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REVIEW ARTICLE

The complement system in *Aspergillus fumigatus* infections and its crosstalk with pentraxins

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Aspergillosis is a life-threatening infection mostly affecting immunocompromised individuals and primarily caused by the saprophytic fungus *Aspergillus fumigatus*. At the host–pathogen interface, both cellular and humoral components of the innate immune system are increasingly acknowledged as essential players in the recognition and disposal of this opportunistic mold. Fundamental hereof is the contribution of the complement system, which deploys all three activation pathways in the battle against *A. fumigatus*, and functionally cooperates with other soluble pattern recognition molecules, including pentraxins. In particular, preclinical and clinical observations point to the long pentraxin PTX3 as a nonredundant and complement-dependent effector with protective functions against *A. fumigatus*. Based on past and current literature, here we discuss how the complement participates in the immune response to this fungal pathogen, and illustrate its crosstalk with the pentraxins, with a focus on PTX3. Emphasis is placed on the molecular mechanisms underlying such processes, the genetic evidence from human epidemiology, and the translational potential of the currently available knowledge.

Keywords: aspergillosis; *Aspergillus fumigatus*; complement; host–pathogen interface; infection immunity; pentraxins; PTX3

Aspergillus fumigatus is an evolutionary ancient filamentous fungus (mold) that flourishes in soil and decomposing vegetation [1]. Traditionally regarded as asexual, the life cycle of this ubiquitous saprophyte actually comprises cryptic forms of sexual reproduction [2] and is hallmarked by several morphotypes with distinct metabolic, compositional, and structural traits [3]. The metabolically inactive and asexual airborne spores (known as dormant or resting conidia) are encased in a robust cell wall mostly made of complex polysaccharides [i.e., α- and β-glucans, chitin, galactomannan (GM), and galactosaminogalactan (GAG)] in addition to lipids, proteins, and melanin pigments [i.e., dihydroxynaphthalene (DHN) melanin] [4]. Small in size (i.e., 2–3 μm across) and enveloped in a layer of hydrophobic rodlet-like proteins (i.e., RodA, that

Abbreviations
AMs, alveolar macrophages; CNPA, chronic necrotizing pulmonary aspergillosis; C4BP, C4-binding protein; CGD, chronic granulomatous disease; CP, LP, and AP, classical, lectin, and alternative pathway of complement; CR, complement receptor; CRP, C-reactive protein; DAF, decay-accelerating factor; DCs, dendritic cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; DHN, dihydroxynaphthalene; FcγR, Fc fragment of IgG receptor; GAG, galactosaminogalactan; GM, galactomannan; HSCT, hematopoietic stem-cell transplantation; IPA, invasive pulmonary aspergillosis; IFN-γ, interferon-γ; IL, interleukin; MAC, membrane attack complex; MASPs, MBL-associated serine proteases; MBL, mannose-binding lectin; NETs, neutrophil extracellular traps; NK, natural killer cells; PAMPs (DAMPs), pathogen (danger)-associated molecular patterns; PRRs (PRMs), pattern recognition receptors (molecules); ROS, reactive oxygen species; SAP, serum amyloid P component; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α.

imparts air buoyancy and, most importantly, immunological inertness [5,6]), conidia of *A. fumigatus* are naturally fit for air/water interfaces, which favors their penetration into the lower respiratory tracts (i.e., lung alveoli) of the mammalian host [7]. Up to hundreds of conidia are inhaled daily by humans; nonetheless, this incessant infectious challenge rarely involves lung colonization and disease in immunocompetent individuals. Indeed, most of the inhaled spores are mechanically removed by the mucociliary movements of the ciliated cells (mainly in the upper airways), and those resisting this mechanical disposal are promptly recognized, phagocytosed, and killed by alveolar epithelial cells (mostly type II pneumocytes) in cooperation with lung-resident and lung-recruited immune cells [i.e., alveolar macrophages (AMs), dendritic cells (DCs), and polymorphonuclear neutrophils] [8,9]. However, in cases of impaired mucociliary clearance (e.g., in cystic fibrosis, asthma, bronchiectasis) and/or defective immunity (e.g., primary and acquired immune deficiencies), conidia of *A. fumigatus* find a favorable ground for germination and hyphal growth, thus turning into an opportunistic pathogen. In fact, a functionally and/or immunologically compromised lung environment sets the scene for initial colonization of the alveolar space by germlings, followed by invasion of the lung parenchyma by filamentous hyphae and, in the most severe cases, dissemination of this angioinvasive morphotype to distant organs and tissues, including the central nervous system, liver, spleen, skin, and bone [7,10]. Depending on fungal strain's virulence and host's immune status, this pathogenic process might lead to a broad spectrum of clinical manifestations, ranging from noninvasive infections, such as allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis, and aspergilloma, to invasive aspergillosis (IA) [11]. The global burden of noninvasive form of the disease has been recently estimated as ~ 5 million cases per year, and up to 10 million individuals are predicted to be at risk of IA worldwide, mostly as a reflection of the increasing number of immunocompromised patients (see below) [12]. With a global incidence of more than 300 000 cases per year and a mortality rate as high as 95% in the worst clinical scenarios, IA is the most severe form of aspergillosis, mainly caused by *A. fumigatus*, which accounts for ~ 90% of cases [13]. To emphasize the opportunistic nature of this pathogen, host factors that predispose to IA notably include hematological malignancies (particularly, acute leukemia), allogeneic hematopoietic stem-cell and solid-organ transplantation, advanced AIDS, chronic granulomatous disease (CGD), preexisting lung diseases (e.g., emphysema, cavitary tuberculosis, cystic fibrosis, asthma, bronchiectasis), and corticosteroid regimens for the treatment of autoimmune diseases [14].

It is increasingly acknowledged that effective recognition and clearance of *A. fumigatus* require major cellular effectors of the innate immune system to be functionally competent [15]. In this regard, the early immune response to *A. fumigatus* infections primarily involves AMs, DCs, and neutrophils, collectively equipped with an arsenal of pattern recognition receptors (PRRs) that recognize a range of pathogen associated molecular patterns (PAMPs) on the fungal surface [9]. In particular, neutrophils are the first-responder, inflammatory cells to migrate toward the infected lung, where they exert phagocytic and killing activities against all *A. fumigatus* morphotypes, *via* production of reactive oxygen species (ROS) [16], formation of neutrophil extracellular traps (NETs) [17], and release of microbicidal components from intracellular granules [18]. Defects either in number, recruitment, or function of neutrophils are known to increase the host's susceptibility to IA, which highlights the importance of these cells in the control of *A. fumigatus* infections [19].

Recognition of the fungal particles is also mediated by soluble pattern recognition molecules (PRMs), including components of the complement system, collectins, and pentraxins [20]. Despite the impressive structural diversity across these humoral PRMs, their general mode of action resembles that of antibodies ('ante-antibodies'), inasmuch as they have opsonic activity and facilitate recognition of pathogens (including *A. fumigatus*) by phagocytes, either directly or *via* complement-dependent pathways [21]. Furthermore, a tight cooperation exists amongst soluble PRMs, which extends to cell-associated PRRs, whereby different multimolecular complexes are formed that have distinct functional properties, depending on the immune status of the host and the mode of presentation of the pathogen (e.g., the morphotype of *A. fumigatus*) [22].

The complement system is a key humoral effector in antifungal immunity and all its three activation pathways are involved, although to varying extents, in recognition and opsonization of the *A. fumigatus* morphotypes (see the 'Pathways of complement activation along the fungal life cycle' section) [23]. Amongst other soluble PRMs, the long pentraxin 3 (PTX3) is recognized as an important host-protective factor in *A. fumigatus* infections [24] and cooperates with components of the complement system to promote clearance of this pathogen [25]. Therefore, complement and pentraxins form an integrated system with crosstalk, synergism, and regulation, thus standing as a paradigm

of the interplay between different PRMs in the mounting and orchestration of the immune response [26]. Here, we present our current understanding of the molecular mechanisms underlying this system and discuss them in the light of the available clinical information. Similarities and differences in the complement/pentraxin responses to another opportunistic fungal pathogen of great clinical relevance, that is, *Candida albicans*, are also discussed.

The complement system in *Aspergillus fumigatus* infections

Evolutionary conserved in as distant species as mammals, birds, reptiles, amphibians, and ascidians, the complement system comprises a panoply of soluble and cell-anchored proteins that collectively and coordinately mediate multiple effector functions [27]. These range from recognition of PAMPs and danger-associated molecular patterns on pathogens and damaged host cells, respectively, and their elimination *via* either direct (i.e., formation of the membrane attack complex, MAC) or indirect (i.e., phagocytosis and phagolysosomal killing) lytic mechanisms [28], to elicitation of inflammatory reactions (i.e., recruitment and activation of immune cells), and regulation of adaptive immunity (i.e., control of B- and T-cell responses) [29,30].

Activation of the system proceeds through feed-forward mechanisms involving a cascade of proteolytic events, conventionally classified in three distinct pathways: classical, lectin, and alternative (CP, LP, and AP, respectively) [28]. The CP initiates with recognition of IgM and IgG clusters (i.e., in immune complexes) by C1q (component of the C1 complex), whereas the LP is triggered by the association of mannose-binding lectin (MBL) and ficolins with carbohydrate patterns exposed on pathogens and damaged cells. These recognition events are followed by activation of associated serine proteases [i.e., C1r/C1s for C1, MBL-associated serine proteases (MASPs) for MBL/ficolins] and cleavage of C4 and C2 to generate the C4b and C2a fragments. These assemble into the CP/LP C3 convertase (C4bC2a) that catalyzes conversion of C3 into C3a and C3b, the latter containing a reactive thioester that form amide and/or ester bonds with proximal nucleophiles (i.e., amines and hydroxyl functions, respectively). Cell-bound C3b can associate with factor Bb (i.e., generated by the factor D-dependent hydrolysis of factor B) to form the AP C3 convertase (C3bBb), which is stabilized by factor P (properdin) and produces additional C3b, therefore acting as an amplification loop for the CP and LP. In addition, the AP is in a constant state of low-level activation ('tickover'), independent of any recognition event, whereby C3 undergoes hydrolysis (i.e., $C3(H_20)$) and forms a convertase with factor Bb ($C3(H_20)Bb$) in solution that cleaves C3 to C3b. The latter can form covalent bonds with proximal surfaces (as discussed above) and, if not inactivated (see below), acts as a scaffold for assembly of the AP C3 convertase, which amplifies complement deposition [28].

All three pathways converge toward enzymatic complexes (i.e., C3 and C5 convertases) that, in turn, generate the anaphylatoxins C3a and C5a (chemoattractants and activators of immune cells), the MAC (C5b-C9, which causes lysis of the target cells) and C3b (that opsonizes pathogens and promotes their phagocytosis). To avoid uncontrolled activation, the complement system is tightly regulated by fluid-phase and cell-associated proteins, whereby C3b and C4b undergo proteolytic inactivation to iC3b and iC4b (that cannot form C3 convertases) by factor I. This enzyme requires, as cofactors, either membrane proteins (membrane cofactor protein, MCP, also known as CD46, and complement receptor 1, CR1, also known as CD35) or fluid-phase regulators (factor H and C4-binding protein, C4BP). Furthermore, the membrane-bound decay-accelerating factor (DAF), also known as CD55 or DAF, CR1, factor H, and C4BP, inhibit formation and accelerate dissociation of the C3 and C5 convertases, and MAC assembly is controlled by the MAC-inhibitory protein (or CD59) and S protein (vitronectin) [28,31]. Functional and/or quantitative alterations in components of the system can lead to defective or excessive complement activation, where this has been associated with diverse pathological conditions, including renal [32], autoimmune [33], neurological [34], hemolytic [35], and inflammatory [36] diseases.

In the following section, we will discuss how and when the three complement pathways undergo activation on *A. fumigatus*, the functional outcomes of this process, and the strategies evolved by this mold to evade the complement attack.

Pathways of complement activation along the fungal life cycle

Inhaled dormant conidia of *A. fumigatus* that escape mucociliary clearance can reach the lung alveoli, where they are faced both by epithelial and innate immune cells (see 'Introduction'). However, recognition of conidia by cell-associated PRRs (e.g., dectin-1 [37] and dectin-2 [38], dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin, DC-SIGN [39],

melanin-sensing C-type lectin receptor [40], and Toll-like receptors (TLR)2 and TLR4 [41]) is hampered by the rodlet and, possibly, melanin layers of the outer cell wall that mask the PAMPs (i.e., mostly polysaccharides) present in the inner cell wall [42,43]. Despite of this shielding effect, dormant conidia are phagocytosed and killed, which highlights the crucial role of soluble PRMs, including the complement system, in the early immune response to this opportunistic pathogen [23]. Furthermore, as conidia swell and germinate, the rodlet and melanin layers are shed, and the polysaccharidic PAMPs not only become exposed but also change in structure and composition, a process that continuously redefines the host–pathogen interface throughout the fungus' life cycle [20]. We, therefore, examine the dynamics of complement activation as this mold transits from dormant conidia to adult hyphae (Fig. 1).

Complement activation on dormant conidia of *A. fumigatus* was first described more than 40 years ago [44] and has been consistently reported in a number of studies since (reviewed in Ref. [45]); however, the relative contribution of the three pathways is still debated, likely due to inherent differences in the applied experimental settings and fungal strains. Using human serum chelated with magnesium/EGTA (to abolish the CP/LP) and AP reconstituted from the purified components, Kozel *et al* reported that complement activation on *A. fumigatus* propagules was primarily mediated by the AP [46]. In the same study, this morphotype was shown to bind intact human C3 and its proteolytic fragments C3b and iC3b, where the presence of the latter is indeed suggestive of an involvement of the AP [46]. This notion was reinforced some 20 years later by Dumestre-Pérard *et al.* [47] who described exclusive activation of the AP on 29 different strains of *Aspergillus* conidia, including 18 environmental and clinical strains of *A. fumigatus*, with the highest deposition of C3 (and its fragments) on clinical isolates from IA patients. Furthermore, by means of MBL depletion and reconstitution experiments, MBL was proposed to mediate direct hydrolysis of C3 to C3b *via* a C2 bypass mechanism, which implied a canonical PRM of the LP (i.e., MBL) to act as trigger for AP activation on dormant conidia [47], and expanded previous evidence of complement activating properties of MBL toward *A. fumigatus* [48]. Additional complexity was brought to the field by the observation that collectin-12 (a member of the collectin family) recognized *A. fumigatus* conidia through its carbohydrate recognition domain (in a Ca^{2+}-independent manner), and promoted activation of the AP only, *via* a properdin-dependent mechanism [49].

Another interesting observation came from the original study of Kozel *et al.* [46] that is, despite of increasing accumulation of C3 as dormant conidia matured to swollen and hyphal morphotypes, the amount of C3 per unit of surface area did not change (as assessed by radioactive protein labeling). These findings were recapitulated by Braem *et al.* [50] who additionally provided confocal microscopy evidence of a 'patchy' (i.e., high-density spots) distribution of the C3 proteins on the conidial surface, whereas these appeared diffusely spread on that of swollen conidia and germlings. Nonetheless, the three morphotypes were equally phagocytosed by human neutrophils that recognize the C3b and iC3b opsonins *via* CR1 and CRs 3 and 4 (CR3, or CD11b/CD18, and CR4, or CD11c/CD18, respectively) [28]. On this point, the authors argued that 'the density of C3b deposition is more important than the total C3b deposition for efficient phagocytosis' [50], which raises the question of how the C3 fragments are presented to their cognate receptors and if this has functional implications on clearance mechanisms (including phagocytosis and, possibly, killing).

As a point of note, in the same experimental model, depletion of either C2 (to abrogate the CP/LP) or factor D (to abolish the AP) reduced C3 deposition on dormant conidia [50], suggesting that the CP and/or LP (in addition to the AP) are involved in complement activation on this morphotype, consistent with previous evidence of C1q [51] and MBL [48] binding to *A. fumigatus*. Furthermore, human neutrophils failed to engulf *A. fumigatus* conidia *in vitro* in the presence of heat-inactivated serum, indicating that this process did not involve IgG-mediated phagocytosis [50]. These observations were expanded and revisited in a study by Rosbjerg *et al.* [52] who reported AP amplification on the dormant conidia of *A. fumigatus* that was, however, conditioned to initiation of the CP/LP. The relative contribution of the CP and LP to deposition of C4b, C3b, and MAC as well as phagocytosis by human neutrophils *in vitro* was primarily set by the serum content of IgM, in that C1q was the main initiator of complement (*via* the CP) in the presence of normal human serum (NHS, that contains physiological levels of IgM), and MBL proved to be the key trigger for complement activation (*via* the LP) in the presence of serum from umbilical cord (UC) and a patient with X-linked agammaglobulinemia in IgG replacement therapy, both IgG-competent but poor in IgM. Furthermore, UC sera with low titers of MBL had reduced complement activation on *A. fumigatus*, as opposed to normal levels of activation in MBL-competent NHS [52]. While questioning the previously proposed C2 bypass mechanism [47] and, possibly,

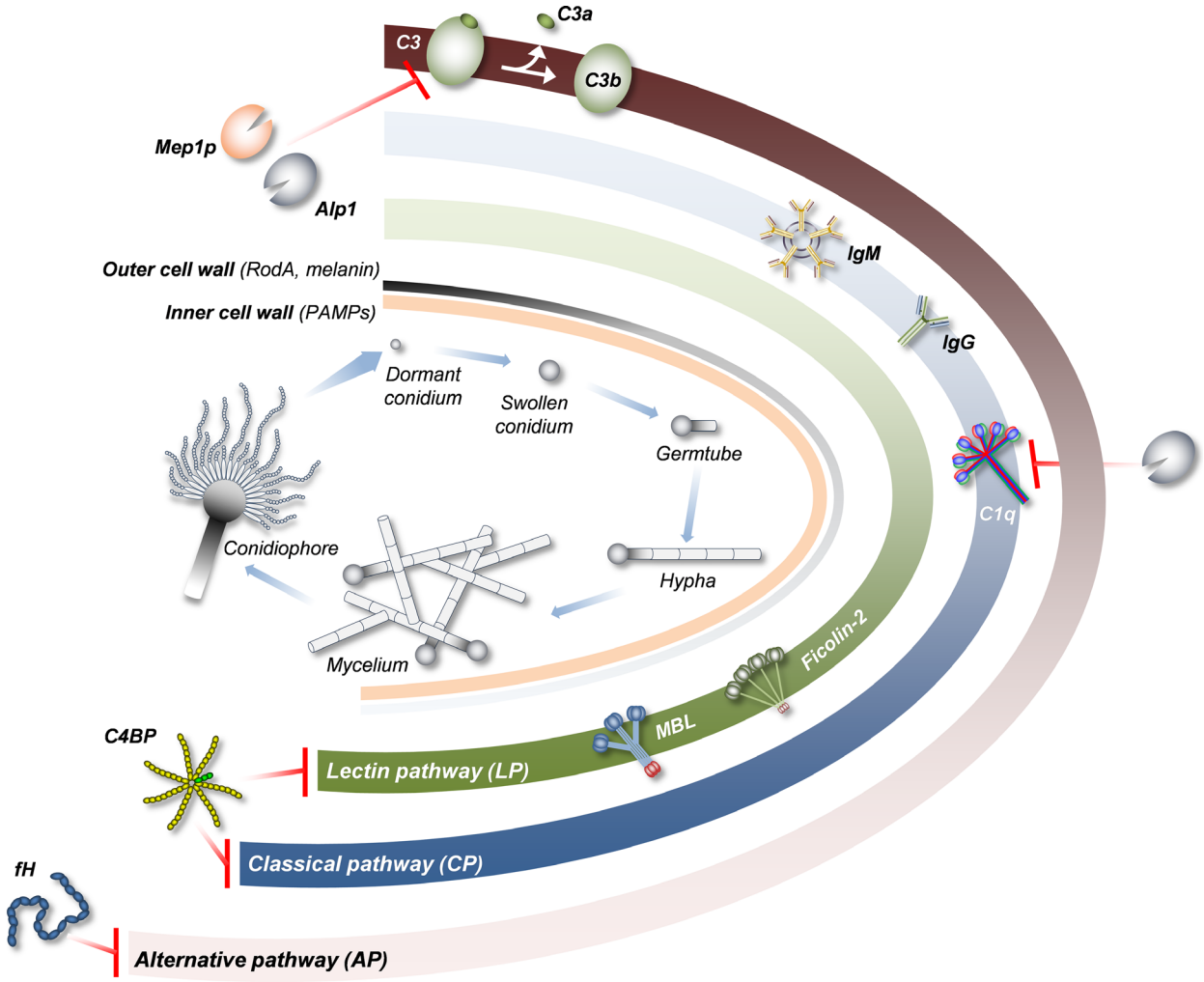

Fig. 1. The life cycle of *Aspergillus fumigatus*: stage-dependent activation of complement pathways and evasion strategies. *A. fumigatus* spreads in the environment in the form of airborne spores (conidia, ~ 3 μm across). These can penetrate into the lung alveoli and initiate a germination program, first evolving into swollen conidia (~ 6 μm across), then generating germlings and filamentous hyphae (mycelium), which eventually form conidiophores that, in turn, make and disseminate new conidia [3]. The fungal cell wall undergoes dramatic modifications, as conidia turn into hyphae, with shedding of the outer layers (that mostly comprise RodA and DHN melanin) and exposure of the inner polysaccharides (i.e., the PAMPs α- and β-glucans, chitin, GM, and GAG) [20]. As a reflection of the changing pathogen surface, activation of the complement system proceeds in a stage-dependent fashion, with varying involvements of the three pathways (LP, CP, and AP) [23]. In this regard, dormant conidia are acknowledged to activate mainly the AP (here schematically depicted as hydrolysis of C3 to C3b, scaffolding unit of the AP C3 convertase, and C3a, potent anaphylatoxin) [46,47], while there is growing activation of the LP/CP as conidia swell and germinate [50]. CP activation is mainly driven by immune complexes (with IgM prevailing over IgG), and the LP is sustained by MBL and, to a lesser extent, ficolins (especially, ficolin-2) [52]. The complement-dependent disposal of the fungal pathogen is mostly mediated by opsonic and phagocytic mechanisms, with direct membrane lysis (i.e., by MAC formation) playing a minor role, due to thickness and resistance of the cell wall [23]. *A. fumigatus* has evolved several strategies to evade the complement response, including secretion of proteases (i.e., Alp1 [94] and Mep1p [96]) that targets key components of the system, such as C3 and C1q (as well as C4 and C5, not shown in the figure), and recruitment of inhibitors of the AP (i.e., factor H, fH) [89] and CP/LP (i.e., C4BP) [90].

underestimating the contribution of the AP (i.e., heat-inactivated conidia were used throughout the study that likely exposed PAMPs as a consequence of thermal damage to the outer cell wall), this report highlighted the role of MBL and C1q as key initiators of the complement response against *A. fumigatus*. In addition, another activator of the LP, that is, ficolin-2 (and to a minor extent, ficolins 1 and 3), was shown to bind the dormant conidia, in agreement with previous observations [53,54]; however, this marginally

contributed to complement activation in conditions of C1q deficiency and MBL competence [52]. In this regard, a synergism was reported between ficolin-2 and PTX3 in the recognition of and complement deposition on dormant conidia (further discussed in the 'PTX3 in the immune response to A. fumigatus and its crosstalk with complement' section), which was mostly apparent in the absence of both C1q and MBL, indicating that ficolin-2, likely *via* a crosstalk with PTX3, participates in the LP response to *A. fumigatus* [55]. Furthermore, ficolin-3 was shown to enhance activation of the LP on the conidial surface *in vitro* [56], and ficolin-2 and its murine orthologue A-ficolin were described to exert immunomodulatory activities by reducing the production of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) in human monocyte-derived macrophages and neutrophils challenged with dormant conidia, in spite of increased fungal uptake and killing [57,58]. It is worth noting here that most of the *in vitro* complement activation studies have been performed using serum (either as a whole, heat-inactivated or genetically/biochemically devoid of selected complement proteins and/or immunoglobulins), whereas the concentration of most complement proteins in the alveolar fluid (i.e., a more physiological milieu for invading conidia of *A. fumigatus*, particularly in the early phases of the infection) is lower than that measured in the serum. However, in conditions of lung inflammation (as those found in the course of an *A. fumigatus* infection) the complement repertoire of the lung, as contributed by local synthesis and systemic circulation, becomes similar (in terms of relative titers, composition, and activity) to that present in the serum, which makes the latter a good alternative to the alveolar fluid as a surrogate source of complement proteins in *in vitro* complement activation assays [59].

As dormant conidia initiate the germination process, the outer wall layers of RodA and DHN-melanin are shed, and inner wall polysaccharides become exposed [20]. Of these, β-glucans are displayed at high levels on germlings and young hyphae, and masked again (i.e., by the exopolysaccharide GAG) in adult hyphae [60]. In addition to dectin-1 (a well-known PRR for β-glucans that mediates essential innate and adaptive immune responses [37]), ficolin-1 was reported to bind β-glucan-containing, alkali-insoluble hyphal cell wall fractions (AIF) of *A. fumigatus* [61], in addition to purified β-glucans, and elicit LP activation with enhanced IL-8 secretion by A594 airway epithelial cells *in vitro* [62].

Galactomannan is another major polysaccharide of *A. fumigatus* that is found both in the hyphal cell wall (conjugated to proteins and glucans [63]) and as a soluble molecule, which makes it a useful tool for diagnosis of aspergillosis [64]. The best-characterized cellular receptors for GM are DC-SIGN [39] and dectin-2 [38], which are involved in recognition and activation of DCs and macrophages. Although not formally proven, GM appears to be recognized by the complement system *via* MBL, where this lectin has been reported to enhance phagocytosis and killing of *A. fumigatus* hyphae by human neutrophils [65].

No complement proteins have been described up to now that bind the α-glucans of *A. fumigatus*, which are found within the outer cell wall of germinating conidia and hyphae, and have been implicated in the inhibition of the TLR2- and TLR4-dependent production of IL-6 by peripheral blood mononuclear cells [66]. As opposed to α-glucans, chitin, the innermost polysaccharide of the fungal cell wall, has been proposed as a ligand of ficolins. In this regard, ficolin-1 recognized chitin (in addition to β-glucans) on the surface of *A. fumigatus* young hyphae [62], and the binding of ficolins 2 and 3 (and murine ficolin-A) to *A. fumigatus* conidia was inhibited by N-acetylglucosamine (GlcNAc, the monosaccharide component of chitin), suggesting that they may recognize chitin and exert complement activating functions on germlings and hyphae (in addition to conidia) [67]. The relevance of chitin as a PAMP for complement activation has been recently highlighted in a study on mutant strains of *A. fumigatus* lacking β-glucans, which synthesized and exposed high levels of chitin (likely through compensatory pathways), and underwent enhanced complement activation and dendritic cell-mediated clearance [68].

Similarly to α-glucans, no evidence is available to suggest an active role of complement against GAG, a peculiar linear hetero-polysaccharide composed of α-(1,4)-linked galactose and N-acetylgalactosamine (GalNAc) that is abundant in the outer cell wall and extracellular matrix (ECM) of hyphae [69], and undergoes partial N-deacetylation of the composing GalNAc residues, which imparts to the polymer a cationic and cell-adhesive nature [70]. However, in addition to concealing immunoreactive cell wall components, GAG has been shown to induce apoptosis of human neutrophils with a mechanism that involves natural killer (NK) cells [71], which raises the possibility that complement components or other PRMs might take part in this process.

Based on current evidence, the opsonophagocytic properties of the complement system are crucial in promoting immune recognition and clearance of *A. fumigatus*. In this regard, the occurrence of a thick cell wall makes it unlikely for MAC to mediate effective

killing of *A. fumigatus* through cell membrane lysis, especially in the case of dormant conidia [23]; however, a contribution of the terminal complex to clearance of the more advanced fungal morphotypes cannot be ruled out, as suggested (but not formally proven) by *in vivo* modeling of aspergillosis. In addition to opsonic and lytic functions, mounting and propagation of appropriate inflammatory reactions (*via* the anaphylatoxins C3a and C5a) are major effector mechanisms of the complement system that mediate vessel permeabilization, recruitment and activation of innate immune cells (primarily neutrophils), and pathogen-tailored adaptive responses [28]. With regard to aspergillosis, these mechanisms are perhaps best appreciated when modeling *A. fumigatus* infections *in vivo* (see 'The complement in animal models of aspergillosis' section). In an effort of synthesis, available literature indicates that the AP contributes to initiation of the complement reaction primarily on dormant conidia, and the CP/LP take over as conidia swell and germinate, with the AP mostly acting as an amplifier (Fig. 1).

The complement in animal models of aspergillosis

The etiopathogenic roles of complement in the immune response to *A. fumigatus* have been addressed in several animal models of aspergillosis. Some 17 years ago, we reported that genetic deficiency of C1q enhanced susceptibility to invasive pulmonary aspergillosis (IPA) in nonimmunocompromised mice, due to enhanced fungal colonization of the lung [24]. Our initial observations have been afterward corroborated by *in vitro* evidence indicating an important role of the CP in guiding complement activation on conidia of *A. fumigatus* in conditions of immunoglobulin competence (discussed above) [50,52]. This information notwithstanding, several noncomplement functions have been attributed to C1q [72], including an active role in maturation of myeloid DCs and polarization of T lymphocytes toward a Th1 phenotype (of potential relevance to the adaptive antifungal immunity) [73], which, however, have not been addressed in experimental models of aspergillosis. Site- and condition-specific roles have been reported for MBL. In this regard, administration of recombinant human MBL to corticosteroid-immunosuppressed mice prolonged their median survival time in a model of IPA, where this was associated with a more pronounced inflammatory reaction in the lung (i.e., higher levels of TNFα and IL-1β, and reduced production of IL-10) [65]. As opposed to this, in a model of systemic aspergillosis, nonimmunosuppressed MBL-deficient mice were protected, arguably due to reduced tissue damage, secondary to defective recruitment, and activation of neutrophils [74]. Furthermore, in a model of *A. fumigatus*-induced asthma, MBL deficiency was associated with lower titers of type-2 cytokines and decreased airway hyper-responsiveness at early times postinfection, which suggests MBL to be involved in the initial phases of the allergic response to this pathogen [75]. Interestingly, a recent study unveiled a mycobiome-dependent protumoral role for MBL, whose complement activating properties were proposed to support oncogenic progression in mouse models of pancreatic ductal adenocarcinoma. Although restricted to *Malassezia* species in the applied experimental setting, this mechanism evokes other pathogenic fungi to promote tumor growth *via* LP pathways [76].

The role of ficolins (ficolin-A and ficolin-B, the latter regarded as the murine orthologue of human ficolin-1) in *A. fumigatus* infections has been studied *in vivo* using an immunocompetent mouse model of IPA [77]. In this model, an increased fungal burden was observed in the double-deficient mice compared to WT animals at early times (i.e., 24 h) from infection, consistent with impaired opsonophagocytosis of dormant conidia [67]. Complement activation and recruitment of inflammatory cells were both unaltered in this setting; however, decreased production of proinflammatory cytokines in the lung of ficolin-A- and ficolin-B-deficient mice was reported, which suggests an early immunomodulatory role for these ficolins *in vivo*, similar to what described *in vitro* [62].

Aspergillus fumigatus infections have not been explicitly modeled in AP-modified animals. However, in murine models of fungal allergy, this pathway has been involved in the chitin-dependent production of C3a in the lung, a process that suppresses regulatory dendritic and T cells, and supports allergy-promoting T cells, *via* C3a receptor (C3aR) [78]. Similarly, repeated intranasal inoculation of immunocompetent mice with *A. fumigatus* conidia exposing high levels of chitin skewed T-helper cells toward a type-2 allergic response [79]. Furthermore, genetic deficiency or functional inhibition of C3aR reduced inflammation and development of chronic rhinosinusitis in a murine model of sinonasal *A. fumigatus* infection [80]. Also, mice carrying a loss-of-function mutation in C5 were described to be more susceptible to *Aspergillus* infections in models of chronic systemic and pulmonary aspergillosis, likely due to defective C5a-dependent recruitment of neutrophils (where effective generation of this anaphylatoxin requires the AP), and, possibly, reduced MAC formation (particularly on germinating conidia and hyphae) [81].

Membrane-born CRs are of paramount importance for recognition, phagocytosis, and killing of C3b/iC3b (and C4b/iC4b)-opsonized pathogens [82]; however, their involvement in the immune reaction to *A. fumigatus* infections has been sparsely addressed *in vivo*. In this regard, functional loss of CD18 (known as leukocyte-adhesion deficiency type 1, LAD1) is a known risk factor for IA, and genetic deficiency of CD11b (that forms the ß2 integrin CR3 along with the common beta subunit CD18) was shown to restrain fungal burden (but not neutrophil infiltration) in a mouse model of IPA [83]. In spite of CR1 and 2 being pivotal for the development of appropriate B-cell responses [30], experimental aspergillosis has not been modeled in CR1/2 gene-targeted animals, possibly due to different gene regulation and protein synthesis in humans and rodents (e.g., humans have two distinct genes, whereas mice carry a single gene, CD21/*Cr2*, that codes for the two receptor proteins *via* alternative splicing mechanisms [84]).

Complement evasion strategies of *Aspergillus fumigatus*

Aspergillus fumigatus has evolved a number of strategies to escape the complement-mediated immune response, which are listed and discussed below.

1 *Hiding*. Aspergillosis is often characterized by the formation of abscesses, mainly if the central nervous system is involved. During an inflammatory reaction, conidia can germinate and invade the brain parenchyma, leukocytes are recruited, and a fibrous capsule forms around the infected area. This is meant to avoid pathogen dissemination, thus acting as a host-defense mechanism; however, it also prevents the access of humoral effectors, including complement components, to the fungal pathogen, which is therefore spared from complement attack and disposal [85].
2 *Masking*. Polysaccharidic PAMPs of the inner cell wall of dormant conidia are masked to complement recognition by the outermost layers of RodA and DHN melanin [5,6,43,86]. A cellular PRR for DHN melanin has been recently identified (see above), which however does not belittle the masking effect of this pigment toward complement activation. Indeed, genetic abrogation of melanin synthesis in *A. fumigatus* led to increased C3-dependent opsonophagocytosis of dormant conidia by professional phagocytes [87,88].
3 *Recruitment of complement inhibitors*. *Aspergillus fumigatus* has been shown to hijack soluble inhibitors of the three pathways, most notably factor H and its splicing variant factor H-like protein 1 (that control the AP) [89] and C4BP (major soluble regulator of the CP/LP) [90]. In particular, conidia-bound factor H was shown to retain its cofactorial activity toward factor I, therefore supporting the factor I-mediated hydrolysis of C3b to iC3b, which inhibited activation and amplification of the AP on the conidial surface [89]. Recently, Aspf2, a protein allergen expressed throughout the life cycle of *A. fumigatus*, has been proposed as a ligand of factor H, FHL-1, and factor H-related protein 1 (an additional member of the factor H family of complement regulators). Genetic deletion of Aspf2 led to decreased binding of these inhibitors, enhanced C3 deposition, and more pronounced phagocytosis and killing of fungal conidia by human neutrophils. Furthermore, Aspf2 was shown to recruit human plasminogen and make use of the newly generated plasmin (i.e., from proteolytic activation of plasminogen) to degrade fibrin and fibrinogen, a mechanism that likely promotes penetration of the fungal pathogen in the lung tissue [91].
4 *Production of complement inhibitors*. In addition to recruiting complement inhibitors, *A. fumigatus* can also make soluble factors with complement inhibiting activity [92]. Of yet uncertain identity, these molecules appear to selectively target the AP and prevent conidia opsonization by C3 [93].
5 *Degradation of complement proteins*. *Aspergillus fumigatus* can synthesize and release proteolytic enzymes that target and degrade selected complement proteins. In this respect, the hyphal morphotype was found to secrete the Alp1 protease that degrades C3, C4, and C5, and has been proposed to exert an important role in cerebral aspergillosis [94,95]. More recently, an *A. fumigatus* metalloprotease has been described (i.e., Mep1p) that is synthesized by the dormant conidia in the presence of collagen (to model the lung ECM), and acts on the same subset of complement components, in addition to their anaphylatoxin products (i.e., C3a, C4a, and C5a) and the LP initiators MBL and ficolin-1 [96]. A mechanism has been proposed here that involves either direct degradation of these proteins or their cleavage to forms that are further processed (and inactivated) in solution by complement proteases. However, an Mep1p-deficient *A. fumigatus* mutant showed similar virulence to that of the WT strain in a immunosuppressed murine model of IPA, suggesting a certain degree of redundancy amongst the proteolytic mechanisms evolved by this fungus [96].

In addition to direct antifungal properties, a close crosstalk has been described between the complement system and other soluble PRMs in the immune response to *A. fumigatus* [22]. The following sections focus on pentraxins, particularly the long pentraxin PTX3 (see Ref. [97] for a review on complement regulation by pentraxins).

The role of pentraxins in *Aspergillus fumigatus* infections

Pentraxins are a family of evolutionary conserved multimeric PRMs, characterized by the presence in their carboxy-terminus of a 200 amino acid long pentraxin domain with a family distinctive signature (HxCxS/TWxS, where x is any amino acid) [98]. Based on primary structure of the composing protomer subunits, these proteins are divided into two groups: short and long pentraxins.

C-reactive protein (CRP) and serum amyloid P component (SAP) are the 'classical' short pentraxins, hallmarked by a common quaternary structure with five or ten identical subunits folding into annular pentameric disks stabilized by noncovalent interactions [99,100]. Likely originating from the duplication of a single ancestral gene, CRP was the first soluble PRM to be identified in the human serum [101], and SAP was afterward described as a close relative of CRP, based on structural similarities [102]. Despite of substantial sequence homology across orthologs in different mammalian species, these pentraxins are remarkably diverse in basal circulating levels and responsiveness to acute phase stimuli (in particular, IL-6). In this regard, CRP and SAP are the main acute phase proteins in human and mouse, respectively; therefore extrapolating clinically relevant information from murine models of disease is somewhat problematic [103]. In addition, the plasmatic levels of CRP increase as much as 1000-fold (from basal values ≤ 3 mg·L^{-1} in the healthy adult) in the course of an acute phase reaction (which makes CRP a useful diagnostic and prognostic tool), whereas those of SAP are constitutively set at 30–50 mg·L^{-1}, mostly due to inherent differences in gene regulation in the liver, major source of both proteins [104].

A number of functions have been attributed to the short pentraxins that involve pathogen (and damaged host cells) recognition, promotion of phagocytosis *via* complement- and/or Fc fragment of IgG receptor (FcγR)-dependent mechanisms, regulation of inflammation, atherogenesis, and amyloidogenesis (reviewed in Ref. [104]). In particular, CRP has been shown to bind a panoply of microbes, including fungi, yeasts, bacteria, and parasites, mostly through phosphorylcholine and carbohydrate structures, and promote phagocytosis, thus supporting resistance to infections [105]. Noticeably, high levels of CRP have been reported in IA patients [106,107], and this pentraxin has been described to recognize yet unknown components of the hyphal cell wall of *A. fumigatus* [108] and promote phagocytosis of germinating (more than dormant) conidia by human neutrophils *in vitro* [109]. Also, SAP has been shown to localize in areas of hyphal dissemination in the lung parenchyma of IPA patients [110] and attenuate some traits (i.e., airway resistance to methacholine, inflammation, and tissue remodeling) of the chronic allergic airway disease in *A. fumigatus*-sensitized mice, possibly *via* modulation of the M2 polarization of macrophages [111]. This information notwithstanding, if and how the short pentraxins participate in the pathogenesis of aspergillosis *in vivo* is currently unclear. This gap in knowledge is surprising, given that both CRP and SAP are ligands of C1q, and can activate the CP cascade [112,113]. Furthermore, CRP and SAP exert complement regulating activities through specific interactions with factor H [114,115] and C4BP [116], respectively, where these inhibitors are regarded as major targets for complement evasion by *A. fumigatus* (see above). Also, SAP is a binder of major ECM components, including type IV collagen [117] and fibronectin [118], which have been implicated in the initial adhesion of dormant conidia to the lung epithelium [119,120], and both short pentraxins recognize FcγRs and participate in the FcγR-dependent phagocytosis of microbes (and apoptotic cells) by phagocytic cells [121].

The long pentraxin PTX3: gene regulation and protein structure

A flourishing literature indicates a pivotal role for the long pentraxin PTX3 in the immune reaction to *A. fumigatus*. Identified in the early 1990s in the secretome of inflamed endothelial cells and fibroblasts, PTX3 is the parent member of the long pentraxin subfamily of soluble PRMs, which are characterized by an N-terminal region linked to a carboxy-terminal pentraxin-like domain [21]. Remarkably conserved across evolutionary distant species, the *PTX3* gene is similarly regulated in humans and mice, which facilitates the modeling of human diseases in the mouse. As opposed to CRP and SAP, whose synthesis mostly occurs in the liver in response to IL-6, PTX3 expression is rapidly and locally induced at sites of inflammation and infection by inflammatory cytokines (e.g., IL-1β, TNF-α), TLR agonists, microbial moieties

(e.g., lipopolysaccharide, outer membrane protein A, lipoarabinomannans), and intact microorganisms (including conidia of *A. fumigatus*) in a number of immune and nonimmune cells (most notably, myeloid DCs, macrophages, endothelial cells, fibroblasts, and alveolar epithelial cells), but not in B and T lymphocytes nor in NK cells [98]. Different to this, IFN-γ, IL-4, 1α,25-dihydroxyvitamin D3, and prostaglandin E2 exert inhibitory effects on *PTX3* transcription [122], and glucocorticoid hormones, like dexamethasone, have differential outcomes on PTX3 expression (i.e., either inhibiting or promoting it in hematopoietic and nonhematopoietic cells, respectively) [123], which is interesting given the association between iatrogenic immunosuppression and the risk of IA (see above). Of major relevance to the pathogenesis of aspergillosis, neutrophils lack *de novo* synthesis of PTX3. In fact, transcription of the *PTX3* gene is confined to promyelocytes and myelocytes/metamyelocytes (i.e., immature myeloid elements) and is absent in bone marrow-segmented and circulating neutrophils (i.e., mature neutrophils). However, the pre-made protein is stocked in a 'ready-to-use' form in lactoferrin^{+} and lactoferrin^{+}/gelatinase^{+} (specific) granules and is promptly released (upon neutrophil degranulation) in response to microbial recognition and/or inflammatory stimuli at sites of infection, where it accumulates in the NETs [124]. This further highlights the differences between this long pentraxin and the short ones as for expression kinetics and localization, which makes PTX3 particularly suited as soluble PRM in the early phases of *A. fumigatus* infections.

In line with the general structural organization of the long pentraxins, the human PTX3 protomer is a glycoprotein containing an N-terminal region (amino acids 18–178), and a C-terminal domain (amino acids 179–381), in addition to a 17 amino acid long signal peptide that targets it to secretion [125]. We have reported a single N-glycosylation site at Asn220 in the C-terminal pentraxin domain that bears fucosylated and sialylated complex type oligosaccharides [126], and regulates the protein's interaction with a number of ligands, including C1q [127], factor H [128], and ficolin-1 [129] (see below). The mature secreted protein is an homo-octamer whose composing protomer subunits are held in place both by noncovalent (most likely, coiled coils contributed by the N-terminal region) and covalent (disulfide bonds formed by cysteine residues both in the N- and C-terminal domain) interactions [130]. Furthermore, based on electron microscopy and X-ray scattering, the PTX3 molecule folds into a large and a small domain with an intervening stalk region, which makes it asymmetric and elongated, a rather unique structural organization amongst pentraxins [131]. This structural complexity (i.e., multidomain organization, post-translational modifications, quaternary structure) sets a frame for the protein's interactome, which is rather broad and supports the numerous and diverse functions attributed to this long pentraxin in innate immunity [132], cancer-related inflammation [133], and, more recently, bone physiology [134]. Here, we focus on the roles exerted by PTX3 in antifungal immunity, which have been extensively studied both *in vitro* and *in vivo*.

PTX3 in the immune response to *Aspergillus fumigatus* and its crosstalk with complement

The first preclinical evidence of an involvement of PTX3 in the pathogenesis of aspergillosis dates back to 2002, when we reported that genetic deficiency of *Ptx3* markedly enhanced susceptibility of otherwise immunocompetent mice to IPA [24]. This was attributed to defective recognition (and killing) of dormant conidia by AMs, impaired activation of DCs, and unbalanced cytokine profiles [with increased levels of interferon-γ (IFN-γ), and IL-12, and reduced titers of IL-4 in the lung of the *Ptx3*$^{-/-}$ mice] that skewed T-helper cells toward a tolerogenic type-2 phenotype. In the same experimental setting, depletion of neutrophils with an antibody (to mimic neutropenia in humans) abrogated the negative effect of *Ptx3* deficiency on survival to IPA [24], indicating that neutrophils and PTX3 cooperate in the handling of *A. fumigatus* infections. This point was further addressed and expanded in a following study, where adoptive transfer of neutrophils from *Ptx3*-deficient (but not competent) mice to littermates (either deficient or competent in *Ptx3*) previously immunosuppressed with cyclophosphamide and infected with *A. fumigatus* failed to restrain the chemotherapy-associated fungal burden in the lung [124]. In another report, PTX3 was described to exert opsonic activity toward this fungal pathogen, thus favoring its recognition, phagocytosis, and killing both by murine and human neutrophils [25]. To further highlight the functional crosstalk between PTX3 (as a soluble PRM) and neutrophils (as innate cellular effectors), here we reiterate that the PTX3 protein is stored in the specific granules of neutrophils [124], and these cells are essential for host resistance to *A. fumigatus* infections [14], a link that is emerging in the clinic too (see the 'Genetic variation in human complement and pentraxin genes and susceptibility to aspergillosis' section).

Interestingly, administration of the exogenous recombinant PTX3 protein rescued resistance to IPA

in *Ptx3*-deficient mice and showed therapeutic efficacy in an IPA model of allogeneic, T cell-depleted, bone marrow transplantation (BMT) [24]. These initial observations have been expanded in animal models of other clinical conditions predisposing to IA. In this regard, PTX3 restored antifungal resistance (*via* promotion of Th1 responses) in an experimental model ($p47^{phox-/-}$ mice) of CGD (a primary immunodeficiency characterized by recurrent bacterial and fungal infections) [135] and proved effective (both as therapeutic and prophylactic) in a cortisone acetate-immunosuppressed rat model of IPA [136], which extended the protective roles of this pentraxin to corticosteroid-induced immunodeficiency, a well-documented IA risk factor in graft-versus-host disease and solid-organ transplantation [14]. Furthermore, PTX3 protected BM-transplanted and murine cytomegalovirus (MCMV)-infected mice from *A. fumigatus* superinfections [137], and had therapeutic activity in animal models of chronic lung infection by *Pseudomonas aeruginosa*, a major cause of morbidity in cystic fibrosis, a condition that predisposes to IPA [138]. Noticeably, the exogenous protein retained its protective properties in the combination therapy, whereby in rodent models of IPA it enhanced or even synergized with clinically established antifungal drugs, including the polyene amphotericin B [139], and the triazole voriconazole [140] and posaconazole [141]. Given the current limitations to the use of antifungals, mostly due to drug–drug interactions and both acute and chronic toxicity [14], these observations are of great clinical significance.

The human PTX3 protein directly recognizes *A. fumigatus via* its N-terminal domain [25]; however, this is restricted to resting, swollen, and germinating conidia, with little or no interaction with fungal hyphae [24]. Based on competition experiments, GM has been proposed as ligand of PTX3 [24]; however, this polysaccharide is enveloped in the layer of RodA and DHN melanin of dormant conidia [6,43] (that are bound by PTX3) and is abundant and exposed in the hyphal wall [69] (that is poorly or not recognized by PTX3), and no direct interaction of PTX3 with purified GM has been reported so far, which suggests that other cell wall components are recognized by this long pentraxin. Indirect binding of PTX3 to *A. fumigatus* conidia has also been reported. In this respect, ficolin-2 (a ligand of *A. fumigatus* [52,53,55]) was shown to bind PTX3 in a calcium-independent manner, and the two proteins recruited each other on the conidial wall, which resulted into amplification of the LP *in vitro* that was apparent, however, only in conditions of C1q and MBL deficiency [55]. PTX3 was described to recognize (*via* the terminal sialic acid residues of its glycosidic moiety) the fibrinogen-like domain of ficolin-1 and activate the LP *in vitro* [129]; nonetheless, the ficolin-1/PTX3 hetero-complex could not form on *A. fumigatus* conidia [142]. Although ficolin-3 has been reported to bind PTX3 [55], the functional implications of this interaction in antifungal immunity have not been explored. Also, PTX3 was reported to interact (in a Ca^{2+}-dependent manner) with the collagen-like domain of MBL, and the resulting complex promoted recruitment of C1q, deposition of C4 and C3, and phagocytosis of *C. albicans*; however, it is not known whether this also applies to *A. fumigatus* [54]. In this regard, in spite of PTX3 being able to recognize C1q and regulate activation of the CP [143–145], C1q proved dispensable for the host-protective activity of this long pentraxin *in vitro* [25] and *in vivo* [24]. It is therefore conceivable that the crosstalk between PTX3 and components of the LP and CP on dormant conidia of *A. fumigatus* is confined to ficolin-2, and this contributes to complement initiation, otherwise dominated by C1q and MBL (Fig. 2).

While PTX3 and ficolin-2 synergize in the promotion of LP activation on *A. fumigatus* conidia, the prophagocytic activity of this long pentraxin strictly requires a functionally competent AP, as demonstrated by Moalli *et al.* [25]. In this study, abrogation of the CP and CP/LP (by depletion of C1q and C4, respectively) did not affect the PTX3-dependent opsonophagocytosis of *A. fumigatus* conidia by human neutrophils *in vitro*. This was lost instead in conditions of AP inactivation (i.e., using factor B-depleted sera) and retained upon *in vitro* reconstitution of the AP (from the purified components). Furthermore, C5 had no role in this setting, suggesting that the C5a-dependent activation of neutrophils is dispensable for the prophagocytic activity of PTX3, which is restricted to the opsonic functions of C3 (and its fragments). On this line, functional depletion (with blocking antibodies) experiments and cytofluorimetric analyses indicated that opsonization of conidia with PTX3-augmented CD11b (component of CR3, major receptor of iC3b) activation, internalization, and recruitment to the phagocytic cup [25]. This mechanism required inside–out activation of CD11b by FcγRIIA (CD32), consistent with the notion that complement and immunoglobulin receptors functionally cooperate in phagocytosis [146]. Based on this and previous reports, PTX3 has been proposed as a functional ancestor of antibodies ('ante-antibody') in that it exerts opsonic activity toward *A. fumigatus*, and enhances recognition, phagocytosis, and killing of fungal conidia by immune cells (mainly neutrophils), *via* complement and Fc receptor pathways (see Ref. [147] and Fig. 2).

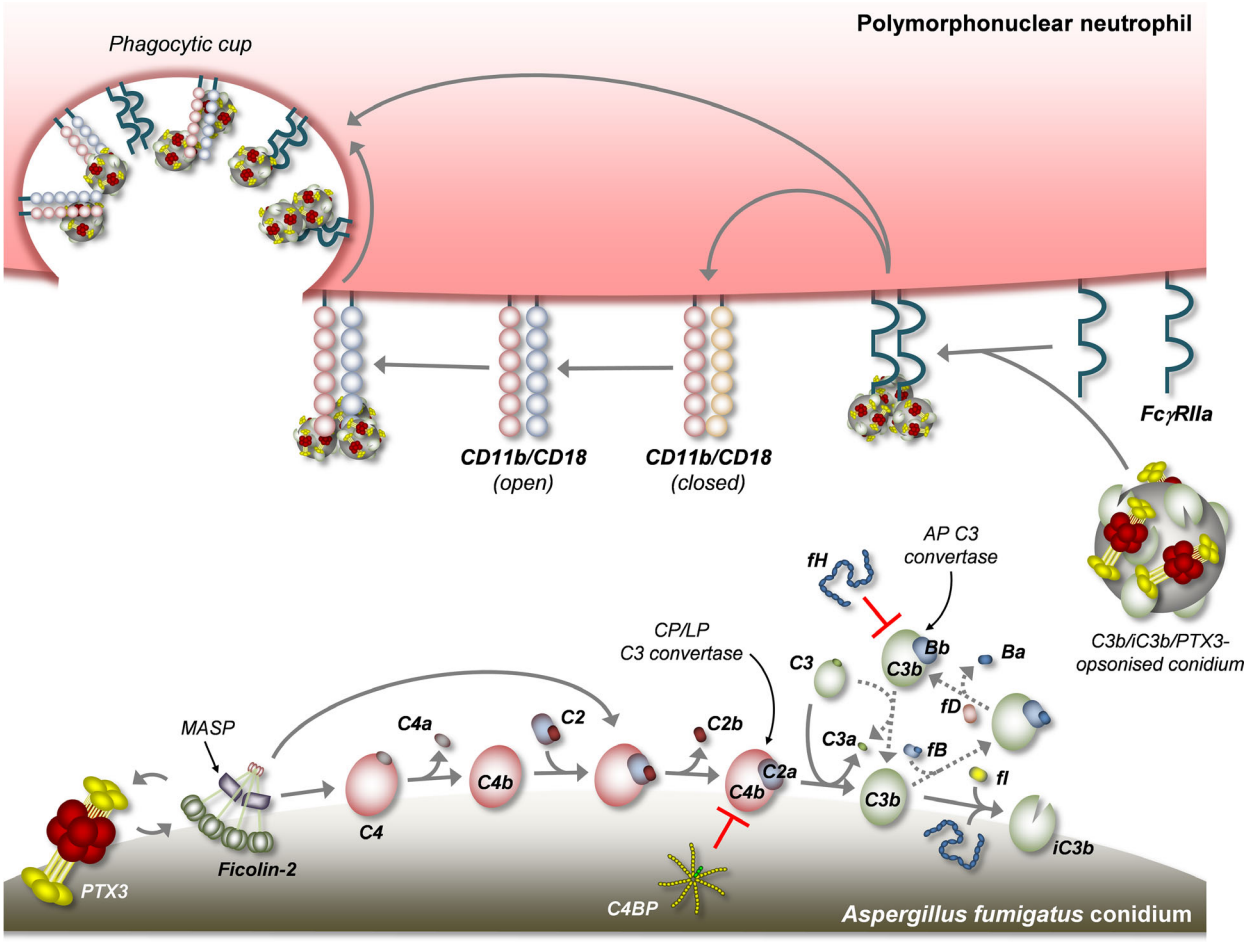

Fig. 2. The molecular crosstalk between complement and the long pentraxin 3 in the opsonophagocytosis of *Aspergillus fumigatus* conidia. The human (and murine) PTX3 protein recognizes resting, swollen, and germinating conidia of *A. fumigatus* (with poor or no binding to hyphae) and favors their recognition, phagocytosis, and killing by neutrophils [24], via complement-dependent mechanisms [25]. In this regard, ficolin-2 and PTX3 recruit each other on the conidial wall and amplify the LP (here exemplified by the MASPs-dependent cleavage of C4 and C2 to generate the CP/LP C3 convertase) [55]. This synergism is apparent in conditions of C1q and MBL deficiency, likely due to these proteins playing a dominant role (over ficolins) in initiating the CP/LP on *A. fumigatus* (Fig. 1) [52]. As the AP (via its components C3, factor B, fB, factor D, fD, and properdin, the latter not shown in the figure) amplifies the complement response, PTX3 promotes phagocytosis of fungal conidia by enhancing internalization and recruitment of CD11b (that forms with CD18 the CR3 complex, major receptor of iC3b) to the phagocytic cup. This proceeds through inside-out activation of CD11b via FcγRIIA (CD32, proposed as a receptor of PTX3), an example of the functional cooperation between complement and immunoglobulin receptors [25]. Shown are the complement inhibitors factor H (fH) and C4BP that are sequestered by the fungus to evade the complement attack [89,90].

Complement-independent mechanisms also have been proposed for the protective antifungal activity of PTX3. In this regard, we have reported that conidia-bound PTX3 recruits myeloid differentiation protein 2 and activate the TLR4/Toll/IL-1R domain-containing adapter inducing IFN-β-dependent signaling pathway that leads to IL-10 synthesis, pointing to a modulatory (in addition to opsonic) role of PTX3 in the immunopathology of aspergillosis [148]. Furthermore, a proinflammatory role has been hypothesized for PTX3 in an *in vitro* model of human cornea infection with *A. fumigatus*, where blocking of endogenous (i.e., corneal) PTX3 synthesis by siRNA inhibited production and release of the inflammatory cytokine IL-1β [149]; however, the pathophysiological implications of this observation have not been explored.

The complement system and pentraxins in *Candida albicans* infections

The evolutionary pressure posed on antifungal immunity by common molecular structures and/or patterns (PAMPs) of yet distant microbial species has led to

selection of remarkably similar effector molecules and mechanisms; nonetheless, species-specific PAMP architectures elicit microbe-tailored immune responses. To highlight this point, here we briefly discuss how the complement system and the pentraxins cooperate in the handling of *C. albicans*, as a further example (in addition to *A. fumigatus*) of human opportunistic fungal pathogen. This commensal yeast is normally present in the gastrointestinal tract of healthy individuals and, in a close similarity to *A. fumigatus*, can turn into an opportunistic pathogen in conditions of altered host's microbiota and immune system, including broad-spectrum antibiotic regimens, surgical and medical interventions that compromise the integrity of the mucosal barriers, and immunosuppressive therapies [150]. Analogous to *A. fumigatus*, the life cycle of *C. albicans* comprises different morphotypes (i.e., yeast cells, pseudohyphae, and hyphae) with stage-specific cell wall composition and structure [151]. In this regard, β-glucans are key polysaccharidic PAMPs of the *C. albicans* inner cell wall that are recognized by cell-borne PRRs, most notably dectin-1 [152] and CR3 [153]. Interestingly, mouse bone marrow-derived neutrophils express more CR3 following recognition of β-glucan particles by dectin-1, and this leads to enhanced particle phagocytosis and ROS production *in vitro*, suggesting that prototypical glucan (i.e., dectin-1) and complement (CR3) receptors act in concert to boost the immune response to *C. albicans* [154]. Consistent with this, CR3 is required for synthesis and release of IL-1β by mouse bone marrow-derived macrophages and DCs, in response to purified β-glucans and heat-killed *C. albicans* [155], and genetic deficiency of CR3 results into higher susceptibility to *C. albicans* infections in experimental models of candidiasis [154].

The outer cell wall of *C. albicans* comprises an array of heavily mannosylated and glycosylphosphatidylinositol-modified proteins that are cross-linked to β-(1,6)-glucans. The N-linked mannans are targeted for immune recognition by several PRRs, including DC-SIGN, mannose receptor, TLR4, dectin-2 and dectin-3, all abundantly represented on the surface of professional phagocytes [20]. The mannan layer of *C. albicans* has been proposed to protect inner PAMPs from AP-dependent complement reactions, based on the observation that chemical removal of mannans from the yeast's cell wall results into enhanced AP activation and neutrophil-mediated phagocytosis [156]. However, these polysaccharides *per se* are targeted for complement activation by MBL, major recognition unit of the LP (see above). In this regard, human MBL has been reported to promote agglutination of *C. albicans* hyphae and inhibit growth of the yeast cells [157]; in addition, it favors C4 and C3 deposition on the fungal pathogen and augments its phagocytosis by neutrophils, but not monocyte-derived DCs [158]. The opsonic and phagocytic activities of MBL toward *C. albicans* have been attributed to formation of hetero-complexes with other serum proteins, most notably PTX3 and SAP, as anticipated above. Interestingly, purified preparations of both pentraxins lack binding to *C. albicans*, and serum MBL acts as a docking system for PTX3 and SAP (but not CRP) on the yeast surface. Furthermore, the interaction of PTX3 with MBL leads to cross-activation of the CP *via* C1q, whereas it is still unclear how SAP/MBL complexes initiate complement activation [54]. This can be regarded as an additional example of functional cooperation within (LP and CP) and between (complement and pentraxins) classes of soluble PRMs [22]. Consistent with these findings, administration of exogenous MBL has been shown to improve the survival of mice challenged intravenously with *C. albicans* in a prophylactic model of invasive candidiasis [159]. Furthermore, genome-wide association studies have identified polymorphisms in the human MBL gene that are associated with increased susceptibility to vulvovaginal candidiasis and frequency of the infection recurrence [160], which suggests an important role for MBL in the mucosal immunity to *C. albicans*.

Genetic variation in human complement and pentraxin genes and susceptibility to aspergillosis

Onset and progression of aspergillosis are characterized by significant interindividual variability, which highlights the relevance of heritable factors in determining human susceptibility to this infection. In this regard, while only a few monogenic defects have been identified that predispose to IPA, the body of common polymorphisms associated to the disease is steadily increasing and mostly comprises genes of the PRR and PRM families, including complement proteins and pentraxins [161].

Mannose-binding lectin is a paradigmatic example of the link between complement gene variation and risk of aspergillosis. Polymorphisms in the human MBL gene have a remarkable effect on production and structure/function of the protein in up to 8% of the general population, yet individuals carrying alleles associated with reduced circulating levels of MBL or defective forms of the protein (i.e., with altered quaternary structure) do not manifest obvious clinical signs [162]. However, in conditions of immune suppression, MBL deficiency is recognized as a major risk factor

for several infections, including IPA. In this regard, the serum levels of this lectin are lower in IPA patients than in immunocompromised control subjects [163], consistent with the dominant role of this PRM in the early complement response to inhaled conidia of *A. fumigatus* [52]. Furthermore, *MBL* haplotypes coding for low levels of the protein have been associated with chronic necrotizing pulmonary aspergillosis (CNPA), with the codon 52 mutant allele (W/M52) being particularly frequent in CNPA patients [164]. These observations substantiate MBL as a potential drug component for treatment and prophylaxis of *A. fumigatus* infections. In spite of available preclinical evidence (see above), the contribution of other complement genes (in particular C1q) to the risk of aspergillosis in humans is yet to be evaluated.

Despite of their involvement in a number of infectious diseases [104], very few clinical records are available to indicate or suggest an etiopathogenic role of the short pentraxins in human aspergillosis. In this regard, CRP has been proposed as a prognostic factor for survival in patients with acute invasive fungal rhinosinusitis [165], and SAP has been observed in human specimens with disseminated aspergillosis in areas of biofilm amyloid accumulation [110], which potentially suggests a role for this protein in the adhesion of *A. fumigatus* (mostly hyphae) to the lung tissue. As opposed to the short pentraxins, common polymorphisms in the *PTX3* gene have been proposed as critical risk factors for IPA in patients undergoing hematopoietic stem-cell transplantation (HSCT) [166]. In particular, an haplotypic block encompassing three single nucleotide polymorphisms in introns 1 and 2, and exon 2 (i.e., Ala/Asp substitution in the protein's N-terminal domain) in HSC donors was associated to defective expression of the protein (i.e., in the lung alveoli), and impaired neutrophil-mediated phagocytosis and clearance of *A. fumigatus* conidia (i.e., due to reduced amounts of the 'pre-made' molecule in neutrophils) [166]. Noticeably, reduced plasma levels of the PTX3 protein have been documented in healthy adults carrying the IPA-associated alleles [167], and these are risk factors for other infections, including *P. aeruginosa* colonization in cystic fibrosis patients [168], urinary tract [169], and *Mycobacterium tuberculosis* [170] infections. In this regard, two enhancers (in addition to the promoter region) have been identified in the human *PTX3* gene that differentially control its transcription in physiological and inflammatory conditions, and the activity of the second (that encompasses exon 2) is affected by the haplotypic variation responsible for decreased synthesis and release of the protein [171], which might explain the genetic link with IPA (and other infections).

The original association between genetic variation in the *PTX3* gene and aspergillosis in HSCT has been validated in a large, independent study on a similar risk population [172] and extended to other clinical conditions that predispose to the disease, including solid-organ (particularly, lung) transplantation [173,174], obstructive pulmonary disease [175], reactivation of latent human cytomegalovirus (HCMV) in HSCT patients [176], and hematological malignancies [177]. Interestingly, this association was lost in conditions of severe neutropenia, as recently reported by Brunel *et al.* [178], who studied IPA amongst acute leukemia patients subjected to intensive chemotherapy, which strongly indicates a close functional cooperation between neutrophils and PTX3 in the immune recognition and clearance of *A. fumigatus in vivo*, in line with the available preclinical and *in vitro* evidence (see above). Furthermore, in an effort to identify a signature of alveolar cytokines associated with the development of IPA, Gonçalves *et al.* [179] reported that gene variation in *PTX3* markedly affects the levels of proinflammatory cytokines in the lung of patients-at-risk, and cannot be ignored when assessing the diagnostic performance of these markers.

Based on available clinical information, genetic variation in *PTX3* is 'the most robust genetic marker for IPA identified to date' [161]. Furthermore, the therapeutic efficacy of the protein in several animal models of aspergillosis, and its additive and/or synergistic effects on established antifungal drugs (as discussed above) strongly encourage the translation of this long pentraxin to the clinic, for prophylaxis and/or treatment of IA, either alone or in the combination therapy.

Conclusions and Perspectives

The incidence of *A. fumigatus* infections has steadily increased over recent years, and invasive forms of these diseases pose a severe threat to a growing number of individuals, mostly those experiencing conditions of immune suppression and deficiency [12]. Humans have evolved immune effector mechanisms to rapidly and effectively recognize and kill invading fungal pathogens, mostly comprising humoral and cellular arms of the innate immune system [8,9]. Preclinical and clinical evidence indicates that complement and other soluble PRMs, in particular the long pentraxin PTX3, functionally cooperate in the immune response to *A. fumigatus* [22]. In an era of extensive iatrogenic immunosuppression, the pharmaceutical pipeline for development of novel antifungal drugs is running dry, and new therapies are needed that are possibly based

on a better understanding of the host/pathogen interplay in the pathogenesis of aspergillosis. In this regard, genetic data from the clinic, traditionally translated into risk stratification and diagnostic strategies, might assist in developing original applications of available therapies (including those targeting complement components, which are nowadays experiencing a renaissance [180]), and in the design and interpretation of clinical trials [161]. Furthermore, it is timely to address fundamental mechanisms of the antifungal immunity, which are expected to provide information on novel molecular targets for pharmacological intervention, thus paving the way to new and much urgent translational efforts in the field. In this regard, further exploitation of the interplay between PTX3 (currently, the best genetic marker for IPA [161]), the AP [25], and TLRs [148] holds promise to deliver new genetic and mechanistic tools for a more effective handling of *A. fumigatus* infections.

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References

1 Peterson SW (2008) Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. *Mycologia* **100**, 205–226.
2 O'Gorman CM, Fuller H and Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* **457**, 471–474.
3 Shapiro RS, Robbins N and Cowen LE (2011) Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol Mol Biol Rev* **75**, 213–267.
4 Latge JP, Beauvais A and Chamilos G (2017) The cell wall of the human fungal pathogen *Aspergillus fumigatus*: biosynthesis, organization, immune response, and virulence. *Annu Rev Microbiol* **71**, 99–116.
5 Carrion Sde J, Leal SM Jr, Ghannoum MA, Aimanianda V, Latge JP and Pearlman E (2013) The RodA hydrophobin on *Aspergillus fumigatus* spores masks dectin-1- and dectin-2-dependent responses and enhances fungal survival *in vivo*. *J Immunol* **191**, 2581–2588.
6 Valsecchi I, Dupres V, Stephen-Victor E, Guijarro JI, Gibbons J, Beau R, Bayry J, Coppee JY, Lafont F, Latge JP *et al.* (2017) Role of hydrophobins in *Aspergillus fumigatus*. *J Fungi* **4**, 2.
7 Dagenais TR and Keller NP (2009) Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. *Clin Microbiol Rev* **22**, 447–465.
8 Croft CA, Culibrk L, Moore MM and Tebbutt SJ (2016) Interactions of *Aspergillus fumigatus* conidia with airway epithelial cells: a critical review. *Front Microbiol* **7**, 472.
9 Erwig LP and Gow NA (2016) Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* **14**, 163–176.
10 Gamaletsou MN, Rammaert B, Bueno MA, Moriyama B, Sipsas NV, Kontoyiannis DP, Roilides E, Zeller V, Prinapori R, Taj-Aldeen SJ *et al.* (2014) *Aspergillus* osteomyelitis: epidemiology, clinical manifestations, management, and outcome. *J Infect* **68**, 478–493.
11 Kosmidis C and Denning DW (2015) The clinical spectrum of pulmonary aspergillosis. *Thorax* **70**, 270–277.
12 Bongomin F, Gago S, Oladele RO and Denning DW (2017) Global and multi-national prevalence of fungal diseases-estimate precision. *J Fungi* **3**.
13 Denning DW and Hope WW (2010) Therapy for fungal diseases: opportunities and priorities. *Trends Microbiol* **18**, 195–204.
14 Segal BH (2009) Aspergillosis. *N Engl J Med* **360**, 1870–1884.
15 Park SJ and Mehrad B (2009) Innate immunity to *Aspergillus* species. *Clin Microbiol Rev* **22**, 535–551.
16 Boyle KB, Stephens LR and Hawkins PT (2012) Activation of the neutrophil NADPH oxidase by *Aspergillus fumigatus*. *Ann N Y Acad Sci* **1273**, 68–73.
17 Papayannopoulos V, Metzler KD, Hakkim A and Zychlinsky A (2010) Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol* **191**, 677–691.
18 Prufer S, Weber M, Stein P, Bosmann M, Stassen M, Kreft A, Schild H and Radsak MP (2014) Oxidative burst and neutrophil elastase contribute to clearance of *Aspergillus fumigatus* pneumonia in mice. *Immunobiology* **219**, 87–96.
19 Mircescu MM, Lipuma L, van Rooijen N, Pamer EG and Hohl TM (2009) Essential role for neutrophils but not alveolar macrophages at early time points following *Aspergillus fumigatus* infection. *J Infect Dis* **200**, 647–656.
20 Snarr BD, Qureshi ST and Sheppard DC (2017) Immune recognition of fungal polysaccharides. *J Fungi* **3**, 47.
21 Bottazzi B, Doni A, Garlanda C and Mantovani A (2010) An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu Rev Immunol* **28**, 157–183.

22 Ma YJ, Lee BL and Garred P (2017) An overview of the synergy and crosstalk between pentraxins and collectins/ficolins: their functional relevance in complement activation. *Exp Mol Med* **49**, e320.
23 Wong SSW and Aimanianda V (2018) Host soluble mediators: defying the immunological inertness of *Aspergillus fumigatus* Conidia. *J Fungi* **4**, 3.
24 Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R, Maccagno A, Riva F, Bottazzi B, Peri G *et al.* (2002) Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* **420**, 182–186.
25 Moalli F, Doni A, Deban L, Zelante T, Zagarella S, Bottazzi B, Romani L, Mantovani A and Garlanda C (2010) Role of complement and Fc{gamma} receptors in the protective activity of the long pentraxin PTX3 against *Aspergillus fumigatus*. *Blood* **116**, 5170–5180.
26 Inforzato A, Doni A, Barajon I, Leone R, Garlanda C, Bottazzi B and Mantovani A (2013) PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Semin Immunol* **25**, 79–85.
27 Ricklin D, Hajishengallis G, Yang K and Lambris JD (2010) Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* **11**, 785–797.
28 Merle NS, Church SE, Fremeaux-Bacchi V and Roumenina LT (2015) Complement system part I - molecular mechanisms of activation and regulation. *Front Immunol* **6**, 262.
29 Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V and Roumenina LT (2015) Complement system part II: role in immunity. *Front Immunol* **6**, 257.
30 Reis ES, Mastellos DC, Hajishengallis G and Lambris JD (2019) New insights into the immune functions of complement. *Nat Rev Immunol* **19**, 503–516.
31 Parente R, Clark SJ, Inforzato A and Day AJ (2017) Complement factor H in host defense and immune evasion. *Cell Mol Life Sci* **74**, 1605–1624.
32 Ricklin D, Reis ES and Lambris JD (2016) Complement in disease: a defence system turning offensive. *Nat Rev Nephrol* **12**, 383–401.
33 Ballanti E, Perricone C, Greco E, Ballanti M, Di Muzio G, Chimenti MS and Perricone R (2013) Complement and autoimmunity. *Immunol Res* **56**, 477–491.
34 Morgan BP (2015) The role of complement in neurological and neuropsychiatric diseases. *Expert Rev Clin Immunol* **11**, 1109–1119.
35 Keragala CB, Draxler DF, McQuilten ZK and Medcalf RL (2018) Haemostasis and innate immunity - a complementary relationship: a review of the intricate relationship between coagulation and complement pathways. *Br J Haematol* **180**, 782–798.
36 Markiewski MM and Lambris JD (2007) The role of complement in inflammatory diseases from behind the scenes into the spotlight. *Am J Pathol* **171**, 715–727.
37 Steele C, Rapaka RR, Metz A, Pop SM, Williams DL, Gordon S, Kolls JK and Brown GD (2005) The beta-glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog* **1**, e42.
38 Loures FV, Rohm M, Lee CK, Santos E, Wang JP, Specht CA, Calich VL, Urban CF and Levitz SM (2015) Recognition of *Aspergillus fumigatus* hyphae by human plasmacytoid dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. *PLoS Pathog* **11**, e1004643.
39 Serrano-Gomez D, Dominguez-Soto A, Ancochea J, Jimenez-Heffernan JA, Leal JA and Corbi AL (2004) Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of *Aspergillus fumigatus* conidia by dendritic cells and macrophages. *J Immunol* **173**, 5635–5643.
40 Stappers MHT, Clark AE, Aimanianda V, Bidula S, Reid DM, Asamaphan P, Hardison SE, Dambuza IM, Valsecchi I, Kerscher B *et al.* (2018) Recognition of DHN-melanin by a C-type lectin receptor is required for immunity to Aspergillus. *Nature* **555**, 382–386.
41 Meier A, Kirschning CJ, Nikolaus T, Wagner H, Heesemann J and Ebel F (2003) Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. *Cell Microbiol* **5**, 561–570.
42 Bayry J, Aimanianda V, Guijarro JI, Sunde M and Latge JP (2012) Hydrophobins–unique fungal proteins. *PLoS Pathog* **8**, e1002700.
43 Pihet M, Vandeputte P, Tronchin G, Renier G, Saulnier P, Georgeault S, Mallet R, Chabasse D, Symoens F and Bouchara JP (2009) Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. *BMC Microbiol* **9**, 177.
44 De Bracco MM, Budzko DB and Negroni R (1976) Mechanisms of activation of complement by extracts of *Aspergillus fumigatus*. *Clin Immunol Immunopathol* **5**, 333–339.
45 Speth C, Rambach G, Wurzner R and Lass-Florl C (2008) Complement and fungal pathogens: an update. *Mycoses* **51**, 477–496.
46 Kozel TR, Wilson MA, Farrell TP and Levitz SM (1989) Activation of C3 and binding to *Aspergillus fumigatus* conidia and hyphae. *Infect Immun* **57**, 3412–3417.
47 Dumestre-Perard C, Lamy B, Aldebert D, Lemaire-Vieille C, Grillot R, Brion JP, Gagnon J and Cesbron JY (2008) *Aspergillus* conidia activate the complement by the mannan-binding lectin C2 bypass mechanism. *J Immunol* **181**, 7100–7105.
48 Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ and Turner MW (2000) Mannose-binding lectin binds to a range of clinically relevant microorganisms and

promotes complement deposition. *Infect Immun* **68**, 688–693.
49 Ma YJ, Hein E, Munthe-Fog L, Skjoedt MO, Bayarri-Olmos R, Romani L and Garred P (2015) Soluble collectin-12 (CL-12) Is a pattern recognition molecule initiating complement activation via the alternative pathway. *J Immunol* **195**, 3365–3373.
50 Braem SG, Rooijakkers SH, van Kessel KP, de Cock H, Wosten HA, van Strijp JA and Haas PJ (2015) Effective neutrophil phagocytosis of *Aspergillus fumigatus* is mediated by classical pathway complement activation. *J Innate Immun* **7**, 364–374.
51 Schonheyder H and Andersen P (1983) Complement-binding antibodies to *Aspergillus fumigatus* in patients with pulmonary aspergillosis. *Acta Pathol Microbiol Immunol Scand B* **91**, 1–7.
52 Rosbjerg A, Genster N, Pilely K, Skjoedt MO, Stahl GL and Garred P (2016) Complementary roles of the classical and lectin complement pathways in the defense against *Aspergillus fumigatus*. *Front Immunol* **7**, 473.
53 Hummelshoj T, Ma YJ, Munthe-Fog L, Bjarnsholt T, Moser C, Skjoedt MO, Romani L, Fujita T, Endo Y and Garred P (2012) The interaction pattern of murine serum ficolin-A with microorganisms. *PLoS ONE* **7**, e38196.
54 Ma YJ, Doni A, Skjoedt MO, Honore C, Arendrup M, Mantovani A and Garred P (2011) Heterocomplexes of mannose-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem* **286**, 3405–3417.
55 Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A, Thielens NM and Garred P (2009) Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem* **284**, 28263–28275.
56 Bidula S, Sexton DW, Yates M, Abdolrasouli A, Shah A, Wallis R, Reed A, Armstrong-James D and Schelenz S (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.
57 Bidula S, Sexton DW, Abdolrasouli A, Shah A, Reed A, Armstrong-James D and Schelenz S (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.
58 Bidula S, Sexton DW and Schelenz S (2016) Serum opsonin ficolin-A enhances host-fungal interactions and modulates cytokine expression from human monocyte-derived macrophages and neutrophils following *Aspergillus fumigatus* challenge. *Med Microbiol Immunol* **205**, 133–142.
59 Bolger MS, Ross DS, Jiang H, Frank MM, Ghio AJ, Schwartz DA and Wright JR (2007) Complement levels and activity in the normal and LPS-injured lung. *Am J Physiol Lung Cell Mol Physiol* **292**, L748–L759.
60 Gravelat FN, Beauvais A, Liu H, Lee MJ, Snarr BD, Chen D, Xu W, Kravtsov I, Hoareau CM, Vanier G *et al.* (2013) *Aspergillus* galactosaminogalactan mediates adherence to host constituents and conceals hyphal beta-glucan from the immune system. *PLoS Pathog* **9**, e1003575.
61 Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M and Latge JP (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J Biol Chem* **275**, 41528.
62 Jensen K, Lund KP, Christensen KB, Holm AT, Dubey LK, Moeller JB, Jepsen CS, Schlosser A, Galgoczy L, Thiel S *et al.* (2017) M-ficolin is present in *Aspergillus fumigatus* infected lung and modulates epithelial cell immune responses elicited by fungal cell wall polysaccharides. *Virulence* **8**, 1870–1879.
63 Henry C, Fontaine T, Heddergott C, Robinet P, Aimanianda V, Beau R, Beauvais A, Mouyna I, Prevost MC, Fekkar A *et al.* (2016) Biosynthesis of cell wall mannan in the conidium and the mycelium of *Aspergillus fumigatus*. *Cell Microbiol* **18**, 1881–1891.
64 Lehrnbecher T, Robinson PD, Fisher BT, Castagnola E, Groll AH, Steinbach WJ, Zaoutis TE, Negeri ZF, Beyene J, Phillips B *et al.* (2016) Galactomannan, beta-D-glucan, and polymerase chain reaction-based assays for the diagnosis of invasive fungal disease in pediatric cancer and hematopoietic stem cell transplantation: a systematic review and meta-analysis. *Clin Infect Dis* **63**, 1340–1348.
65 Kaur S, Gupta VK, Thiel S, Sarma PU and Madan T (2007) Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. *Clin Exp Immunol* **148**, 382–389.
66 Chai LY, Vonk AG, Kullberg BJ, Verweij PE, Verschueren I, van der Meer JW, Joosten LA, Latge JP and Netea MG (2011) *Aspergillus fumigatus* cell wall components differentially modulate host TLR2 and TLR4 responses. *Microbes Infect* **13**, 151–159.
67 Bidula S, Kenawy H, Ali YM, Sexton D, Schwaeble WJ and Schelenz S (2013) Role of ficolin-A and lectin complement pathway in the innate defense against pathogenic *Aspergillus* species. *Infect Immun* **81**, 1730–1740.
68 Steger M, Bermejo-Jambrina M, Yordanov T, Wagener J, Brakhage AA, Pittl V, Huber LA, Haas H, Lass-Florl C, Posch W *et al.* (2018) beta-1,3-glucan-lacking *Aspergillus fumigatus* mediates an efficient antifungal immune response by activating complement and dendritic cells. *Virulence* **10**, 957–969.

69 Beauvais A, Fontaine T, Aimanianda V and Latge JP (2014) *Aspergillus* cell wall and biofilm. *Mycopathologia* **178**, 371–377.
70 Sheppard DC (2011) Molecular mechanism of *Aspergillus fumigatus* adherence to host constituents. *Curr Opin Microbiol* **14**, 375–379.
71 Robinet P, Baychelier F, Fontaine T, Picard C, Debre P, Vieillard V, Latge JP and Elbim C (2014) A polysaccharide virulence factor of a human fungal pathogen induces neutrophil apoptosis via NK cells. *J Immunol* **192**, 5332–5342.
72 Kouser L, Madhukaran SP, Shastri A, Saraon A, Ferluga J, Al-Mozaini M and Kishore U (2015) Emerging and novel functions of complement protein C1q. *Front Immunol* **6**, 317.
73 Csomor E, Bajtay Z, Sandor N, Kristof K, Arlaud GJ, Thiel S and Erdei A (2007) Complement protein C1q induces maturation of human dendritic cells. *Mol Immunol* **44**, 3389–3397.
74 Clemons KV, Martinez M, Tong AJ and Stevens DA (2010) Resistance of MBL gene-knockout mice to experimental systemic aspergillosis. *Immunol Lett* **128**, 105–107.
75 Hogaboam CM, Takahashi K, Ezekowitz RA, Kunkel SL and Schuh JM (2004) Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile. *J Leukoc Biol* **75**, 805–814.
76 Aykut B, Pushalkar S, Chen R, Li Q, Abengozar R, Kim JI, Shadaloey SA, Wu D, Preiss P, Verma N *et al.* (2019) The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature* **574**, 264–267.
77 Genster N, Praestekjaer Cramer E, Rosbjerg A, Pilely K, Cowland JB and Garred P (2016) Ficolins promote fungal clearance *in vivo* and modulate the inflammatory cytokine response in host defense against *Aspergillus fumigatus*. *J Innate Immun* **8**, 579–588.
78 Roy RM, Paes HC, Nanjappa SG, Sorkness R, Gasper D, Sterkel A, Wuthrich M and Klein BS (2013) Complement component 3C3 and C3a receptor are required in chitin-dependent allergic sensitization to *Aspergillus fumigatus* but dispensable in chitin-induced innate allergic inflammation. *mBio* **4**, e00162-13.
79 O'Dea EM, Amarsaikhan N, Li H, Downey J, Steele E, Van Dyken SJ, Locksley RM and Templeton SP (2014) Eosinophils are recruited in response to chitin exposure and enhance Th2-mediated immune pathology in *Aspergillus fumigatus* infection. *Infect Immun* **82**, 3199–3205.
80 Mulligan JK, Patel K, Williamson T, Reaves N, Carroll W, Stephenson SE, Gao P, Drake RR, Neely BA, Tomlinson S *et al.* (2018) C3a receptor antagonism as a novel therapeutic target for chronic rhinosinusitis. *Mucosal Immunol* **11**, 1375–1385.
81 Hector RF, Yee E and Collins MS (1990) Use of DBA/2N mice in models of systemic candidiasis and pulmonary and systemic aspergillosis. *Infect Immun* **58**, 1476–1478.
82 van Lookeren Campagne M, Wiesmann C and Brown EJ (2007) Macrophage complement receptors and pathogen clearance. *Cell Microbiol* **9**, 2095–2102.
83 Teschner D, Cholaszczynska A, Ries F, Beckert H, Theobald M, Grabbe S, Radsak M and Bros M (2019) CD11b regulates fungal outgrowth but not neutrophil recruitment in a mouse model of invasive pulmonary Aspergillosis. *Front Immunol* **10**, 123.
84 Kurtz CB, O'Toole E, Christensen SM and Weis JH (1990) The murine complement receptor gene family. IV. Alternative splicing of Cr2 gene transcripts predicts two distinct gene products that share homologous domains with both human CR2 and CR1. *J Immunol* **144**, 3581–3591.
85 Rambach G, Maier H, Vago G, Mohsenipour I, Lass-Florl C, Defant A, Wurzner R, Dierich MP and Speth C (2008) Complement induction and complement evasion in patients with cerebral aspergillosis. *Microbes Infect* **10**, 1567–1576.
86 Chai LY, Netea MG, Sugui J, Vonk AG, van de Sande WW, Warris A, Kwon-Chung KJ and Kullberg BJ (2010) *Aspergillus fumigatus* conidial melanin modulates host cytokine response. *Immunobiology* **215**, 915–920.
87 Tsai HF, Washburn RG, Chang YC and Kwon-Chung KJ (1997) *Aspergillus fumigatus* arp1 modulates conidial pigmentation and complement deposition. *Mol Microbiol* **26**, 175–183.
88 Tsai HF, Chang YC, Washburn RG, Wheeler MH and Kwon-Chung KJ (1998) The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. *J Bacteriol* **180**, 3031–3038.
89 Behnsen J, Hartmann A, Schmaler J, Gehrke A, Brakhage AA and Zipfel PF (2008) The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement system. *Infect Immun* **76**, 820–827.
90 Vogl G, Lesiak I, Jensen DB, Perkhofer S, Eck R, Speth C, Lass-Florl C, Zipfel PF, Blom AM, Dierich MP *et al.* (2008) Immune evasion by acquisition of complement inhibitors: the mould *Aspergillus* binds both factor H and C4b binding protein. *Mol Immunol* **45**, 1485–1493.
91 Dasari P, Shopova IA, Stroe M, Wartenberg D, Martin-Dahse H, Beyersdorf N, Hortschansky P, Dietrich S, Cseresnyes Z, Figge MT *et al.* (2018) Aspf2 from *Aspergillus fumigatus* recruits human immune

regulators for immune evasion and cell damage. *Front Immunol* **9**, 1635.
92 Washburn RG, DeHart DJ, Agwu DE, Bryant-Varela BJ and Julian NC (1990) *Aspergillus fumigatus* complement inhibitor: production, characterization, and purification by hydrophobic interaction and thin-layer chromatography. *Infect Immun* **58**, 3508–3515.
93 Washburn RG, Hammer CH and Bennett JE (1986) Inhibition of complement by culture supernatants of *Aspergillus fumigatus*. *J Infect Dis* **154**, 944–951.
94 Behnsen J, Lessing F, Schindler S, Wartenberg D, Jacobsen ID, Thoen M, Zipfel PF and Brakhage AA (2010) Secreted *Aspergillus fumigatus* protease Alp1 degrades human complement proteins C3, C4, and C5. *Infect Immun* **78**, 3585–3594.
95 Rambach G, Dum D, Mohsenipour I, Hagleitner M, Wurzner R, Lass-Florl C and Speth C (2010) Secretion of a fungal protease represents a complement evasion mechanism in cerebral aspergillosis. *Mol Immunol* **47**, 1438–1449.
96 Shende R, Wong SSW, Rapole S, Beau R, Ibrahim-Granet O, Monod M, Guhrs KH, Pal JK, Latge JP, Madan T *et al*. (2018) *Aspergillus fumigatus* conidial metalloprotease Mep1p cleaves host complement proteins. *J Biol Chem* **293**, 15538–15555.
97 Haapasalo K and Meri S (2019) Regulation of the complement system by pentraxins. *Front Immunol* **10**, 1750.
98 Garlanda C, Bottazzi B, Magrini E, Inforzato A and Mantovani A (2018) Ptx3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiol Rev* **98**, 623–639.
99 Thompson D, Pepys MB and Wood SP (1999) The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* **7**, 169–177.
100 Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, Blundell TL, Pepys MB and Wood SP (1994) Structure of pentameric human serum amyloid P component. *Nature* **367**, 338–345.
101 Tillet WS and T.j. Francis, (1930) Serological reactions in pneumonia with a non protein somatic fraction of pneumococcus. *J Exp Med* **52**, 561–585.
102 Thompson AR and Enfield DL (1978) Human plasma P component: isolation and characterization. *Biochemistry* **17**, 4304–4311.
103 Daigo K, Inforzato A, Barajon I, Garlanda C, Bottazzi B, Meri S and Mantovani A (2016) Pentraxins in the activation and regulation of innate immunity. *Immunol Rev* **274**, 202–217.
104 Pepys MB (2018) The Pentraxins 1975–2018: serendipity, diagnostics and drugs. *Front Immunol* **9**, 2382.
105 Szalai AJ (2002) The antimicrobial activity of C-reactive protein. *Microbes Infect* **4**, 201–205.
106 Chai L, Netea MG, Teerenstra S, Earnest A, Vonk AG, Schlamm HT, Herbrecht R, Troke PF and Kullberg BJ (2010) Early proinflammatory cytokines and C-reactive protein trends as predictors of outcome in invasive Aspergillosis. *J Infect Dis* **202**, 1454–1462.
107 Roques M, Chretien ML, Favennec C, Lafon I, Ferrant E, Legouge C, Plocque A, Golfier C, Duvillard L, Amoureux L *et al*. (2016) Evolution of procalcitonin, C-reactive protein and fibrinogen levels in neutropenic leukaemia patients with invasive pulmonary aspergillosis or mucormycosis. *Mycoses* **59**, 383–390.
108 Jensen TD, Schonheyder H, Andersen P and Stenderup A (1986) Binding of C-reactive protein to *Aspergillus fumigatus* fractions. *J Med Microbiol* **21**, 173–177.
109 Richardson MD, Gray CA and Shankland GS (1991) Opsonic effect of C-reactive protein on phagocytosis and intracellular killing of virulent and attenuated strains of *Candida albicans* by human neutrophils. *FEMS Microbiol Immunol* **3**, 341–344.
110 Garcia-Sherman MC, Lundberg T, Sobonya RE, Lipke PN and Klotz SA (2015) A unique biofilm in human deep mycoses: fungal amyloid is bound by host serum amyloid P component. *NPJ Biofilms Microbiomes* **1**, 15009.
111 Moreira AP, Cavassani KA, Hullinger R, Rosada RS, Fong DJ, Murray L, Hesson DP and Hogaboam CM (2010) Serum amyloid P attenuates M2 macrophage activation and protects against fungal spore-induced allergic airway disease. *J Allergy Clin Immunol* **126**, 712–721, e7.
112 Sjowall C, Wettero J, Bengtsson T, Askendal A, Almroth G, Skogh T and Tengvall P (2007) Solid-phase classical complement activation by C-reactive protein (CRP) is inhibited by fluid-phase CRP-C1q interaction. *Biochem Biophys Res Commun* **352**, 251–258.
113 Sorensen IJ, Nielsen EH, Andersen O, Danielsen B and Svehag SE (1996) Binding of complement proteins C1q and C4bp to serum amyloid P component (SAP) in solid contra liquid phase. *Scand J Immunol* **44**, 401–407.
114 Okemefuna AI, Nan R, Miller A, Gor J and Perkins SJ (2010) Complement factor H binds at two independent sites to C-reactive protein in acute phase concentrations. *J Biol Chem* **285**, 1053–1065.
115 Swinkels M, Zhang JH, Tilakaratna V, Black G, Perveen R, McHarg S, Inforzato A, Day AJ and Clark SJ (2018) C-reactive protein and pentraxin-3 binding of factor H-like protein 1 differs from complement factor H: implications for retinal inflammation. *Sci Rep* **8**, 1643.
116 Garcia de Frutos P and Dahlback B (1994) Interaction between serum amyloid P component and C4b-binding

protein associated with inhibition of factor I-mediated C4b degradation. *J Immunol* **152**, 2430–2437.
117 Zahedi K (1996) Characterization of the binding of serum amyloid P to type IV collagen. *J Biol Chem* **271**, 14897–14902.
118 de Beer FC, Baltz ML, Holford S, Feinstein A and Pepys MB (1981) Fibronectin and C4-binding protein are selectively bound by aggregated amyloid P component. *J Exp Med* **154**, 1134–1139.
119 Bouchara JP, Tronchin G and Chabasse D (1994) [Mechanisms and implications of the adhesion phenomenon in *Aspergillus fumigatus*]. *Pathol Biol* **42**, 640–646.
120 Escobar N, Ordonez SR, Wosten HA, Haas PJ, de Cock H and Haagsman HP (2016) Hide, keep quiet, and keep low: properties that make *Aspergillus fumigatus* a successful lung pathogen. *Front Microbiol* **7**, 438.
121 Mold C, Gresham HD and Du Clos TW (2001) Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs. *J Immunol* **166**, 1200–1205.
122 Doni A, Michela M, Bottazzi B, Peri G, Valentino S, Polentarutti N, Garlanda C and Mantovani A (2006) Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN-gamma. *J Leukoc Biol* **79**, 797–802.
123 Doni A, Mantovani G, Porta C, Tuckermann J, Reichardt HM, Kleiman A, Sironi M, Rubino L, Pasqualini F, Nebuloni M *et al.* (2008) Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and non-hematopoietic cells. *J Biol Chem* **283**, 29983–29992.
124 Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, Garlanda C, Romani L, Gascan H, Bellocchio S *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.
125 Inforzato A, Jaillon S, Moalli F, Barbati E, Bonavita E, Bottazzi B, Mantovani A and Garlanda C (2011) The long pentraxin PTX3 at the crossroads between innate immunity and tissue remodelling. *Tissue Antigens* **77**, 271–282.
126 Inforzato A, Reading PC, Barbati E, Bottazzi B, Garlanda C and Mantovani A (2013) The "sweet" side of a long pentraxin: how glycosylation affects PTX3 functions in innate immunity and inflammation. *Front Immunol* **3**, 407.
127 Inforzato A, Peri G, Doni A, Garlanda C, Mantovani A, Bastone A, Carpentieri A, Amoresano A, Pucci P, Roos A *et al.* (2006) Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. *Biochemistry* **45**, 11540–11551.
128 Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, Jokiranta TS, Mantovani A and Meri S (2008) Binding of the long pentraxin PTX3 to Factor H: Interacting domains and function in the regulation of complement activation. *J Immunology* **181**, 8433–8440.
129 Gout E, Moriscot C, Doni A, Dumestre-Perard C, Lacroix M, Perard J, Schoehn G, Mantovani A, Arlaud GJ and Thielens NM (2011) M-ficolin interacts with the long pentraxin PTX3: a novel case of cross-talk between soluble pattern-recognition molecules. *J Immunol* **186**, 5815–5822.
130 Inforzato A, Rivieccio V, Morreale AP, Bastone A, Salustri A, Scarchilli L, Verdoliva A, Vincenti S, Gallo G, Chiapparino C *et al.* (2008) Structural characterization of PTX3 disulfide bond network and its multimeric status in cumulus matrix organization. *J Biol Chem* **283**, 10147–10161.
131 Inforzato A, Baldock C, Jowitt TA, Holmes DF, Lindstedt R, Marcellini M, Rivieccio V, Briggs DC, Kadler KE, Verdoliva A *et al.* (2010) The angiogenic inhibitor long pentraxin PTX3 forms an asymmetric octamer with two binding sites for FGF2. *J Biol Chem* **285**, 17681–17692.
132 Porte R, Davoudian S, Asgari F, Parente R, Mantovani A, Garlanda C and Bottazzi B (2019) The Long Pentraxin PTX3 as a humoral innate immunity functional player and biomarker of infections and sepsis. *Front Immunol* **10**, 794.
133 Doni A, Stravalaci M, Inforzato A, Magrini E, Mantovani A, Garlanda C and Bottazzi B (2019) The long pentraxin PTX3 as a link between innate immunity, tissue remodeling, and cancer. *Front Immunol* **10**, 712.
134 Grcevic D, Sironi M, Valentino S, Deban L, Cvija H, Inforzato A, Kovacic N, Katavic V, Kelava T, Kalajzic I *et al.* (2018) The long pentraxin 3 plays a role in bone turnover and repair. *Front Immunol* **9**, 417.
135 D'Angelo C, De Luca A, Zelante T, Bonifazi P, Moretti S, Giovannini G, Iannitti RG, Zagarella S, Bozza S, Campo S *et al.* (2009) Exogenous pentraxin 3 restores antifungal resistance and restrains inflammation in murine chronic granulomatous disease. *J Immunol* **183**, 4609–4618.
136 Lo Giudice P, Campo S, Verdoliva A, Rivieccio V, Borsini F, De Santis R and Salvatori G (2010) Efficacy of PTX3 in a rat model of invasive aspergillosis. *Antimicrob Agents Chemother* **54**, 4513–4515.
137 Bozza S, Bistoni F, Gaziano R, Pitzurra L, Zelante T, Bonifazi P, Perruccio K, Bellocchio S, Neri M, Iorio AM *et al.* (2006) Pentraxin 3 protects from MCMV infection and reactivation through TLR sensing

pathways leading to IRF3 activation. *Blood* **108**, 3387–3396.

138 Moalli F, Paroni M, Veliz Rodriguez T, Riva F, Polentarutti N, Bottazzi B, Valentino S, Mantero S, Nebuloni M, Mantovani A *et al.* (2011) The therapeutic potential of the humoral pattern recognition molecule PTX3 in chronic lung infection caused by *Pseudomonas aeruginosa*. *J Immunol* **186**, 5425–5434.

139 Gaziano R, Bozza S, Bellocchio S, Perruccio K, Montagnoli C, Pitzurra L, Salvatori G, De Santis R, Carminati P, Mantovani A *et al.* (2004) Anti-*Aspergillus fumigatus* efficacy of pentraxin 3 alone and in combination with antifungals. *Antimicrob Agents Chemother* **48**, 4414–4421.

140 Lo Giudice P, Campo S, De Santis R and Salvatori G (2012) Effect of PTX3 and voriconazole combination in a rat model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* **56**, 6400–6402.

141 Marra E, Sousa VL, Gaziano R, Pacello ML, Arseni B, Aurisicchio L, De Santis R and Salvatori G (2014) Efficacy of PTX3 and posaconazole combination in a rat model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* **58**, 6284–6286.

142 Ma YJ, Doni A, Romani L, Jurgensen HJ, Behrendt N, Mantovani A and Garred P (2013) Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. *J Immunol* **191**, 1324–1333.

143 Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, Spreafico F, Pausa M, D'Ettorre C, Gianazza E *et al.* (1997) 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* **272**, 32817–32823.

144 Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, Gingras AR, Tzima S, Vivanco F, Egido J *et al.* (2003) Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol* **33**, 465–473.

145 Bally I, Inforzato A, Dalonneau F, Stravalaci M, Bottazzi B, Gaboriaud C and Thielens NM (2019) Interaction of C1q With pentraxin 3 and IgM revisited: mutational studies with recombinant C1q variants. *Front Immunol* **10**, 461.

146 Jongstra-Bilen J, Harrison R and Grinstein S (2003) Fcgamma-receptors induce Mac-1 (CD11b/CD18) mobilization and accumulation in the phagocytic cup for optimal phagocytosis. *J Biol Chem* **278**, 45720–45729.

147 Moalli F, Jaillon S, Inforzato A, Sironi M, Bottazzi B, Mantovani A and Garlanda C (2011) Pathogen recognition by the long pentraxin PTX3. *J Biomed Biotechnol* **2011**, 830421.

148 Bozza S, Campo S, Arseni B, Inforzato A, Ragnar L, Bottazzi B, Mantovani A, Moretti S, Oikonomous V, De Santis R *et al.* (2014) PTX3 binds MD-2 and promotes TRIF-dependent immune protection in aspergillosis. *J Immunol* **193**, 2340–2348.

149 Zhang J, Zhao G, Lin J, Che C, Li C, Jiang N, Hu L and Wang Q (2018) Role of PTX3 in corneal epithelial innate immunity against *Aspergillus fumigatus* infection. *Exp Eye Res* **167**, 152–162.

150 Shoham S and Levitz SM (2005) The immune response to fungal infections. *Br J Haematol* **129**, 569–582.

151 Tsui C, Kong EF and Jabra-Rizk MA (2016) Pathogenesis of *Candida albicans* biofilm. *Pathog Dis* **74**, ftw018.

152 Brown GD, Taylor PR, Reid DM, Willment JA, Williams DL, Martinez-Pomares L, Wong SY and Gordon S (2002) Dectin-1 is a major beta-glucan receptor on macrophages. *J Exp Med* **196**, 407–412.

153 Rubin-Bejerano I, Abeijon C, Magnelli P, Grisafi P and Fink GR (2007) Phagocytosis by human neutrophils is stimulated by a unique fungal cell wall component. *Cell Host Microbe* **2**, 55–67.

154 Li X, Utomo A, Cullere X, Choi MM, Milner DA Jr, Venkatesh D, Yun SH and Mayadas TN (2011) The beta-glucan receptor Dectin-1 activates the integrin Mac-1 in neutrophils via Vav protein signaling to promote *Candida albicans* clearance. *Cell Host Microbe* **10**, 603–615.

155 Ganesan S, Rathinam VAK, Bossaller L, Army K, Kaiser WJ, Mocarski ES, Dillon CP, Green DR, Mayadas TN, Levitz SM *et al.* (2014) Caspase-8 modulates dectin-1 and complement receptor 3-driven IL-1beta production in response to beta-glucans and the fungal pathogen, *Candida albicans*. *J Immunol* **193**, 2519–2530.

156 Boxx GM, Kozel TR, Nishiya CT and Zhang MX (2010) Influence of mannan and glucan on complement activation and C3 binding by *Candida albicans*. *Infect Immun* **78**, 1250–1259.

157 Ip WK and Lau YL (2004) Role of mannose-binding lectin in the innate defense against *Candida albicans*: enhancement of complement activation, but lack of opsonic function, in phagocytosis by human dendritic cells. *J Infect Dis* **190**, 632–640.

158 van Asbeck EC, Hoepelman AI, Scharringa J, Herpers BL and Verhoef J (2008) Mannose binding lectin plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells. *BMC Microbiol* **8**, 229.

159 Lillegard JB, Sim RB, Thorkildson P, Gates MA and Kozel TR (2006) Recognition of *Candida albicans* by mannan-binding lectin *in vitro* and *in vivo*. *J Infect Dis* **193**, 1589–1597.

160 Nedovic B, Posteraro B, Leoncini E, Ruggeri A, Amore R, Sanguinetti M, Ricciardi W and Boccia S

(2014) Mannose-binding lectin codon 54 gene polymorphism and vulvovaginal candidiasis: a systematic review and meta-analysis. *Biomed Res Int* **2014**, 738298.

161 Cunha C and Carvalho A (2019) Genetic defects in fungal recognition and susceptibility to invasive pulmonary aspergillosis. *Med Mycol* **57**, S211–S218.

162 Sprong T and van Deuren M (2008) Mannose-binding lectin: ancient molecule, interesting future. *Clin Infect Dis* **47**, 517–518.

163 Lambourne J, Agranoff D, Herbrecht R, Troke PF, Buchbinder A, Willis F, Letscher-Bru V, Agrawal S, Doffman S, Johnson E *et al.* (2009) Association of mannose-binding lectin deficiency with acute invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* **49**, 1486–1491.

164 Crosdale DJ, Poulton KV, Ollier WE, Thomson W and Denning DW (2001) Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. *J Infect Dis* **184**, 653–656.

165 Cho HJ, Jang MS, Hong SD, Chung SK, Kim HY and Dhong HJ (2015) Prognostic factors for survival in patients with acute invasive fungal rhinosinusitis. *Am J Rhinol Allergy* **29**, 48–53.

166 Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, Loffler J, Maertens JA, Bell AS, Inforzato A *et al.* (2014) Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. *N Engl J Med* **370**, 421–432.

167 Barbati E, Specchia C, Villella M, Rossi ML, Barlera S, Bottazzi B, Crociati L, d'Arienzo C, Fanelli R, Garlanda C *et al.* (2012) Influence of pentraxin 3 (PTX3) genetic variants on myocardial infarction risk and PTX3 plasma levels. *PLoS ONE* **7**, e53030.

168 Chiarini M, Sabelli C, Melotti P, Garlanda C, Savoldi G, Mazza C, Padoan R, Plebani A, Mantovani A, Notarangelo LD *et al.* (2010) PTX3 genetic variations affect the risk of *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients. *Genes Immun* **11**, 665–670.

169 Jaillon S, Moalli F, Ragnarsdottir B, Bonavita E, Puthia M, Riva F, Barbati E, Nebuloni M, Cvetko Krajinovic L, Markotic A *et al.* (2014) The humoral pattern recognition molecule PTX3 is a key component of innate immunity against urinary tract infection. *Immunity* **40**, 621–632.

170 Olesen R, Wejse C, Velez DR, Bisseye C, Sodemann M, Aaby P, Rabna P, Worwui A, Chapman H, Diatta M *et al.* (2007) DC-SIGN (CD209), Pentraxin 3 and Vitamin D Receptor gene variants associate with pulmonary tuberculosis risk in West-Africans. *Genes Immun* **8**, 456–467.

171 Rubino M, Kunderfranco P, Basso G, Greco CM, Pasqualini F, Serio S, Roncalli M, Laghi L, Mantovani A, Papait R *et al.* (2017) Epigenetic regulation of the extrinsic oncosuppressor PTX3 gene in inflammation and cancer. *Oncoimmunology* **6**, e1333215.

172 Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, Martin PJ, Warren EH, Boeckh M and Hansen JA (2017) Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood* **129**, 2693–2701.

173 Cunha C, Monteiro AA, Oliveira-Coelho A, Kuhne J, Rodrigues F, Sasaki SD, Schio SM, Camargo JJ, Mantovani A, Carvalho A *et al.* (2015) PTX3-based genetic testing for risk of aspergillosis after lung transplant. *Clin Infect Dis* **61**, 1893–1894.

174 Wojtowicz A, Lecompte TD, Bibert S, Manuel O, Rueger S, Berger C, Boggian K, Cusini A, Garzoni C, Hirsch H *et al.* (2015) PTX3 polymorphisms and invasive mold infections after solid organ transplant. *Clin Infect Dis* **61**, 619–622.

175 He Q, Li H, Rui Y, Liu L, He B, Shi Y and Su X (2018) Pentraxin 3 gene polymorphisms and pulmonary aspergillosis in chronic obstructive pulmonary disease patients. *Clin Infect Dis* **66**, 261–267.

176 Campos CF, Leite L, Pereira P, Vaz CP, Branca R, Campilho F, Freitas F, Ligeiro D, Marques A, Torrado E *et al.* (2019) PTX3 polymorphisms influence cytomegalovirus reactivation after stem-cell transplantation. *Front Immunol* **10**, 88.

177 Herrero-Sanchez MC, Angomas EB, de Ramon C, Telleria JJ, Corchete LA, Alonso S, Ramos MDC, Penarrubia MJ, Marquez S, Fernandez N *et al.* (2018) Polymorphisms in receptors involved in opsonic and nonopsonic phagocytosis, and correlation with risk of infection in oncohematology patients. *Infect Immun* **86**, e00709-18.

178 Brunel AS, Wojtowicz A, Lamoth F, Spertini O, Neofytos D, Calandra T, Marchetti O and Bochud PY (2018) Pentraxin-3 polymorphisms and invasive mold infections in acute leukemia patients with intensive chemotherapy. *Haematologica* **103**, e527–e530.

179 Goncalves SM, Lagrou K, Rodrigues CS, Campos CF, Bernal-Martinez L, Rodrigues F, Silvestre R, Alcazar-Fuoli L, Maertens JA, Cunha C *et al.* (2017) Evaluation of bronchoalveolar lavage fluid cytokines as biomarkers for invasive pulmonary aspergillosis in at-risk patients. *Front Microbiol* **8**, 2362.

180 Mastellos DC, Ricklin D and Lambris JD (2019) Clinical promise of next-generation complement therapeutics. *Nat Rev Drug Discov* **18**, 707–729.