia, whereas ficolin-A was shown to play a redundant role to MBL-C [19,26,27]. Consequently, opsonisation by L-ficolin, ficolin-A, and H-ficolin has been demonstrated to enhance the phagocytosis of conidia by primary macrophages, neutrophils, and the type II epithelial cell line (A549), but it is only following interaction with the macrophages and neutrophils where significant fungal killing is observed [25,26,29].

Furthermore the inflammatory response elicited by ficolin-opsonised conidia is dependent upon the cell type involved. Following cell challenge with ficolin-opsonised conidia, a MAPK-dependent increase in IL-8 production was observed from epithelial cells, whereas down-regulation of IL-1β, IL-6, IL-8, IL-10, and TNF-α production was observed from macrophages and neutrophils via currently uncharacterized mechanisms [25,26,29]. These observations have raised some interesting questions; however, the implications of ficolins in disease models have yet to be elucidated, and our understanding of the role of ficolins in antifungal immunity are in their infant stages.

**Diagnostic and Therapeutic Potential of Soluble PRRs**

Antifungal drug resistance and a lack of conclusive diagnostics are two of the major challenges limiting the cure of aspergillosis, and many opsonins demonstrate therapeutic potential.

It has been demonstrated that administration of recombinant MBL is protective in a murine model of invasive *A. fumigatus* infection and can significantly reduce mortality [18]. The therapeutic potential of SP-D has also been explored in mice, and administration of native and recombinant SP-D is associated with decreased fungal burden in the lungs and increased levels of antifungal IFN-γ in IA [30].

Administration of recombinant PTX3 can ameliorate infection and increase survival in a pulmonary model of *A. fumigatus* infection in mice [13]. An interesting caveat of PTX3 is its ability to have an additive effect on the efficacy of commonly used antifungals such as amphotericin and voriconazole [3]. Importantly, in combination with PTX3, the antifungal dose could be lowered whilst maintaining efficacy, which could lead to the reduced risk of drug-related side effects. This would be especially beneficial for severely immunocompromised patients [3].

Unlike MBL, PTX3, and SP-D, the therapeutic potential of ficolins is yet to be explored. Unsurprisingly, H-ficolin BAL concentrations are increased during *A. fumigatus* infection, but
as L-ficolin is not produced directly in the lung, it was hypothesised that it enters the alveolar space following vascular damage and may be useful as a diagnostic marker in combination with other fungal specific markers such as galactomannan [25,26]. As ficolins have been observed to dampen pro-inflammatory cytokine production by phagocytic cells, it could be hypothesised that they may have the potential to be exploited therapeutically [25,29].

To date, there has been no indication that soluble PRRs can be exploited for their diagnostic potential, albeit the presence of PRRs such as L-ficolin and MBL in the lung during inflammation and infection highlights the necessity to investigate soluble PRRs as potential diagnostic tools. Moreover, further larger-scale clinical trials are needed to assess the full diagnostic potential of ficolins and other PRRs in combination with current fungal and host biomarkers in order to evaluate their role in diagnostics and possible impact on patient outcomes.

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References

1. Schelenz S, Barnes RA, Barton RC, Cleverley JR, Lucas SB, et al. (2015) British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. Lancet Infect Dis 15: 461–474. doi: 10.1016/S1473-3099(15)70006-X PMID: 25771341
2. Margalit A, Kavanagh K (2015) The innate immune response to Aspergillus fumigatus at the alveolar surface. FEMS Microbiol Rev 39: 670–687. doi: 10.1093/femsre/fuv018 PMID: 25934117
3. Gaziano R, Bozza S, Belloccchio S, Perruccio K, Montagnoli C, et al. (2004) Anti-Aspergillus fumigatus efficacy of pentraxin 3 alone and in combination with antifungals. Antimicrob Agents Chemother 48: 4414–4421. PMID: 15504871
4. Speth C, Rambach G, Wurzner R, Lass-Florl C (2008) Complement and fungal pathogens: an update. Mycoses 51: 477–496. doi: 10.1111/j.1439-0507.2008.01597.x PMID: 18705662
5. Doni A, Garlanda C, Bottazzi B, Meri S, Garred P, et al. (2012) Interactions of the humoral pattern recognition molecule PTX3 with the complement system. Immunobiology 217: 1122–1128. doi: 10.1016/j.imbio.2012.07.004 PMID: 22964239
6. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, et al. (2007) The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J Exp Med 204: 793–804. PMID: 17389238
7. Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, et al. (2002) Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. Nature 420: 182–186. PMID: 12432394
8. Inforzato A, Reading PC, Barbati E, Bottazzi B, Garlanda C, et al. (2012) The “sweet” side of a long pentraxin: how glycosylation affects PTX3 functions in innate immunity and inflammation. Front Immunol 3: 407. doi: 10.3389/fimmu.2012.00407 PMID: 23316195
9. Bozza S, Campo S, Arseni B, Inforzato A, Ragnar L, et al. (2014) PTX3 binds MD-2 and promotes TRIF-dependent innate immune protection in aspergillosis. J Immunol 193: 2340–2348. doi: 10.4049/jimmunol.1400814 PMID: 25049357
10. Moalli F, Doni A, Deban L, Zelante T, Zagarella S, et al. (2010) Role of complement and Fc(epsilon) receptors in the protective activity of the long pentraxin PTX3 against Aspergillus fumigatus. Blood 116: 5170–5180. doi: 10.1182/blood-2009-12-283576 PMID: 20928938
11. Cunha C, Aversa F, Lacerta JF, Busca A, Kurzai O, et al. (2014) Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. N Engl J Med 370: 421–432. doi: 10.1056/NEJMoai1211161 PMID: 24476432
12. Wojtowicz A, Leompe TD, Bilbert S, Manuel O, Rueger S, et al. (2015) PTX3 Polymorphisms and Invasive Mold Infections After Solid Organ Transplant. Clin Infect Dis 61: 619–622. doi: 10.1093/cid/civ396 PMID: 25977268
13. Salvatori G, Campo S (2012) Current understanding of PTX3 protective activity on Aspergillus fumigatus infection. Med Mycol 50: 225–233. doi: 10.3109/13693788.2011.648215 PMID: 22309253
14. Summerfield JA (1993) The role of mannose-binding protein in host defence. Biochem Soc Trans 21: 473–477. PMID: 8395913
15. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, et al. (2000) Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun 68: 688–693. PMID: 10639434
16. Lambourne J, Agranoff D, Herbrecht R, Troke PF, Buchbinder A, et al. (2009) Association of mannose-binding lectin deficiency with acute invasive aspergillosis in immunocompromised patients. Clin Infect Dis 49: 1486–1491. doi: 10.1086/644619 PMID: 19827955
17. Crosdale DJ, Poulton KV, Ollier WE, Thomson W, Denning DW (2001) Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. J Infect Dis 184: 653–656. PMID: 11474427
18. Kaur S, Gupta VK, Thiel S, Sarma PU, Madan T (2007) Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. Clin Exp Immunol 148: 382–389. PMID: 17335555
19. Bidula S, Kenawy H, Ali Y, Sexton D, Schwaeble W, et al. (2013) Role of ficolin-A and lectin complement pathway in the innate defense against pathogenic Aspergillus species. Infect Immun 81: 1730–1740. doi: 10.1128/IAI.00032-13 PMID: 23478320
20. Clemens KV, Martinez M, Tong AJ, Stevens DA (2010) Resistance of MBL gene-knockout mice to experimental systemic aspergillosis. Immunol Lett 128: 105–107. doi: 10.1016/j.imlet.2009.12.021 PMID: 20064561
21. Geunes-Boyer S, Heitman J, Wright JR, Steinbach WJ (2010) Surfactant protein D binding to Aspergillus fumigatus hyphae is calcineurin-sensitive. Med Mycol 48: 580–588. doi: 10.3109/13693780903401682 PMID: 20141481
22. Madan T, Kishore U, Singh M, Strong P, Hussain EM, et al. (2001) Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. Infect Immun 69: 2728–2731. PMID: 11254642
23. Madan T, Reid KB, Clark H, Singh M, Nayak A, et al. (2010) Susceptibility of mice genetically deficient in SP-A or SP-D gene to invasive pulmonary aspergillosis. Mol Immunol 47: 1923–1930. doi: 10.1016/j.molimm.2010.02.027 PMID: 20881660
24. Vaid M, Kaur S, Sambatacou H, Madan T, Denning DW, et al. (2007) Distinct alleles of mannose-bind- ing lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Clin Chem Lab Med 45: 183–186. PMID: 17311505
25. Bidula S, Sexton DW, Abdolrasouli A, Shah A, Reed A, et al. (2015) The serum opsonin L-ficolin is detected in lungs of human transplant recipients following fungal infections and modulates inflammation and killing of Aspergillus fumigatus. J Infect Dis 212: 234–246. doi: 10.1093/infdis/jiv027 PMID: 25612732
26. Bidula S, Sexton DW, Yates M, Abdolrasouli A, Shah A, et al. (2015) H-ficolin binds Aspergillus fumigatus leading to activation of the lectin complement pathway and modulation of lung epithelial immune responses. Immunology 146: 281–291. doi: 10.1111/imm.12501 PMID: 26133042
27. Ma YJ, Doni A, Hummelshøj T, Honore C, Bastone A, et al. (2009) Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. J Biol Chem 284: 28263–28275. doi: 10.1074/jbc.M109.009225 PMID: 19632990
28. Hummelshøj T, Ma YJ, Munthe-Fog L, Bjarnsholt T, Moser C, et al. (2012) The interaction pattern of murine serum ficolin-A with microorganisms. PLoS ONE 7.
29. Bidula S, Sexton DW, Schelenz S (2015) Serum opsonin ficolin-A enhances host-fungal interactions and modulates cytokine expression from human monocye-derived macrophages and neutrophils following Aspergillus fumigatus challenge. Med Microbiol Immuno.
30. Strong P, Reid KB, Clark H (2002) Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. Clin Exp Immunol 130: 19–24. PMID: 12296848