The Serum Opsonin L-ficolin Is Detected in Lungs of Human Transplant Recipients Following Fungal Infections and Modulates Inflammation and Killing of Aspergillus fumigatus

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Background. Invasive aspergillosis (IA) is a life-threatening systemic fungal infection in immunocompromised individuals that is caused by Aspergillus fumigatus. The human serum opsonin, L-ficolin, has been observed to recognize A. fumigatus and could participate in fungal defense.

Methods. Using lung epithelial cells, primary human monocyte-derived macrophages (MDMs), and neutrophils from healthy donors, we assessed phagocytosis and killing of L-ficolin–opsonized live A. fumigatus conidia by flow cytometry and microscopy. Additionally, cytokines were measured by cytometric bead array, and L-ficolin was measured in bronchoalveolar lavage (BAL) fluid from lung transplant recipients by enzyme-linked immunosorbent assay.

Results. L-ficolin opsonization increased conidial uptake and enhanced killing of A. fumigatus by MDMs and neutrophils. Opsonization was also shown to manifest an increase in interleukin 8 release from A549 lung epithelial cells but decreased interleukin 1β, interleukin 6, interleukin 10, and tumor necrosis factor α release from MDMs and neutrophils 24 hours after infection. The concentration of L-ficolin in BAL fluid from patients with fungal infection was significantly higher than that for control subjects (P = .00087), and receiving operating characteristic curve analysis highlighted the diagnostic potential of L-ficolin for lung infection (area under the curve, 0.842; P < .0001).

Conclusions. L-ficolin modulates the immune response to A. fumigatus. Additionally, for the first time, L-ficolin has been demonstrated to be present in human lungs.

Keywords. L-ficolin; Aspergillus fumigatus; macrophage; neutrophil; epithelial; phagocytosis; cytokines; lung transplant.

Aspergillus fumigatus is a major pathogenic mold that is prevalent worldwide and the primary cause of invasive pulmonary aspergillosis (IA) in immunocompromised hosts [1]. In individuals at risk, such as persons with leukemia, solid-organ and hematopoietic transplant recipients, or people with neutropenia, IA is associated with a mortality rate of up to 30% among patients receiving treatment and 100% in untreated patients [2–6]. Infection is initiated following the inhalation of small hydrophobic conidia from the environment, which have the propensity to germinate into filamental (hyphal) structures. These invade local tissues, causing thrombosis, necrosis, and dissemination of the fungus to other organs, such as the skin and brain, ultimately leading to death [7–9].

Alveolar macrophages, neutrophils, complement, and pattern-recognition proteins, such as the ficolins...
and collectins, all work synergistically to remove *Aspergillus*. The process of phagocytosis by macrophages is an integral aspect in innate host defense against *A. fumigatus* conidia [10,11]. Neutrophils have also been observed to be important in the early stages of conidial removal, but they are essential in the destruction of the large hyphal structures following degranulation and in the production of neutrophil extracellular traps [12–14].

Ficolins are a family of proteins composed of an N-terminal collagen-like domain and a C-terminal fibrinogen-like domain with lectin activity (highly specific for N-acetylglicosamine). Human serum L-ficolin has the potential to enhance phagocytosis via direct binding to pathogens [15], but the protective roles of ficolins in *Aspergillus* defense are still poorly characterized.

We have recently demonstrated that L-ficolin is able to enhance the binding of *Aspergillus* conidia to the lung epithelium, but little is known about the functional consequences following ficolin opsonization [16]. We therefore used L-ficolin to investigate its roles in phagocytosis and killing of *A. fumigatus* by phagocytes, in addition to its role in modulating cytokine production. This study is also the first to show that L-ficolin is present in bronchoalveolar lavage (BAL) fluid from lung transplant patients with fungal pneumonia, compared with uninfected controls. Additionally, we highlight the potential of L-ficolin as a tool for the diagnosis of fungal infections following lung transplantation.

**MATERIALS AND METHODS**

**Patients and Ethical Approval**

Evidence of fungal infection was based on clinical European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [17]. BAL fluid sampling of lung transplant recipients from the Royal Brompton Hospital and the Harefield National Health Service (NHS) Trust was performed with ethical approval from an affiliated biomedical research unit (RBH/AS1).

Ethical approval for blood donation by healthy participants was obtained from the Faculty of Health Research Ethics Committee (Ref. Mechanisms of airway diseases – 2008042). Blood was acquired through venipuncture of healthy participants, who gave informed consent at the time of collection. No donors were receiving medication at the time of collection.

Informed consent was obtained from patients, and human experimentation guidelines of the US Department of Health and Human Services were adhered to in the conduct of clinical research.

**Cells and Reagents**

All experiments were conducted using the A549 adenocarcinomic human alveolar basal epithelial cell line, human monocyte-derived macrophages (MDMs), or peripheral blood neutrophils. MDMs and neutrophils were isolated from healthy donor blood specimens via a 68% Percoll gradient, using a modified version of the methods described Walsh et al [18]. Monocytes were selected on the basis of adherence to tissue culture plastic ware for 1 hour and were differentiated in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% autologous serum, 50 U mL⁻¹ penicillin, and 50 µg mL⁻¹ streptomycin over 5–9 days. A549 cells and neutrophils were briefly maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 I. U mL⁻¹ penicillin, and 50 µg mL⁻¹ streptomycin. Polymorphonuclear preparations containing >90% neutrophils and exhibiting >98% viability (as determined by trypan blue staining) were placed in culture. All experiments were performed in serum-free conditions. Recombinant L-ficolin was purchased from R&D Systems. Fluorescein isothiocyanate (FITC) was purchased from Sigma-Aldrich. A clinical *A. fumigatus* strain isolated from a respiratory specimen was used in all experiments and maintained/harvested as previously described [16].

**Detection of Infection and L-ficolin in BAL Fluid**

BAL fluid was collected from lung transplant recipients at the Royal Brompton Hospital and the Harefield NHS Trust by instilling 200 mL of sterile saline into distal airway segments under flexible bronchoscopy. Recovered BAL fluid was centrifuged at 425g for 10 minutes. *Aspergillus* antigens, which are indicative of IA, were detected via a lateral-flow device as previously described [19] and/or via detection of galactomannan by using a Platelia *Aspergillus* antigen kit (Bio-Rad). For BAL samples, an index of <0.5 was considered negative for galactomannan, and an index of ≥0.5 was considered positive for galactomannan [20]. Samples were tested for a panel of respiratory viruses by use of a multiplex polymerase chain reaction and for bacteria by means of culture (BS7, UK Standards for Microbiology Investigations) [21]. Images yielded by high-resolution computed tomography of the chest were reviewed for evidence of findings consistent with fungal infection [21]. The presence of L-ficolin in BAL fluid from lung transplant recipients was detected using a ficolin-2 human ELISA kit (Hyclut). Patients were categorized as having possible, probable, or proven invasive fungal infection according to revised EORTC/MSG criteria [17].

**Phagocytosis Assays**

FITC-labeled live *A. fumigatus* conidia were opsonized with 5 µg mL⁻¹ L-ficolin as previously described [16]. MDMs or human neutrophils were seeded in 24-well plates (Nunc) before challenge with 5 × 10⁵ ficolin-opsonized FITC-labeled *A. fumigatus* conidia (conidia to cell ratio, 5:1) for 2 hours at 37°C. Adherent cells were subsequently removed by the use of trypsin/ethylenediaminetetraacetic acid, gentle trituration, and scraping. Neutrophils in suspension were pelleted at 300 g for 5 minutes. Cells were fixed in 4% phosphate-buffered saline.
(PBS)/formaldehyde for 10 minutes at room temperature before resuspension in PBS. Phagocytosis was analyzed by flow cytometry (excitation λ, 488 nm; emission λ, 533/30 nm) on a BD Accuri C6 flow cytometer with BD CFlow Software (BD Biosciences) collecting 5000 events. To yield quantitative counts, phagocytic cells that had ingested conidia were identified and are expressed as a percentage of all phagocytes present, and the relative abundance of conidia contained within the phagocytic cells was determined by the relative fluorescence intensity (FL1-A) of these cells.

Cytokine Concentration Determination
Cytokine protein concentrations in the supernatants of A. fumigatus–challenged A549 cells, MDMs, and neutrophils were determined using a BD cytometric bead array Human Inflammatory Cytokines kit (BD Biosciences). Data were obtained by flow cytometry (excitation λ, 488 nm; emission λ, 585/40 nm) on a BD Accuri C6 flow cytometer with BD CFlow Software, collecting 1800 events as outlined in the protocol.

Statistical Analysis
Results were expressed as mean ± SD. Descriptive and 2-tailed Student t tests were performed using GraphPad Prism software (version 5). One-way analyses of variance were performed using SigmaStat software (version 3.5). A P value of < .05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was conducted using MedCalc (version 13.1.1).

RESULTS
L-ficolin Opsonization Enhances Phagocytosis and Killing of A. fumigatus by Human MDMs
We and others have previously acknowledged that L-ficolin is capable of binding to A. fumigatus [16, 22]. Here, we verify again that L-ficolin can recognize live A. fumigatus conidia (P = 2.7 × 10⁻⁵; Figure 1A), and we demonstrate enhanced binding in an acidic pH of 5.7 (P = .00089; Figure 1B).

Figure 1. L-ficolin binding to live Aspergillus fumigatus conidia. A total of 5 × 10⁵ live A. fumigatus conidia were opsonized with 5 µg mL⁻¹ L-ficolin in the presence or absence of Ca²⁺ and at a range of pH's (3.7–10.7) before staining with mouse monoclonal anti-human L-ficolin primary antibodies and anti-mouse-FITC conjugated secondary antibodies and flow cytometric analysis. A, Binding of L-ficolin to A. fumigatus in the presence of absence of Ca²⁺. Bovine serum albumin (BSA) was used as a negative control for binding. B, Binding of L-ficolin to A. fumigatus in pH 3.7–10.7 conditions. Results are representative of the average of all data points gained from 3 independent experiments. Error bars represent the SD, and significance was determined via a 2-tailed Student t test. *P < .05. Abbreviations: FITC, fluorescein isothiocyanate; MFI, median fluorescence intensity; +, present; −, absent.
We have shown that the phagocytosis of conidia by the airway epithelial cell line A549 is enhanced following L-ficolin opsonization [16]. Another integral cell type involved in the early defense against *Aspergillus* conidia is the macrophage.

Initially, the ability of L-ficolin to enhance phagocytosis was investigated using FITC-labeled *A. fumigatus* conidia opsonized with L-ficolin before incubation with adherent human MDMs for 2 hours. MDMs were gated (Figure 2A), and the percentage of FITC-negative and FITC-positive MDMs were used to

![Image](https://via.placeholder.com/150)

**Figure 2.** Phagocytosis and fungal viability following incubation of L-ficolin-opsonized *Aspergillus fumigatus* conidia with monocyte-derived macrophages (MDMs). A total of $5 \times 10^5$ fluorescein isothiocyanate (FITC)-labeled or live freshly harvested *A. fumigatus* conidia were opsonized with 5 µg mL$^{-1}$ L-ficolin before incubation with MDMs (conidia to MDM ratio, 5:1) at pH 5.7 or 7.4 for 2 or 24 hours for phagocytosis and viability assays, respectively. A, Gate P1 on MDMs used for the analyses in panels B–F. Some points were removed for clarity. B and C, Representative flow cytometry data depicting the percentage of phagocytosing MDMs in the absence of FITC-labeled *A. fumigatus* conidia (Q1; B) or in the presence of FITC-labeled *A. fumigatus* conidia (Q1; C). D, The percentage of MDMs phagocytosing conidia at pH’s of 5.7 or 7.4 in the presence or absence of L-ficolin. E, Representative histogram depicting the uptake of conidia at pH 5.7 in the presence or absence of L-ficolin. F, The relative number of phagocytosed FITC-labeled conidia (based on the median fluorescence intensity [MFI]) either unopsonized or opsonized by L-ficolin.
Figure 2 continued. G and H, Hyphal germination following incubation of unopsonized conidia at pH 5.7 (G) and pH 7.4 (H). The black arrows point to macrophages containing conidia, which make macrophages appear dark. The white arrowheads trace single hyphae from MDMs. Many hyphae are visible, some of which are blurry because they are growing in 3 dimensions and are out of the focal plane. I and J, Hyphal germination following incubation of L-ficolin–opsonized conidia at pH 5.7 (I) and pH 7.4 (J). Hyphae are present, although growth is much less dense. K, Gate P2 on MDMs and *A. fumigatus* used for the analyses in panels L and M. L, Representative histogram depicting the killing of conidia at pH 5.7 in the presence or absence of L-ficolin. Increased relative fluorescence intensity (FL1-A) depicts enhanced killing. M, Viability of unopsonized or L-ficolin–opsonized conidia after incubation with MDMs. Results are representative of the average of all data points gained from 3 independent experiments. Error bars represent the SD, and significance was determined via a 2-tailed Student t test. *P* < .05. Abbreviations: AF, *A. fumigatus*; FSC-A, forward scatter; SSC-A, side scatter.
identify phagocytic cells (Figure 2B and 2C). The proportion of phagocytic MDMs was unaffected in physiological conditions (pH 7.4) or acidic conditions (pH 5.7; Figure 2D). However, the number of FITC-labeled L-ficolin–opsonized conidia ingested per MDM (based on the median fluorescence intensity of phagocytic MDMs) was significantly enhanced in inflammatory conditions (pH 5.7) but not at pH 7.4 \((P = 6.6 \times 10^{-5};\) Figure 2E and 2F).

Additionally, light microscopy demonstrated that MDMs inhibited conidial germination following opsonization by L-ficolin in inflammatory conditions (Figure 2G–J). L-ficolin in the absence of phagocytes had no effect on A. fumigatus growth (data not shown). Moreover, following gating (Figure 2K), fungal viability assays demonstrated a significant increase in fungal killing following opsonization by L-ficolin in these conditions, as quantitated by flow cytometry \((P = 0.00249;\) Figure 2L and 2M). When the ingested A. fumigatus and free A. fumigatus populations were gated separately, the death-associated green-yellow fluorescence emitted by A. fumigatus within MDMs was significantly greater than that for unassociated fungi, highlighting potent intracellular killing (Supplementary Figure 1).

**L-ficolin Opsonization Enhances Phagocytosis and Killing of A. fumigatus by Human Neutrophils**

Neutropenia poses a significant risk factor for developing aspergillosis, which led us to investigate the importance of neutrophils in the recognition and removal of A. fumigatus conidia following ficolin opsonization.

The association of L-ficolin–opsonized conidia with human neutrophils was investigated as per MDM protocols. In this case, neutrophils were gated (Figure 3A), and the percentage of FITC-negative and FITC-positive neutrophils were used to identify phagocytic cells (Figure 3B and 3C). Again, the percentage of cells phagocytosing was unaffected in physiological conditions (pH 7.4) or acidic conditions (pH 5.7; Figure 3D). However, as for the macrophages, flow cytometry indicated a significant increase in the number of conidia phagocytosed per neutrophil following L-ficolin opsonization, but only at pH 5.7 \((P = 0.01056;\) Figure 3E and 3F).

Light microscopy demonstrated that, in the absence of L-ficolin opsonization at pH 5.7 and 7.4 or L-ficolin opsonization at pH 7.4, hyphal growth appeared very dense (Figure 3G, 3H, and 3J). Following opsonization by L-ficolin at pH 5.7, hyphal growth appeared significantly less dense, and clumping was observed (Figure 3I). Following gating (Figure 3K), the viability assays demonstrated a significant decrease in fungal viability following opsonization by L-ficolin in these conditions \((P = 0.04324;\) Figure 3L and 3M). As for the MDMs, the death-associated green-yellow fluorescence emitted by A. fumigatus within neutrophils was significantly greater than that for unassociated fungi (Supplementary Figure 2A). Conversely, the fluorescence of the free A. fumigatus in the presence of neutrophils was significantly greater than that for A. fumigatus in the absence of neutrophils, suggesting augmentation of extracellular killing mechanisms (Supplementary Figure 2B).

**L-ficolin Opsonization Modulates the Secretion of Inflammatory Cytokines in Response to A. fumigatus**

We used cytometric bead arrays to investigate the concentration of interleukin 8 (IL-8), IL-1β, interleukin 6 (IL-6), interleukin 10 (IL-10), and TNF-α secreted from A549 type II alveolar cells, MDMs, and human neutrophils following challenge by L-ficolin–opsonized A. fumigatus conidia. IL-8 was the only cytokine significantly modulated in A549 cells in response to L-ficolin (Supplementary Figure 3). L-ficolin opsonization induced a significant increase in the secretion of proinflammatory IL-8, compared with challenge with unopsonized conidia after 8 hours and 24 hours (Supplementary Figure 3). L-ficolin in the absence of conidia induced a significant spike in IL-8 secretion at 8 hours, which was maintained up to 24 hours (Supplementary Figure 3).

L-ficolin opsonization also modulated cytokine secretion from MDMs. Following MDM challenge with conidia opsonized by L-ficolin, an antiinflammatory effect was observed. The secretion of IL-8, IL-1β, IL-6, IL-10, and TNF-α from MDMs 24 hours after infection were decreased (Figure 4A–E). Again, L-ficolin alone appeared capable of significantly increasing the cytokine concentrations of all tested (Figure 4A–E).

Additionally, L-ficolin opsonization led to significantly decreased secretion of IL-8, IL-1β, IL-6, and TNF-α from neutrophils, compared with unopsonized conidia (Figure 5A–E). We observed that IL-10 was only secreted at baseline levels regardless of any challenges (Figure 5D). L-ficolin was also observed to have the ability to increase the secretion of IL-8, IL-1β, and TNF-α in the absence of A. fumigatus (Figure 5A, 5B, and 5E).

**L-ficolin Is Present in the BAL Fluid of Lung Transplant Recipients With Fungal Pneumonia**

Based on our recent observations [16], it was important to investigate whether L-ficolin was detectable in the lungs of patients with invasive fungal infections, particularly as L-ficolins have never formally been described to be present in lungs. Here, we used an L-ficolin–specific ELISA to detect the presence of L-ficolin in BAL fluid samples from lung transplant recipients. In patients with a diagnosis of probable or proven invasive pulmonary fungal infection, based on EORTC/MSG criteria, and/or the presence of fungal biomarkers (galactomannan and Aspergillus antigens [by lateral-flow analysis]), L-ficolin was detected at significantly higher concentrations \((P = 0.00087;\) Figure 6A), compared with uninfected control patients. L-ficolin was only detected once in the BAL samples that tested negative.
for fungal growth or fungal radiology features, albeit at a very low concentration (Figure 6A). A ROC curve analysis was conducted to investigate whether the detection of L-ficolin could be used as a potential biomarker/diagnostic tool for fungal infection in the lung. The area under the curve was calculated to be 0.842, suggesting an 84.2% chance that fungus-infected

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**Figure 3.** Phagocytosis and fungal viability following incubation of ficolin-opsonized *Aspergillus fumigatus* conidia with human neutrophils. A total of $5 \times 10^5$ fluorescein isothiocyanate (FITC)-labeled or live freshly harvested *A. fumigatus* conidia were opsonized with 5 µg mL$^{-1}$ L-ficolin before incubation with neutrophils (conidia to neutrophil ratio, 5:1) at pH 5.7 or 7.4 for 2 or 24 hours for phagocytosis and viability assays, respectively. A, Gate P1 on neutrophils used for the analyses in panels B–F. B and C, Representative flow data depicting the percentage of neutrophils phagocytosing in the absence of FITC-labeled *A. fumigatus* conidia (Q1; B) or in the presence of FITC-labeled *A. fumigatus* conidia (Q1; C). D, The percentage of neutrophils phagocytosing conidia at pH 5.7 or 7.4 in the presence or absence of L-ficolin. E, Representative histogram depicting the uptake of conidia at pH 5.7 in the presence or absence of L-ficolin. F, The relative number of phagocytosed FITC-labeled conidia (based on the median fluorescence intensity [MFI]) either unopsonized or opsonized by L-ficolin. G and H, Hyphal germination following incubation of unopsonized conidia at pH 5.7 (G) or 7.4 (H). I and J, Hyphal germination following incubation of L-ficolin–opsonized conidia at pH 5.7 (I) or 7.4 (J). K, Gate P2 on neutrophils and *A. fumigatus* used for the analyses in panels L and M. L, Representative histogram depicting the killing of conidia at pH 5.7 in the presence or absence of L-ficolin. Increased relative fluorescence intensity (FL1-A) depicts enhanced killing. M, Viability of unopsonized conidia or L-ficolin–opsonized conidia after incubation with neutrophils. Results are representative of the average of all the data points gained from 3 independent experiments. Error bars represent the SD, and significance was determined via a 2-tailed Student t test. *P<.05. Abbreviations: AF, *A. fumigatus*; FSC-A, forward scatter; SSC-A, side scatter.
Figure 3 continued.
transplant recipients would have L-ficolin present in their BAL fluid ($P < .0001$; Figure 6B).

**DISCUSSION**

Our study focused on the functional consequences of L-ficolin opsonization of *A. fumigatus*, particularly its effect on *Aspergillus*-phagocyte interactions. To translate our in vitro findings to clinical infections, we also investigated whether L-ficolin is present in human lungs during fungal pneumonia. As a result, a number of new observations have been made. First, L-ficolin opsonization led to enhanced uptake of *A. fumigatus* conidia by MDMs and neutrophils under inflammatory conditions. Second, this opsonization led to enhanced inhibition of hyphal formation and an increase in *A. fumigatus* killing by MDMs and neutrophils. Third, opsonization of *A. fumigatus* conidia by L-ficolin evoked an antiinflammatory cytokine response from MDMs and neutrophils. Finally, we have provided the first evidence that L-ficolin is present in the BAL fluid of lung transplant recipients with a diagnosis of fungal infection, which could potentially be used as a diagnostic tool for fungal infection in a clinical setting.

Initially, we showed that L-ficolin bound to *A. fumigatus* at low pH (5.7), which was similar to ficolin-A [16]. The ability of such pattern-recognition molecules to function at decreased pH is important in the defense against microorganisms, with pH at the local site of infection being observed to drop as low as 5.5 during inflammation [23].

Another key participant during infection-induced inflammation is the macrophage, which is the most prominent phagocyte in the lung in the early stages of *A. fumigatus* infection [24]. We have demonstrated here that L-ficolin enhances conidial uptake by primary MDMs from healthy donors. Opsonophagocytosis was enhanced at an inflammatory pH, which is also optimal for ficolin binding.

The other essential phagocyte in the defense against *Aspergillus* is the neutrophil, which is known to prevent fungal growth, although the mechanism has not been fully elucidated [25]. We
have shown here that L-ficolin enhances neutrophil function by increasing conidial uptake following opsonization.

Our observations add to the knowledge of previous reports on L-ficolin enhancing the opsonophagocytosis not only of bacteria such as Salmonella typhimurium and Streptococcus agalactiae, but also of fungi [26, 27]. It is likely that ficolins work together with other pattern-recognition molecules (SP-A, SP-D, and mannos-binding lectin [MBL]) and receptors (dectin-1 and Toll-like receptor 2), which have also been observed to bind A. fumigatus conidia and enhance phagocytic uptake [11, 16, 28–32]. Although binding and phagocytosis is important, ultimately, killing of the fungi is crucial to sterilize infected tissues.

Macrophages are usually able to kill conidia in their acidic phagolysosomes [33], but if conidia escape this process and germinate into hyphae, they become too large to be phagocytosed. Neutrophils are then recruited to the site of infection (in response to IL-8), where they assist the inhibition of fungal invasion by degranulating and by producing fungistatic neutrophil extracellular traps following adherence to the hyphal cell wall [14, 24, 34].

We observed that L-ficolin opsonization potentiated the ability of macrophages and neutrophils to significantly enhance fungal killing. Macrophages appeared more capable than neutrophils of inhibiting germination of conidia. Gating separately on the neutrophil/MDM populations containing A. fumigatus conidia (Neut and AF), and neutrophils with L-ficolin–opsonized A. fumigatus conidia (Neut, AF, and L-ficolin). Results are representative of the average of all the data points gained from 3 independent experiments. Error bars represent the SD. Significance was determined via 1-way analysis of variance, and pair-wise comparisons were conducted using the Student-Newman–Keuls method. *P<.05. Abbreviation: AF, A. fumigatus.

Figure 5. Inflammatory cytokine release from neutrophils following challenge by unopsonized or L-ficolin–opsonized conidia. Supernatants were collected 8 and 24 hours after challenge with 5 × 10⁵ live Aspergillus fumigatus conidia that were either unopsonized or L-ficolin–opsonized (5 µg mL⁻¹) before the conduction of cytometric bead arrays. Concentrations of interleukin 8 (IL-8; A), interleukin 1β (IL-1β; B), interleukin 6 (IL-6; C), interleukin 10 (IL-10; D), and tumor necrosis factor α (TNF-α; E) secreted by neutrophils alone (Neut), neutrophils with L-ficolin (Neut and L-ficolin), neutrophils with unopsonized A. fumigatus conidia (Neut and AF), and neutrophils with L-ficolin–opsonized A. fumigatus conidia (Neut, AF, and L-ficolin). Results are representative of the average of all the data points gained from 3 independent experiments. Error bars represent the SD. Significance was determined via 1-way analysis of variance, and pair-wise comparisons were conducted using the Student-Newman–Keuls method. *P<.05. Abbreviation: AF, A. fumigatus.
Aspergillus infections, further emphasizing that humoral pattern-recognition molecules play an important role in the defense against fungi [32, 36]. However, we are currently investigating the role of L-ficolin in the in vivo defense against aspergillosis.

As indicated earlier, we have previously observed that ficolin-A opsonization leads to an increase in IL-8 secretion from A549 cells, a cytokine that is crucial for the recruitment of neutrophils during Aspergillus infection. It is known that, in response to A. fumigatus, a plethora of cytokines are secreted from various host cells, including interleukin 2, interleukin 5, IL-6, IL-8, interleukin 13, interleukin 17A, interleukin 22, interferon γ, TNF-α, granulocyte macrophage colony-stimulating factor, and monocyte chemotactic protein 1 [24, 37–42].

In the present study, we found that L-ficolin–opsonized conidia were also capable of inducing an increase in IL-8, as previously observed for ficolin-A–opsonized conidia [16]. In contrast, opsonization of A. fumigatus by L-ficolin led to a significant decrease in IL-8, IL-1β, IL-6, IL-10, and TNF-α production by MDMs and neutrophils. In support of our observations, it was recently observed that ficolin-A could act in an antiinflammatory manner by binding to lipopolysaccharide (LPS) and inhibiting LPS-mediated proinflammatory responses on murine mast cells [43]. Additionally, the pattern-recognition proteins SP-A and SP-D modulate an antiinflammatory cytokine profile in response to viruses, LPS-induced cytokine and nitric oxide production, and allergens [44–46].

Our work represents the first observations that unbound ficolins may have the potential to increase cytokine secretion. The mechanisms of this interaction are still not fully understood, but they most likely depend on the orientation of ficolin binding. Interestingly, both SP-A and SP-D have been observed to function in both an antiinflammatory and proinflammatory manner, depending upon the interaction of their globular heads with SIRPα or their collagenous tails with calreticulin/CD91, respectively [47]. Some data suggest that L-ficolin binds to calreticulin, but there has been no demonstrable binding to SIRPα [48]. Another caveat to be aware of is that, in its native state, L-ficolin normally exists as quiescent polymers, but the recombinant form used in this study was in a depolymerized state and may not be completely representative of normal in vivo function. This is an area of research that is currently ongoing in our laboratory.

The most important clinical observation of our study was the detection of the serum L-ficolin in BAL fluid from the lungs of patients with a diagnosis invasive A. fumigatus infection. Moreover, L-ficolin could also be detected in the lungs of recipients infected with Aspergillus flavus, Penicillium species, Acremonium species, and Scedosporium apiospermum and at very low concentration in 1 case of S. aureus infection. This ficolin has, until now, not been reported to be present in the lung. We postulate that L-ficolin, which is normally produced by the liver, enters the alveolar space during infection from the bloodstream, similar to the related acute phase protein MBL (a serum collectin), which has also been found in BAL fluid from infected lungs [49]. Although the current sample size is small (39 patients), ROC analysis has indicated that the presence of L-ficolin in the lungs of transplant recipients could be linked with fungal infection, but this diagnostic potential will need to be further investigated in larger clinical trials.

In conclusion, L-ficolin is present in fungus-infected lungs of transplant recipients and has immunomodulatory properties that highlight an important role in the innate defense against Aspergillus through enhancing opsonophagocytosis by macrophages and neutrophils, increasing fungal killing, and manifesting an antiinflammatory cytokine profile after infection. Future
research will be concerned with understanding the signaling pathways involved in immune defense and with using ficolin-deficient transgenic animal models to elucidate the function of ficolins in the defense against Aspergillus in vivo.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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