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Role of Ficolin-A and Lectin Complement Pathway in the Innate Defense against Pathogenic *Aspergillus* Species

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***Aspergillus* species are saprophytic molds causing life-threatening invasive fungal infections in the immunocompromised host. Innate immune recognition, in particular, the mechanisms of opsonization and complement activation, has been reported to be an integral part of the defense against fungi. We have shown that the complement component ficolin-A significantly binds to *Aspergillus* conidia and hyphae in a concentration-dependent manner and was inhibited by *N*-acetylglucosamine and *N*-acetylgalactosamine. Calcium-independent binding to *Aspergillus fumigatus* and *A. terreus* was observed, but binding to *A. flavus* and *A. niger* was calcium dependent. Ficolin-A binding to conidia was increased under low-pH conditions, and opsonization led to enhanced binding of conidia to A549 airway epithelial cells. In investigations of the lectin pathway of complement activation, ficolin-A-opsonized conidia did not lead to lectin pathway-specific C4 deposition. In contrast, the collectin mannose binding lectin C (MBL-C) but not MBL-A led to efficient lectin pathway activation on *A. fumigatus* in the absence of ficolin-A. In addition, ficolin-A opsonization led to a modulation of the proinflammatory cytokine interleukin-8. We conclude that ficolin-A may play an important role in the innate defense against *Aspergillus* by opsonizing conidia, immobilizing this fungus through enhanced adherence to epithelial cells and modulation of inflammation. However, it appears that other immune pattern recognition molecules, i.e., those of the collectin MBL-C, are involved in the *Aspergillus*-lectin complement pathway activation rather than ficolin-A.**

Aspergillus species are ubiquitous saprophytic molds causing life-threatening invasive fungal infections (invasive aspergillosis) in the immunocompromised host. Among the human-pathogenic species, the most common causative agent of human infections is *Aspergillus fumigatus*, followed by *A. flavus*, *A. terreus*, and *A. niger* (1). Patients at risk include leukemia, transplant, and neutropenic patients, and the infection-associated mortality rate has been described to be as high as 90% (2–5). The ubiquitous fungi are present in soil or decaying matter, with primary infection mainly caused via the production of small conidia that are inhaled by the host (6, 7).

Upon inhalation, conidia are immediately challenged by the host's innate immune system, comprising lung epithelial cells, alveolar macrophages, complement, and collectins (CLs) such as mannose binding lectin (MBL). In particular, the processes of phagocytosis and complement activation play a key role in the removal of inhaled conidia by MBL via the association with MBL-associated serine protease-2 (MASP-2) and subsequent activation of the lectin complement pathway (8, 9). MBL, however, is not at all the only pathogen recognition molecule capable of driving the lectin activation pathway of complement; CL-11, another collectin with close structural similarity to MBL, and ficolins, a family of proteins composed of both collagen-like long thin stretches and fibrinogen-like globular domains with lectin activity (usually specific for *N*-acetylglucosamine [GlcNAc]), also associate with MASPs and activate the lectin complement pathway (10). Human plasma contains three different ficolins called Ficolin-1 (FCN-1), or M-ficolin, Ficolin-2 (FCN-2), or L-ficolin, and Ficolin-3 (FCN-3), or H-ficolin, while mouse plasma contains only an orthologue of human FCN-2, i.e., ficolin-A (9, 10). The protective roles of ficolins in the defense against clinically relevant fungi, however, have not been well characterized to date.

Previously, it has been observed that complement can be activated on the conidial and hyphal surface of *Aspergillus*, resulting in opsonization by the classical complement compound iC3b and enhanced uptake by phagocytic leukocytes (8, 11). However, the role of ficolins as opsonins, and in the activation of the lectin pathway of complement, in the innate defense against *Aspergillus* is poorly understood. Therefore, we have utilized the rat orthologue of human serum L-ficolin, ficolin-A, and investigated its ability to function as an opsonin and modulator of the immune defense against *Aspergillus*.

MATERIALS AND METHODS

Fungal pathogens. Isolates of *A. fumigatus*, *A. niger*, *A. terreus*, and *A. flavus* were obtained from clinical specimens and stored in sterile water at 4°C. Resting conidia were obtained after *Aspergillus* spp. were subcultured on Sabouraud dextrose agar at 37°C for 7 days, and conidia were harvested using sterile physiological saline (Oxoid, United Kingdom). Resting live conidia were used immediately or fixed in 4% phosphate-buffered saline (PBS)–formaldehyde for 10 min at room temperature (RT), washed, and resuspended in PBS. Fixed *Aspergillus* spp. were stored at 4°C for up to 1 month until further use.

For hyphal experiments, conidia were grown in 10 ml RPMI 1640 media containing 2.05 mM L-glutamine (Gibco, United Kingdom) sup-

Received 11 January 2013 Returned for modification 29 January 2013
Accepted 1 March 2013
Published ahead of print 11 March 2013
Editor: G. S. Deepe, Jr.
Address correspondence to Silke Schelenz, sschelenz@doctors.org.uk.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/IAI.00032-13.

doi:10.1128/IAI.00032-13

plemented with 10% (vol/vol) heat-inactivated fetal calf serum (FCS; PAA Laboratories Ltd., United Kingdom) and a mix of 50 U ml^{-1} penicillin and 50 $\mu g\ ml^{-1}$ streptomycin (Gibco) at 37°C for 24 h. Following growth, hyphae were either sonicated to produce fragments or used unfragmented (pending on experiment) prior to two washes in PBS supplemented with 0.9 mM $CaCl_2$ and 0.1% (wt/vol) bovine serum albumin (BSA) before resuspension in the same buffer. Unless stated otherwise, 5×10^5 *Aspergillus* conidia/hyphal fragments were used for each experiment.

Ficolin-A and L-ficolin. Rat recombinant ficolin-A was produced from CHO cells and purified by affinity chromatography on *N*-acetylglucosamine (GlcNAc)-Sepharose columns as previously described (12). Ficolin-A protein was eluted with 50 mM Tris-HCl (pH 7.5) containing 150 mM NaCl and 300 mM GlcNAc (Sigma-Aldrich, United Kingdom). Finally, GlcNAc was removed by dialysis and ficolin-A presence confirmed by SDS-PAGE.

Recombinant L-ficolin and polyclonal mouse anti-L-ficolin antibodies were a kind gift from T. Hummelshøj (Rigshospitatalet, Department of Clinical Immunology, Copenhagen, Denmark).

Determination of ficolin binding to *Aspergillus* spp. Freshly harvested live *Aspergillus* conidia, formalin/PBS-fixed resting conidia, live swollen conidia, or live sonicated hyphae were washed twice in ficolin binding buffer (FBB) (5.0 mM $CaCl_2$, 155.0 mM NaCl, 25.0 mM HEPES, 0.5% BSA, 0.1% Triton X-100). The fungi were then incubated with 5 $\mu g\ ml^{-1}$ of ficolin-A (or L-ficolin) for 1 h at 37°C. Fungi were washed as described above before prior to staining of the *Aspergillus*-ficolin A/L-ficolin complex with polyclonal rabbit anti-ficolin-A antibodies (kind gift of R. Wallis, University of Leicester) at a dilution of 1:1,000 or polyclonal mouse anti-L-ficolin antibodies (1:200 dilution) for 30 min at 4°C. Following washing, the secondary antibody (goat anti-rabbit Ig-fluorescein isothiocyanate [FITC] or goat anti-mouse Ig-FITC; Beckman Coulter, United Kingdom) at a 1:200 dilution was incubated with the complex for 30 min at room temperature (RT). Following washing, the *Aspergillus*-ficolin complexes were fixed with 4% PBS–formaldehyde for 10 min at RT, washed, and resuspended in sterile PBS.

Concentration-dependent binding of ficolin-A was assessed by using increasing concentrations (0.1, 1, 10, and 100 $\mu g\ ml^{-1}$) of ficolin-A. Calcium-dependent binding was assessed in the presence of 5 mM EGTA. For testing the influence of the pH on ficolin-A binding, the pH of FBB was adjusted from pH 3.7 to 10.7.

The carbohydrate-recognition characteristics of ficolin-A were determined using a ligand inhibition assay prior to *Aspergillus* binding. Concentrations (0.75, 3.1, 12.5, 25, 50, 100 mM) of various carbohydrates (glucose, GlcNAc, galactose, and *N*-acetylgalactosamine [GalNAc]) and mannan (0.75 to 100 $\mu g\ ml^{-1}$) were incubated with 5 $\mu g\ ml^{-1}$ of ficolin-A for 2 h at RT. Following the ligand inhibition, the ficolin/sugar complex was then added to the *Aspergillus* spp. and analyzed for binding as described above.

Ficolin-A binding to conidia or hyphae was analyzed by flow cytometry (laser excitation, 488 nm; emission detection, 533/30 nm) using a BD Accuri C6 flow cytometer with BD CFlow software (BD Accuri Cytometers).

Immunofluorescence microscopy. Unsonicated hyphae were washed once in 1 ml FBB prior to centrifugation at 775 × *g* and resuspension in FBB containing 5 $\mu g\ ml^{-1}$ ficolin-A or FBB alone. Following 1 h of incubation at 37°C, the hypha–ficolin-A complexes were stained with 100 μl of polyclonal rabbit anti-ficolin-A at a dilution of 1:1,000 and incubated for 30 min at 4°C. The organisms were washed twice again as described above prior to the addition of secondary anti-rabbit Ig-FITC (Beckman Coulter, United Kingdom) at a dilution of 1:500 for 30 min at RT. Following further washes, organisms were fixed for 10 min in 4% PBS–formaldehyde before they were mounted on microscope slides using Fluoromount-G (Southern Biotech) and images analyzed using AxioVision 4.8 software (Zeiss, Germany).

Epithelium cell interaction. Human A549 type II alveolar adenocarcinoma cells were seeded on 96-well plates (Nunc, United Kingdom) in supplemented RPMI 1640 and grown to semiconfluence at 37°C in a 5% CO_2 atmosphere. FITC-labeled conidia (FL1-A) of *A. fumigatus* were opsonized with 5 $\mu g\ ml^{-1}$ BSA–ficolin-A for 1 h at 37°C. Ficolin-A-opsonized *A. fumigatus* conidia (1×10^5) were incubated for 16 h with adherent A549 cells (ratio of conidia to cells of 15:1) at pH 5.7 or pH 7.4 at 37°C in a 5% CO_2 atmosphere. Following incubation, nonadherent conidia and A549 cells were removed and the adherent cells were washed with warm supplemented RPMI 1640. Adherent cells were subsequently removed by the use of trypsin-EDTA and gentle trituration. Cells were fixed in 4% PBS–formaldehyde for 10 min at RT and analyzed by flow cytometry using a BD Accuri C6 flow cytometer with BD CFlow software. The percentage of A549 cells associating with *A. fumigatus* conidia was determined by gating on the A549 cell population (forward scatter channel/side scatter channel [FSC/SSC]) and calculating the percentage of A549 cells staining positive for fluorescence, indicating association with FL1-A.

Murine sera. Ficolin-A-deficient mice were generated by targeting *Fcna* with a conventional replacement vector as previously described (13) and utilized as previously described (14). MBL-null mice were purchased from MMRRC, Bar Harbor, ME. Blood was collected from these animals via cardiac puncture prior to serum preparation and storage at −80°C.

Solid-phase binding assays. Maxisorb microtiter 96-well plates (Nunc) were coated with *A. fumigatus* conidial suspensions (optical density at 550 nm [OD_{550}] of 0.5) or 10 μg/well zymosan (Sigma-Aldrich) in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6). The plates were then incubated overnight at 4°C prior to blocking residual protein binding sites with 1% (wt/vol) BSA–Tris-buffered saline (TBS) for 2 h at RT. Plates were then washed three times with wash buffer (TBS, 0.05% Tween 20, 5 mM $CaCl_2$).

To detect binding of MBL-A and -C, serial dilutions of wild-type serum were added to the plates and incubated for 90 min at RT. Plates were washed as described above prior to staining with monoclonal rat anti-mouse MBL-A (Hycult, United Kingdom) or rat anti-mouse MBL-C (Hycult). Bound antibody was then detected using the colorimetric substrate p-nitrophenylphosphate (pNPP; Sigma-Aldrich).

Complement activation assays. Maxisorb microtiter 96-well plates were coated with *A. fumigatus* conidial suspensions (OD_{550} of 0.5), 10 μg/well mannan (Sigma-Aldrich), and *N*-acetylated bovine serum albumin (N-BSA; Promega, United Kingdom) or zymosan in coating buffer. The plates were then incubated, blocked, and washed as described above for the solid-phase binding assays.

To quantify C3 deposition, wild-type or ficolin-deficient mouse serum was diluted in barbital-buffered saline (BBS; 4 mM barbital [Sigma-Aldrich], 145 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$), added to antigen coated wells, and incubated overnight at 4°C. Following a washing step, wells were stained with the primary antibody, polyclonal rabbit anti-human C3c complement antibody (Dako, United Kingdom), at a dilution of 1:5,000 and incubated for 1 h at 37°C. Following washes, the secondary antibody, goat anti-rabbit IgG-alkaline phosphatase conjugate (Sigma-Aldrich), was added at a dilution of 1:10,000 and incubated for 1.5 h at RT. The assay was developed using pNPP.

To quantify C4 deposition, MBL-A- and -C-deficient serum diluted in MBL binding buffer (20 mM Tris-HCl, 10 mM $CaCl_2$, 1 M NaCl, pH 7.4) was added to antigen-coated wells and incubated overnight at 4°C. Following washing, 1 μg/well of human C4 in BBS (pH 7.4) was added and incubated for 1.5 h at 37°C. Following washing, the primary antibody, chicken anti-human C4c antibody (Agrisera, United Kingdom), was added at a dilution of 1:2,000 and the reaction mixture incubated for a further 1.5 h at 37°C. The plates were washed again prior to the addition of the secondary antibody (anti-chicken IgY-alkaline phosphatase conjugate; Sigma-Aldrich) in a dilution of 1:10,000 and incubated for 1 h at RT. The assay was developed using pNPP. N-BSA was used as a positive control for C4 deposition by ficolins.

Interleukin-8 (IL-8) ELISA. Live resting *A. fumigatus* conidia were opsonized with ficolin-A (5 $\mu g\ ml^{-1}$) in FBB at pH 5.7 under optimal

binding conditions before semiconfluent A549 cells were challenged with unopsonized or ficolin-A-opsonized conidia (ratio of conidia to A549 cells, 15:1). Conidia were allowed 2 h for adherence/phagocytosis at 37°C in a 5% CO_2 atmosphere before excess unbound conidia were removed and A549 cells washed and reincubated in warm supplemented RPMI 1640 medium. Supernatants from the interaction between A549 cells and associated conidia were collected after the 8-h and 24-h time points, spun down, and stored at −80°C. Supernatants were assayed for the presence of IL-8 using the human IL-8 enzyme-linked immunosorbent assay (ELISA) Ready-SET-Go! kit protocol (eBioscience, United Kingdom) per the manufacturer's instructions. In brief, Corning Costar 9018 ELISA plates (Corning, United Kingdom) were coated with 100 μl/well of capture antibody in 1× ELISA coating buffer (1:250) before the plate was sealed and incubated overnight at 4°C. Wells were then aspirated and washed 5 times with 250 μl/well wash buffer (1× PBS, 0.05% Tween 20), allowing a 1 min soaking time per wash. Wells were then blocked with 200 μl/well 1× assay diluent at RT for 1 h. Wells were washed as described above prior to the addition of 100 μl/well of the IL-8 standards and sample supernatants before incubation at RT for 2 h. Wells were washed as described before prior to the addition of 100 μl/well detection antibody diluted in 1× assay diluent (1:250) and incubation at RT for 1 h. Following washes as described above, 100 μl/well of avidin-horseradish peroxidase (HRP) conjugate diluted in 1× assay diluent (1:250) was added and the wells were incubated at RT for 30 min. Washes were conducted as described above prior to the addition of 1× 3,3′,5,5′-tetramethylbenzidine (TMB) substrate for 15 min at RT. Following development, 50 μl/well of 2N H_2SO_4 was added to stop any further color development before the absorbance at 450 nm was read in a mini-plate reader (Dynatech, United Kingdom) and IL-8 concentrations were analyzed based on the standard curve.

Statistical analysis. Data were expressed as means ± standard deviations (SD). Descriptive and 2-tailed *t*-test analyses were performed using GraphPad prism software (version 5). One-way analyses of variance (ANOVA) were performed using SigmaStat software (version 3.5). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of ficolin binding to *Aspergillus* species. Resting conidia from four species of *Aspergillus* (*A. fumigatus*, *A flavus*, *A. terreus*, and *A. niger*) were incubated with purified rat recombinant ficolin-A, and the bound ficolin-*Aspergillus* complex was detected using rabbit anti-ficolin-A antibody followed by a goat anti-rabbit Ig-FITC-conjugated antibody. Flow cytometry analysis demonstrated a significant shift in the fluorescence for conidia from all fungal isolates, indicating that 74.0%, 94.2%, 60.4%, 78.2%, and 70.4% of *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and *A. fumigatus*, respectively, stained positive for ficolin-A binding (Fig. 1A to D). Ficolin-A binding occurred to formalin-fixed as well as live conidia, with a significant preference for the latter ($P = 0.00128$; see Fig. S1 in the supplemental material). Interestingly, the human recombinant L-ficolin also bound significantly to live or fixed *A. fumigatus* conidia but with no difference in intensity ($P = 0.6719$; see Fig. S2 in the supplemental material).

The binding of ficolin-A to conidia of all *Aspergillus* spp. tested was observed to be concentration dependent (Fig. 2). Binding was detected at low ficolin-A concentrations (0.1 μg ml^{-1}) and increased for all species in a dose-dependent manner, where *A. flavus* conidia showed binding with ficolin-A that was significantly higher than that seen with the other three *Aspergillus* species. Ficolin-A binding to *Aspergillus* conidia demonstrated saturated binding at 100 μg ml^{-1} ficolin-A for all *Aspergillus* strains, and significant binding was observed to take place at 0.1 μg ml^{-1} of ficolin-A ($P = <0.001$; Fig. 2).

The absence of calcium had no statistically significant effect on

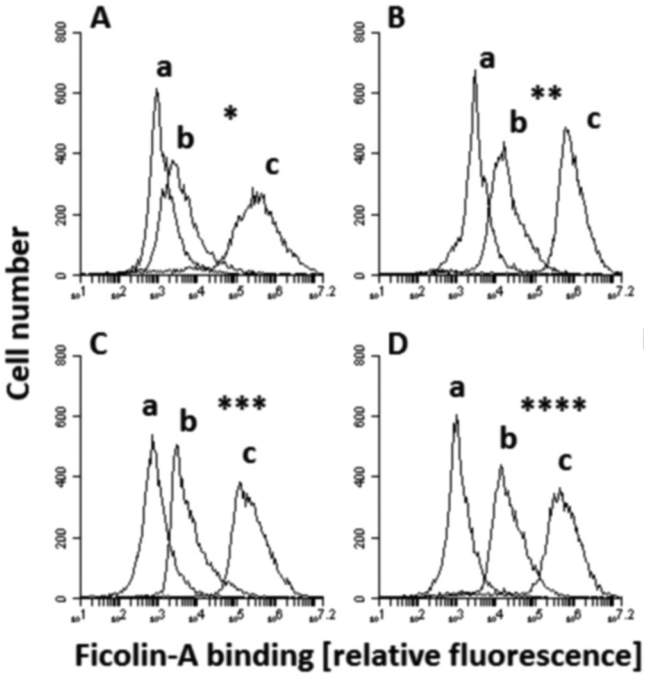

FIG 1 Flow cytometry analysis of ficolin-A binding to conidia of *Aspergillus* spp. Ficolin-A (5 μg ml^{-1}) was incubated with 5×10^5 conidia from clinical isolates of four *Aspergillus* species. Peaks a represent unstained *Aspergillus* controls. Peaks b represent *Aspergillus* spp. that were stained with polyclonal rabbit anti-ficolin-A antibodies followed by secondary goat anti-rabbit Ig-FITC-labeled antibodies. Peaks c represent ficolin-opsonized *Aspergillus* spp. that were stained with the primary and secondary antibodies. (A) *A. fumigatus*. (B) *A. flavus*. (C) *A. terreus*. (D) *A. niger*. The data show results representative of 10 experiments. *P* values are representative of the differences between stained unopsonized conidia and stained ficolin-A-opsonized conidia. Significance was determined via paired Student *t* tests (*, $P = 0.00101$; **, $P = 0.0002$; ***, $P = 0.01471$; ****, $P = 8.32 \times 10^{-7}$).

binding of ficolin-A to either *A. fumigatus* or *A. terreus*, suggesting a calcium-independent interaction (*A. fumigatus* ficolin-A binding median fluorescence intensity of 159,056 ± 31,831 versus 110,586 ± 48,835 for *A. fumigatus* binding in EGTA [$P = 0.223$]; *A. terreus* ficolin-A binding median fluorescence intensity of 173,478 ± 72,745 versus 86,059 ± 42,430 for *A. terreus* binding in EGTA [$P = 0.147$]) (Fig. 3). However, the absence of calcium had a statistically significant effect on binding of ficolin-A to conidia of *A. flavus* and *A. niger*, suggesting a calcium-dependent interaction with these species (*A. flavus* ficolin-A binding median fluorescence intensity of 436,288 ± 57,335 versus 301,354 ± 33,800 for *A. flavus* binding in EGTA [$P = 0.025$]; *A. niger* ficolin-A median fluorescence intensity of 363,004 ± 12,104 versus 202,911 ± 31,078.6 for *A. niger* binding in EGTA [$P = 0.001$]) (Fig. 3).

To assess the specificity of the carbohydrate binding of the ficolin-A fibrinogen-like (FBG) domain with the carbohydrate-rich cell wall of *Aspergillus* spp., ficolin-A was preincubated with a range of carbohydrate ligands at concentrations from 0.75 mM to 100 mM prior to binding to the fungus. The acetylated carbohydrates GlcNAc and GalNAc demonstrated concentration-dependent inhibition of ficolin-A binding to conidia from all species of *Aspergillus*, with significant inhibition of ficolin-A binding occurring at an inhibitor concentration of 12.5 mM for *A. fumigatus* and *A. flavus* ($P = 0.029$ and $P = <0.001$, respectively) and at an

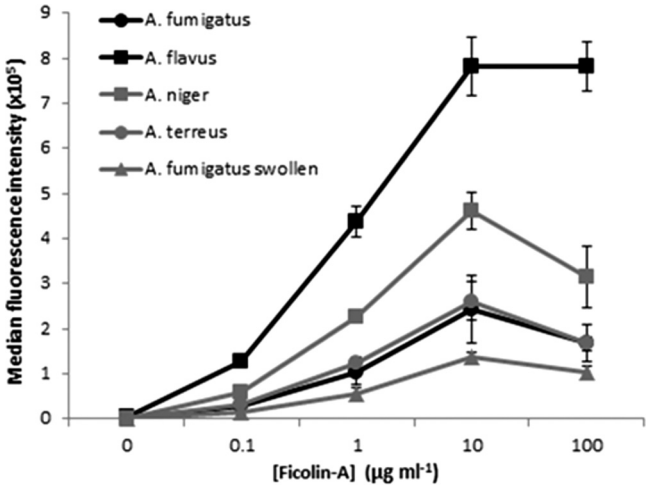

FIG 2 Concentration-dependent binding of ficolin-A to *Aspergillus* spp. Conidia of *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and swollen *A. fumigatus* were incubated with various concentrations (0.1, 1, 10, and 100 μg ml^{-1}) of ficolin-A. Unstained *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and swollen *A. fumigatus* negative controls had median fluorescence intensities of 0.1×10^4, 0.3×10^4, 0.1×10^4, 0.8×10^3, and 0.4×10^3, respectively. All results represent 5×10^5 conidia stained with rabbit anti-ficolin-A- and goat anti-rabbit Ig-FITC-labeled antibodies and are representative of the averages of all the data points gained from three independent experiments. Error bars represent the SD. Significance was determined via one-way ANOVA and was observed at 0.1 μg ml^{-1} (*, $P = <0.001$).

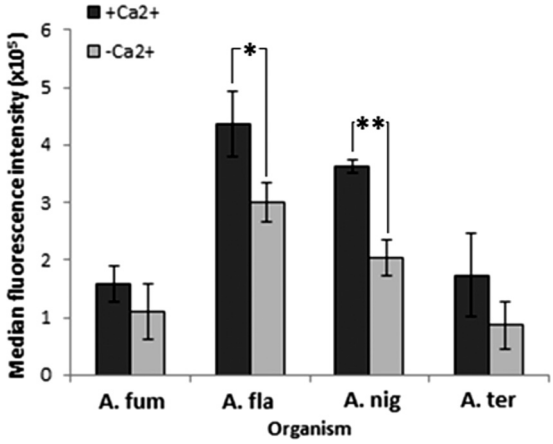

FIG 3 Calcium binding dependency of ficolin-A for *Aspergillus* spp. Conidia of *A. fumigatus* (A. fum), *A. flavus* (A. fla), *A. niger* (A. nig), and *A. terreus* (A. ter) were incubated with 5 μg ml^{-1} ficolin-A in the presence (+Ca^{2+}) or absence (-Ca^{2+}) of calcium and with the addition of 5 mM EGTA. All results represent 5×10^5 conidia stained with rabbit anti-ficolin-A- and goat anti-rabbit Ig-FITC-labeled antibodies and are representative of the averages of all the data points gained from three independent experiments. Error bars represent the SD. Significance was determined via paired Student *t* tests (*, $P = 0.025$; **, $P = 0.001$).

inhibitor concentration of 3.15 mM for *A. terreus* and *A. niger* ($P = 0.009$ and $P = 0.010$, respectively). Ficolin-A binding to *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger* conidia was maximally inhibited at a carbohydrate concentration of 100 mM, with inhibition at 92.3 ± 4.7%, 85.3 ± 2.3%, 91.9 ± 2.6%, and 86.8 ± 3.4%, respectively, for GlcNAc and 93 ± 1.2%, 85.6 ± 1.7%, 95.2 ± 0.6%, and 88.4% ± 1%, respectively, for GalNAc (Fig. 4A to D).

Glucose and galactose demonstrated inhibition that was on average 5-fold lower than that seen with their N-acetylated-amine counterparts (11.6 ± 10.1% and 26.6 ± 5.6%, respectively, for *A. fumigatus*, 34.2 ± 5.5% and 7.6 ± 2.1%, respectively, for *A. flavus*, 18.4 ± 6.6% and 21.1 ± 5.6%, respectively, for *A. terreus*, and 8 ± 7.8% and 19.4 ± 7.9%, respectively, for *A. niger* at a carbohydrate concentration of 100 mM) (Fig. 4A to D). Similar results were observed for the control, mannan, inhibiting ficolin binding by 25.4 ± 12.5%, 4.1 ± 6.8%, 17.9 ± 13.9%, and 12.2 ± 21.2% for *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*, respectively, at a high concentration of 100 μg ml^{-1} (Fig. 4E).

As ficolins are likely to interact with pathogens at the local site of infection, where the pH often becomes more acidic due to increased inflammation, binding was analyzed at different pHs. The binding of ficolin-A to *A. fumigatus* was observed to increase significantly by almost 23-fold at an acidic pH of 5.7 compared to binding under physiological conditions (pH 7.4) (median fluorescence intensity of 136,175.8 ± 18,236 versus 5,963.3 ± 1,171.6, respectively; $P = 0.00025$) (Fig. 5).

Binding of ficolin-A to hyphae of *A. fumigatus*. Flow cytometry analysis showed significant binding of ficolin-A to *A. fumigatus* hyphae (Fig. 6A and B; $P = 0.00592$). This binding was confirmed by immunofluorescence microscopy, whereby a high intensity of fluorescence was observed within the hyphal mass and on individual hyphae following opsonization with ficolin-A in comparison to unopsonized hyphae (see Fig. S3 in the supplemental material). The binding detected by flow cytometry analysis was calcium independent (median binding fluorescence intensity of 5,138.3 ± 832.8 versus 5,828.7 ± 724.6 for binding in EGTA [$P = 0.3397$]) but was unaffected by a change in the pH level (median fluorescence intensity of ficolin-A binding of 5,138.3 ± 832.8 at pH 5.7 versus median fluorescence intensity of 6,042.5 ± 387 at pH 7.4 [$P = 0.16331$]) (Fig. 6B).

Following preincubation with 100 mM glucose, galactose, GlcNAc, or GalNAc or 100 μg ml^{-1} mannan prior to ficolin-A–hypha binding, GlcNAc and GalNAc demonstrated the greatest inhibition of ficolin-A binding to hyphae, with inhibition at 52.2 ± 6.8% and 53.4 ± 4.1% for GlcNAc and GalNAc, respectively, which correlates with reduced ficolin binding (Fig. 6C). Nonacetylated glucose and galactose demonstrated inhibition that was significantly lower than that seen with their N-acetylated-amine counterparts (30.8 ± 6.8% and 32.8 ± 6.8% for glucose and galactose, respectively; $P = 0.01842$ and $P = 0.01068$ compared to their acetylated counterparts) (Fig. 6C). Similar inhibition results were observed for mannan, inhibiting ficolin binding by 39% ± 11% (Fig. 6C).

Adherence of ficolin-A-opsonized *A. fumigatus* to A549 lung epithelial cells. Primary infection with *Aspergillus* spp. is mainly due to the inhalation of small hydrophobic conidia; therefore, the lung epithelial cells play an important role in initiating host defenses. We have investigated whether ficolin-A plays a modulatory role in the interaction with *A. fumigatus* conidia by human A549 type II pneumocytes.

FITC-labeled *A. fumigatus* conidia (either preopsonized with 5 μg ml^{-1} ficolin-A or not preopsonized) were incubated with adherent A549 type II pneumocytes. Following 16 h of incubation at pH 5.7 or 7.4, the majority of A549 cells were associated with unopsonized *A. fumigatus* (93.7% or 96.8%, respectively) and ficolin-A-opsonized *A. fumigatus* (93.3% or 94.2%, respectively)

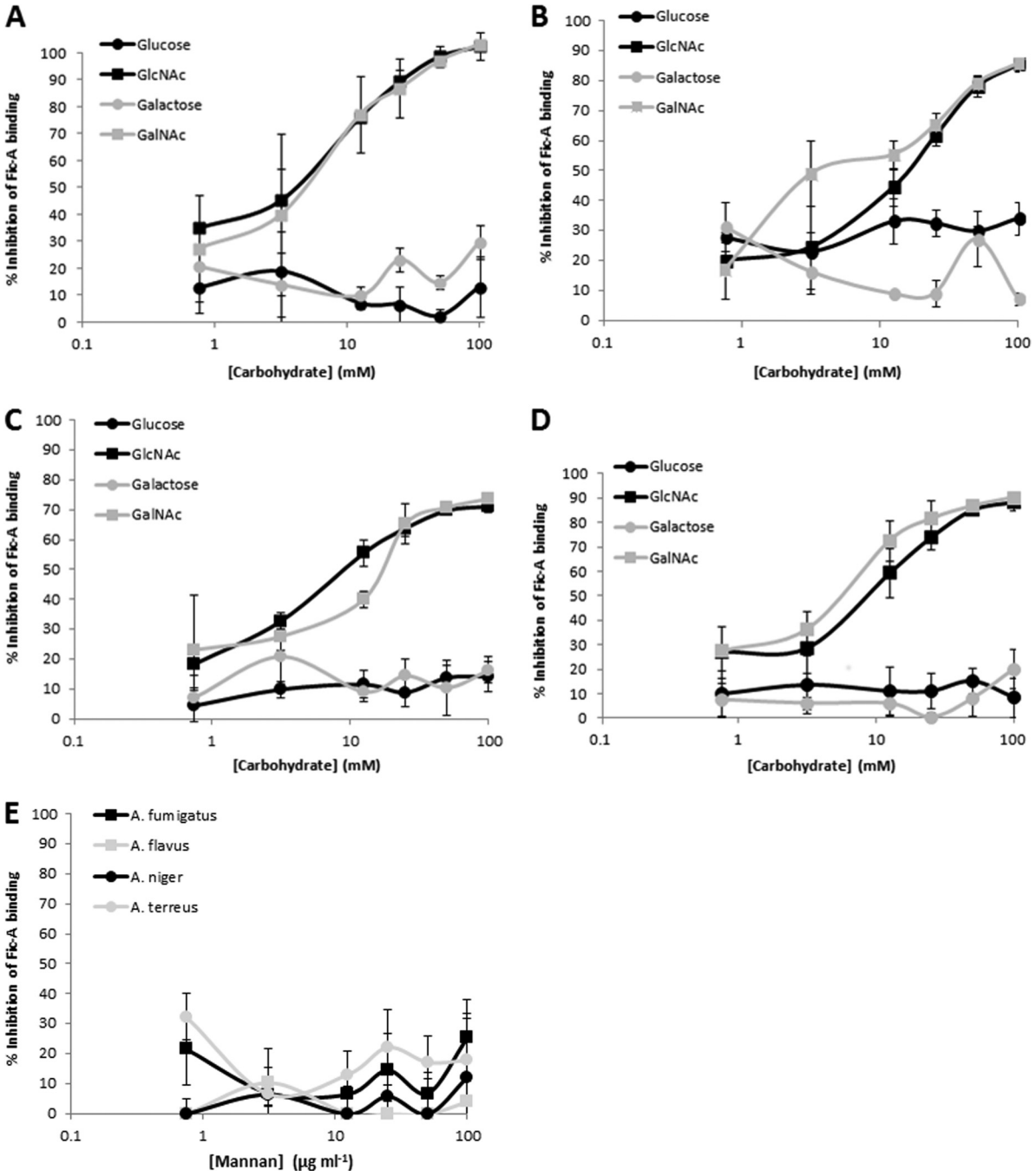

FIG 4 Carbohydrate specificity of ficolin-A (Fic-A) binding. Conidia of *Aspergillus* spp. were incubated with 5 μg ml^{-1} ficolin-A preincubated with different carbohydrates or mannan at various concentrations. (A) *A. fumigatus*. (B) *A. flavus*. (C) *A. terreus*. (D) *A. niger*. (E) All *Aspergillus* spp. and mannan. All results represent 5×10^5 conidia stained with rabbit anti-ficolin-A- and goat anti-rabbit Ig-FITC-labeled antibodies and are representative of the averages of all the data points gained from three independent experiments. Error bars represent the SD. Significance was determined via one-way ANOVA. Significant inhibition was observed following the use of 12.5 mM inhibitor for *A. fumigatus* and *A. flavus*, and significant inhibition was observed following the use of 3.15 mM inhibitor for *A. terreus* and *A. niger* ($P = <0.05$).

(Fig. 7A). At physiological pH (7.4), there was no significant difference between the numbers of opsonized or unopsonized *A. fumigatus* conidia associating per A549 cell ($P = 0.470$; Fig. 7B). However, following ficolin-A opsonization, the number of *Aspergillus* conidia associated per A549 cell (based on the median fluorescence intensity) at pH 5.7 was significantly higher than that seen with the unopsonized controls ($P = 0.014$; Fig. 7B). A control experiment using BSA-preincubated *A. fumigatus* conidia also did not significantly increase the association of conidia with A549 cells ($P = 0.861$; Fig. 7B). Additional experiments in which *A. fumigatus* was opsonized with 5 μg ml^{-1} L-ficolin prior to incubation with A549 cells under acidic conditions

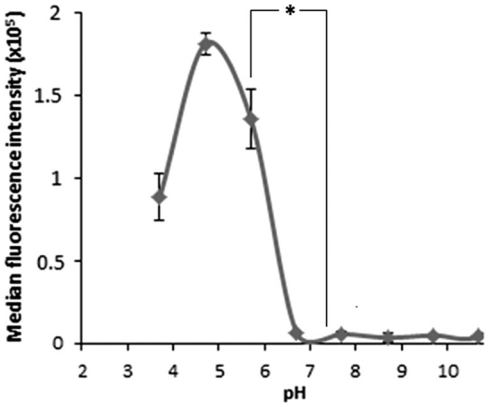

FIG 5 pH-dependent binding of ficolin-A. *A. fumigatus* conidia were incubated with 5 μg ml^{-1} ficolin-A at different pH values (3.7 to 10.7). All results represent 5 × 10^5 conidia stained with rabbit anti-ficolin-A- and goat anti-rabbit Ig-FITC-labeled antibodies and are representative of the averages of all the data points gained from three independent experiments. Error bars represent the SD. Significance was determined via paired Student *t* tests (*, P = 0.00025).

yielded the same observations as with ficolin-A. The number of A549 cells associating with conidia was not significantly increased by L-ficolin opsonization in comparison to unopsonized conidia (P = 0.07328; see Fig. S4 in the supplemental material). However, the number of L-ficolin-opsonized conidia associating per A549 was again (as with ficolin-A) significantly increased in comparison to the numbers of both unopsonized and BSA-opsonized conidia (P = 0.00013 and P = 0.00028, respectively; see Fig. S4 in the supplemental material).

***A. fumigatus* conidia do not induce the lectin complement pathway activation via ficolin-A.** The lectin pathway is known to be activated by complement recognition molecules such as MBLs and ficolins. We therefore firstly confirmed the ability of MBL to bind to *A. fumigatus*. Our data have shown that it is MBL-C which binds *A. fumigatus* significantly and not MBL-A (Fig. 8A; P = 0.015).

Following this observation, we studied the ability of ficolin-A and MBL bound to *Aspergillus* conidia to activate the lectin complement pathway, initially using a C3 deposition assay. The C3 deposition on microbial cell surface is a key indicator of the extent of complement activation. We therefore coated microtiter plate wells with *A. fumigatus* conidia and assessed C3c deposition in an ELISA using both wild-type and ficolin-A-deficient (MBL-A- and -C-sufficient) murine sera. C3 deposition was observed in all cases regardless of pH or the presence or absence of ficolin-A and occurred significantly at the lowest concentration of sera used (P = 0.03).

To further define the role of ficolins in the lectin pathway of complement activation on *A. fumigatus*, a C4c deposition assay was conducted using MBL-A- and -C-deficient serum containing ficolin-A and, potentially, collectin-11 (CL-11). C4 deposition was observed on the positive control, N-BSA (Fig. 8C). However, there was no C4 deposition observed on *A. fumigatus* and this occurred regardless of the pH conditions used (Fig. 8C).

Ficolin-A-opsonized conidia enhance the production of IL-8 by A549 lung epithelial cells. Cytokine activation is a key step in the regulation of inflammatory pathways and recruitment of polymorph nuclear cells upon challenge by *Aspergillus* spp. Therefore,

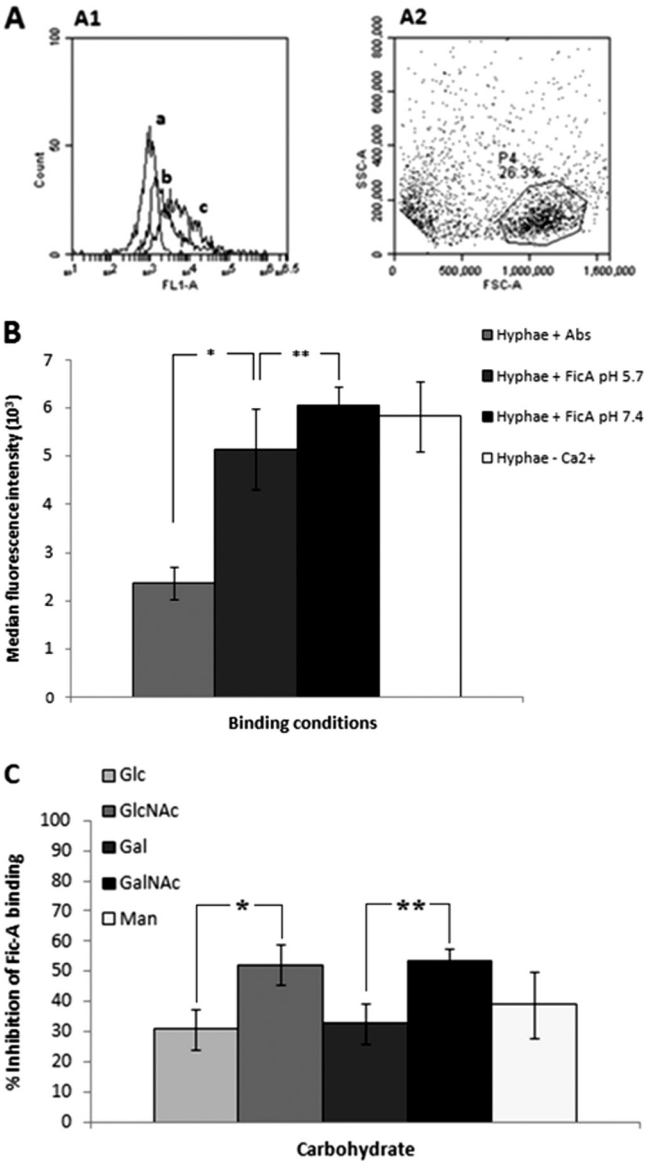

FIG 6 Characteristics of binding of ficolin-A (FicA) to *A. fumigatus* hyphae. (A) For panel A1, an unstained *A. fumigatus* control (peak a), *A. fumigatus* stained with antibodies alone (peak b), and ficolin-opsonized *A. fumigatus* stained with antibodies (peak c) were analyzed by flow cytometry (FL1-A = fluorescence). For panel A2, hyphae were gated on P4 and debris was removed (FSC-A, forward scatter; SSC-A, side scatter). (B) Ficolin-A was incubated with hyphal fragments under pH 5.7, pH 7.4, or Ca^{2+}-deficient conditions. Unstained controls had a median fluorescence of 9 × 10^2 (*, P = 0.00592; **, P = 0.16331). (C) Hyphal fragments were incubated with ficolin-A preincubated with 100 mM glucose, GlcNAc, galactose, or GalNAc or 100 μg ml^{-1} mannan (*, P = 0.01842; **, P = 0.01608). All results represent 5 × 10^5 conidia with or without ficolin-A (5 μg ml^{-1}) stained with rabbit anti-ficolin-A- and goat anti-rabbit Ig-FITC-labeled antibodies and are representative of the averages of all the data points gained from three independent experiments. All data are representative of median fluorescence intensities observed except for the sugar specificity assays, which represent percent inhibition of ficolin-A binding compared to maximal ficolin binding in the absence of inhibitors. Error bars represent the SD. Significance was determined via paired Student *t* tests.

we investigated whether ficolin-A opsonization could be important in the regulation of the proinflammatory cytokine and neutrophil chemotactic factor interleukin-8 (IL-8) upon challenge with live *A. fumigatus* conidia.

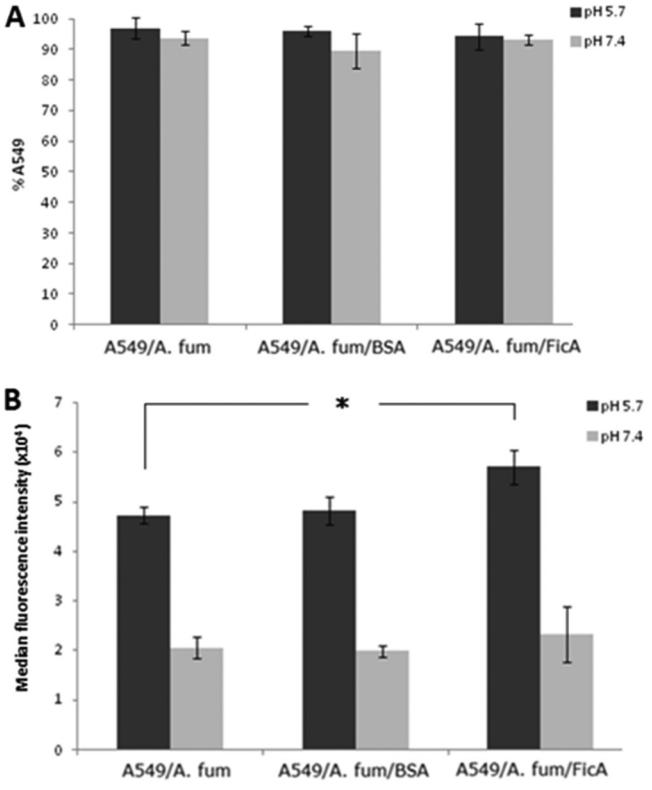

FIG 7 Adherence of ficolin-A-opsonized *A. fumigatus* conidia to A549 type II pneumocytes. (A) The percentage of A549 cells associated with *A. fumigatus* conidia alone (A549/A. fum) or with BSA-opsonized *A. fumigatus* conidia (A549/A. fum/BSA) or with ficolin-A-opsonized *A. fumigatus* conidia (A549/A. fum/FicA). (B) The relative numbers of *A. fumigatus* conidia associated with A549 cells as either unopsonized *A. fumigatus* conidia (A549/A. fum), BSA-opsonized *A. fumigatus* conidia (A549/A. fum/BSA), or ficolin-A-opsonized *A. fumigatus* conidia (A549/A. fum/FicA) incubated at pH 5.7 or pH 7.4 for 16 h (expressed as median fluorescence intensity) (*, $P = 0.014$). Data are representative of the averages of all the data points gained from three independent experiments. Error bars represent the SD. Significance was determined via paired Student *t* tests.

After 8 h, IL-8 production was detected which also coincided with conidial germination (data not shown). At this stage, the use of unopsonized as well as ficolin-A-opsonized conidia led to similar levels of IL-8 production (Fig. 9). After 24 h, it was observed that opsonization by ficolin-A did lead to the production of significantly more IL-8 by A549 lung epithelial cells compared to unopsonized controls (Fig. 9; $P = 0.02197$). Interestingly, ficolin-A itself was observed to stimulate significant quantities of IL-8 production following an incubation of ficolin-A alone with A549 cells (Fig. 9; $P = 0.00826$). A549 cells stimulated with 10 ng ml^{-1} IL-1β as a positive control demonstrated potent IL-8 production, whereas medium alone was devoid of significant amounts of IL-8 (Fig. 9).

DISCUSSION

Our study focused on the immunomodulatory role of ficolin-A against the human fungal pathogen *Aspergillus* and resulted in several important observations. First, rat ficolin-A (a homologue of human serum protein, L-ficolin) bound conidia from clinical isolates of *Aspergillus* species in a specific, concentration-dependent manner at a low pH reflecting inflammatory conditions. Ficolin-A also recognized the filamentous hyphal form of the fungus, and that recognition was specific but not dependent on acidic conditions. Second, the opsonization of *Aspergillus* conidia with ficolin-A led to enhanced adherence to lung epithelial cells compared to unopsonized controls. Third, using wild-type or ficolin-A- or MBL-deficient mouse sera, we found that ficolin-A-bound conidia did not activate the lectin complement pathway whereas complement deposition was mediated via the binding of MBL-C. Finally, we observed that the use of ficolin-A-bound conidia led to significantly increased production of IL-8 protein by A549 lung epithelial cells within 24 h in comparison to unopsonized conidia or ficolin-A alone.

We therefore postulate that ficolin-A may play an important role in the innate defense against *Aspergillus* by opsonizing conidia and immobilizing this fungus through enhanced adherence to epithelial cells at the initial site of infection in the lungs which in turn modulates inflammation. In addition, it appears that other immune pattern recognition molecules, i.e., those of the collectin MBL-C, are involved in the *Aspergillus*-lectin complement pathway activation rather than ficolin-A.

There are now a number of studies that have confirmed that ficolin-A or its human homologue L-ficolin forms an integral part of the innate immune response to several bacteria and viruses (14–17). Previous research has highlighted that L-ficolin opsonization of the bacterial species *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Streptococcus agalactiae* (*S. agalactiae*) led to better phagocytosis and lectin complement pathway activation (14, 15). While ficolins have also been shown to interact with viruses such as influenza virus and hepatitis C virus by inhibiting their replication or leading to complement activation, the role of ficolins in the defense against medically important fungal pathogens has not been greatly investigated to date (16, 17).

We have recently demonstrated the specific recognition of ficolin-A using the yeast of *Cryptococcus neoformans* (*C. neoformans*) (a major pathogen causing life-threatening disease in the immunocompromised patient) (18). These findings prompted our current investigation in assessing whether ficolins also modulate the immune recognition of the medically important mold *Aspergillus*, which is one of the most common life-threatening causes of invasive mycosis in neutropenic cancer and transplant patients.

Here we observed that resting conidia (which represent the initial fungal spores inhaled into the lungs) from a variety of clinically important *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*) are being recognized by the FBG domains of ficolin-A with high affinity. The binding of ficolin-A to conidia of all *Aspergillus* species was observed to be concentration dependent, occurring at levels within normal physiological serum ranges equivalent to L-ficolin of 1 to 12.2 μg ml^{-1} (19). These data were in line with our previous observation for acapsular *C. neoformans* and recent work involving *A. fumigatus* (18, 20). We have also been able to demonstrate that the human equivalent (L-ficolin) also recognized *Aspergillus* conidia, suggesting that ficolins may play a key role in fungal host defense in humans.

Following a preincubation of ficolin-A with a number of sugars, our data show that GlcNAc and GalNAc in the cell wall of *Aspergillus* conidia are likely to be important in the recognition of conidia and that nonacetylated carbohydrates potentially play a lesser role. Recently, the mouse and rat ficolin-A carbohydrate binding specificities have been confirmed to represent recognition

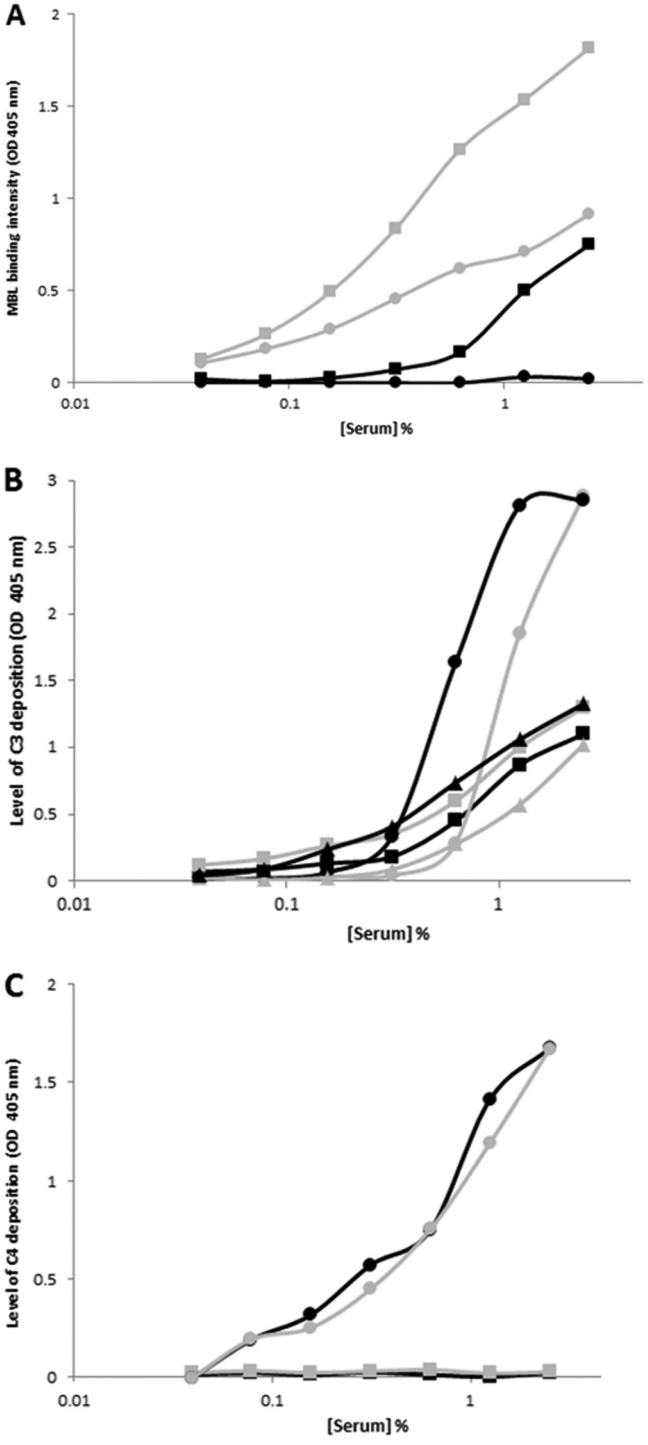

FIG 8 Binding of the lectin pathway pattern recognition molecules (MBL-A and -C) to and activation of complement on *A. fumigatus* conidia. (A) MBL-C, but not MBL-A, of wild-type (WT) murine serum was able to bind to *A. fumigatus* (MBL-A, *A. fumigatus*, black filled circles; MBL-C, *A. fumigatus*, gray filled circles). Zymosan coating was used as a positive control (MBL-A, zymosan, black filled squares; MBL-C, zymosan, gray filled squares). Significant binding was observed at the lowest concentration of serum used ($P = <0.05$). (B) C3 deposition was observed on *A. fumigatus* in the absence or presence of serum ficolin-A. Serum dilutions were incubated with Maxisorb microtiter 96-well coated plates, and deposited C3 was detected by using rabbit anti-human C3c complement antibody and goat anti-rabbit IgG-alkaline phosphatase conjugate (WT serum, zymosan, black filled squares; WT serum, *A. fumigatus*, gray filled squares; ficolin-A-deficient serum, mannan at pH 5.7, gray filled circles; ficolin-A-deficient serum, mannan at pH 7.4, black filled circles; ficolin-A-deficient serum, *A. fumigatus* at pH 5.7, gray filled triangles; ficolin-A-deficient serum, *A. fumigatus* at pH 7.4, black filled triangles). Mannan and zymosan coating were used as positive controls for C3 deposition. Significant C3 deposition was observed at the lowest concentration of serum used ($P = <0.05$). (C) C4 deposition on *A. fumigatus* in the absence of MBL-A and -C using doubly MBL-A- and -C-deficient mouse serum. Serum dilutions were incubated with Maxisorb microtiter 96-well coated plates prior to the addition of human C4 (1 μg/well). C4 deposition was not observed on *A. fumigatus* at pH 5.7 (black filled squares) or at pH 7.4 (gray filled squares); however, C4 was deposited on the positive-control coated wells (N-BSA at pH 5.7 (black filled circles) and N-BSA at pH 7.4 (gray filled circles). N-BSA was used as a positive control for C4 deposition by ficolins. All data are represented as mean absorbance measured at 405 nm and are representative of the averages of all data points gained from three independent experiments. Significance was determined via one-way ANOVA.

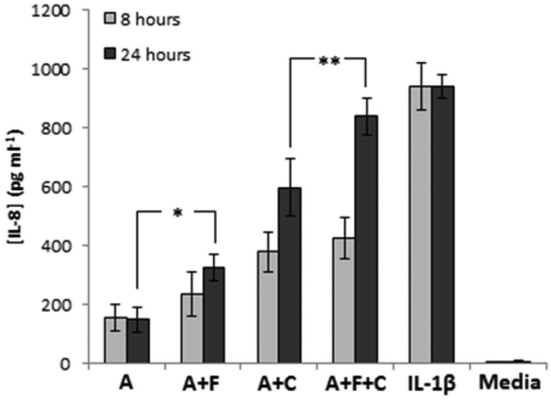

FIG 9 IL-8 production by A549 cells in response to ficolin-A and *Aspergillus* conidia. Supernatants from A549 cells were tested for IL-8 by ELISA after 8 and 24 h of challenge with ficolin-A (5 μg ml^{-1}) alone (A+F), conidia (10^5) alone (A+C), or ficolin-A (5 μg ml^{-1})-opsonized *A. fumigatus* (10^5 conidia) (A+F+C). Media from A549 cells without stimulus (A) or following prior stimulation with IL-1β (10 ng ml^{-1} for 24 h) (IL-1 β) or culture media in the absence of A549 (Media) were used as controls. All data are represented as the average concentration of IL-8 produced (pg ml^{-1}) and are representative of the averages of all data points gained from three independent experiments. Error bars represent the SD. Significance was determined via paired Student *t* tests (*, $P = 0.00826$; **, $P = 0.02197$).

of GlcNAc; the latter is also important for *C. neoformans* cell wall recognition (18, 21). X-ray crystallography analysis of L-ficolin suggests that ligand binding is likely to involve a number of sites (see Fig. S1 to S4 in the supplemental material) simultaneously, with the S3 site being responsible for binding acetylated structures and the S2 site having an affinity for galactose and GlcNAc (22–24).

Interestingly, the intensities of ficolin-A binding were notably different for different *Aspergillus* species. The strongest ficolin-A binding was observed with conidia of the less pathogenic species (*A. flavus* and *A. niger*), whereas ficolin-A showed weak binding affinity for the most common and invasive fungus, *A. fumigatus*. This could potentially have been due to the expression of different cell wall polysaccharides by the different strains or possibly due to factors on the cell surface of *A. fumigatus* conidia that allow them to evade immune recognition. The cell walls of all the fungi contain the carbohydrates glucose, galactose, mannose, and GlcNAc and a small amount of GalNAc (25, 26). It has been observed that

there is high expression of GlcNAc in the cell walls of clinical isolates of *A. flavus* and *A. niger* recovered from the brain and lungs of invasive aspergillosis patients (27). It was found that, compared to *A. flavus* and *A. niger*, *A. fumigatus* contains half as much GlcNAc and low levels of GalNAc in the cell wall (28). The differences in these acetylated carbohydrates, which are strong ligands for ficolin-A, may therefore explain the decreased binding observed with *A. fumigatus* and could potentially be important for the immune evasion.

The binding of ficolin-A to *Aspergillus* was also noted to be influenced by the presence of Ca^{2+} for some species (*A. flavus* and *A. niger*) but not for others (*A. fumigatus* and *A. terreus*). This discrepancy may be explained by the fact that ficolin trimers which have multiple FBG binding sites often involve other accessory sites in the recognition of ligands, some of which can be close to the one Ca^{2+} binding site (29). However, in the majority of cases, ligand recognition occurs without the need for Ca^{2+} presence as described above for L-ficolin and, more recently, for rat ficolin-A in the interaction with GlcNAc or *C. neoformans* (18, 22).

On the basis of our recent observation that ficolin-A binding to fungi was dependent on acidic conditions, we have here shown that the ficolin-A affinity to *A. fumigatus* conidia was again significantly higher in an acidic environment (pH 5.7) than under physiological conditions (pH 7.4) (18). A similar relationship has also been observed for L-ficolin binding to acute-phase proteins (30). The pH is an important aspect of immune defense in response to microorganisms, with particularly acidic pH present at the site of local infection which has been observed to drop to as low as pH 5.5 during inflammation (31). Our findings suggest that the acidic conditions observed at the site of inflammation and infection may be essential for optimizing ficolin-A binding and opsonization at an early stage of infection.

Interestingly. once the initially inhaled resting conidia escaped immune recognition and started to germinate into filamentous hyphae (which then invaded tissue and blood vessels), we found that ficolin-A binding was lower and was independent of pH compared to the levels seen with the conidia, suggesting that ficolin-A may play a lesser role at a later stage of this infection via direct binding, with defense against this stage being stimulated during early infection. Microscopy also indicated that not all hyphae appeared to be stained, in particular, those with longer hyphal filaments. This diminished binding may have been due to the changing expression of GlcNAc and GalNAc in the constantly remodelled polar growing hyphal tips (32). Nevertheless, our observations are the first to characterize the binding of ficolins to hyphae of a clinically relevant fungus. The importance of this binding in a clinical sense has yet to be explored further, but it may be that other groups of pattern recognition molecules such as those of the collectins SP-D may play an additional role in immune modulation, as they have also been found to bind to *A. fumigatus* hyphae (33, 34).

One of the main biological functions of opsonization is the enhanced phagocytosis or uptake by host cells. Previous research has highlighted that L-ficolin is able to function as an opsonin and enhance phagocytosis of *S.* Typhimurium and *S. agalactiae* (14, 15). Due to the small size of the conidia, they can penetrate deep into the lungs, where they initially encounter the lung epithelium, in particular, type II pneumocytes (35). Ficolin-A mRNA has been observed to be most highly expressed in the liver and spleen; however, ficolin-A mRNA has also been observed to be expressed in the lung, suggesting the potential for its role in lung defense (36). Much like MBL, L-ficolin has been observed to be present in the bronchoalveolar lavage fluid following inflammation (J. D. Chalmers, personal communication; 37). L-Ficolin gene polymorphisms and insufficiencies have been acknowledged to have implications for allergic inflammation and recurrent respiratory infections (38, 39). We therefore investigated whether ficolin-A and L-ficolin could modulate the association of *A. fumigatus* conidia with A549 airway epithelial cells. Our study has demonstrated that both ficolins significantly increase the association of conidia with A549 cells under acidic conditions. The opsonization with either L-ficolin or ficolin-A specifically increased the number of conidia associating with each A549 cell in comparison to unopsonized or BSA-opsonized controls, suggesting that ficolins may aid the immobilization of the fungus in the lung.

In addition to the opsonizing activity, ficolins (like the collectin MBL) are also known to activate the lectin complement pathway via interaction with MASP-2 (9). Previously, binding of MBL has been observed to several bacteria and the fungi *Candida albicans*, *C. neoformans*, and *A. fumigatus* (40, 41), promoting lectin pathway complement deposition on *S. aureus*, *C. albicans*, and *C. neoformans* (40, 41). Similarly, L-ficolin/MASP complexes from patients were shown to recognize lipoteichoic acid from Gram-positive bacteria such as *S. aureus*, *Streptococcus pyogenes*, and *S. agalactiae* to initiate lectin pathway-dependent C4 turnover (42, 43). More recently, complement deposition on N-acetylated BSA and GlcNAc by recombinant ficolin-A and an increased susceptibility of ficolin-A knockout mice to *Streptococcus pneumoniae* have been observed, suggesting a potential importance of ficolin-A in complement activation (10).

However, little is known about the complement-activating roles of ficolin-A in the defense against fungal pathogens. We therefore analyzed the involvement of ficolin-A in the activation of the lectin pathway upon binding to conidia of *A. fumigatus*. Our experiments established that, using MBL-A and MBL-C double-knockout (KO) murine sera containing just ficolin-A and CL-11 (data not shown), there was no C4 deposition detected on *A. fumigatus*. Furthermore, using ficolin-A-deficient mouse serum, significant complement activation via C3 deposition on the surface of *A. fumigatus* was observed. In addition, this C3 deposition was not significantly different from that observed with the wild type. To clarify the role of MBL, we undertook MBL binding studies and were able to establish that binding of MBL to *A. fumigatus* conidia and the lectin complement activation which has also been previously reported (8, 10) were due to MBL-C and not MBL-A. Therefore, we postulate that *A. fumigatus* activates the lectin-complement pathway in rodents via MBL-C and not ficolin-A or MBL-A. The importance of CL-11 has yet to be established, but our experiments suggest that this lectin is also unlikely to be important in the *Aspergillus* lectin pathway activation. In addition to our observation, others have demonstrated a synergistic effect of L-ficolin with acute-phase proteins in the activation of complement in response to *A. fumigatus* which may indicate a more complex interaction with the complement system (44). All in all, it is likely that several mechanisms, including the alternative pathway, play a role *in vivo*, as other groups have suggested the involvement of the recently proposed fourth pathway involving the alternative pathway that bypasses C2 of the lectin pathway, leading directly to C3 deposition (45).

Cytokines are key regulators of inflammation and leukocyte

recruitment and thus represent critical components in the defense against microbial challenges. Upon challenge by *A. fumigatus*, a number of cytokines, including IL-5, IL-6, IL-8, IL-13, and gamma interferon (IFN-γ), and the chemokine monocyte chemotactic protein-1 (MCP-1) have been observed to be induced (46–48). The role of opsonins in cytokine activation has also been acknowledged, with surfactant protein-D eliciting cytokine responses from A549 cells in response to allergens (49). Therefore, we were interested in investigating whether ficolin-A opsonization could play a further immunomodulatory role in the defense against *Aspergillus* spp. via the modulation of the proinflammatory cytokine IL-8.

Our results suggest that the opsonization of conidia by ficolin-A leads to significant production of IL-8 over a 24-h period. IL-8 is a key cytokine for the recruitment of polymorph nuclear cells to the site of inflammation (50) which are essential in the defense against pulmonary aspergillosis (51). This enhanced cytokine production may be accelerated by an early increased association of conidia with type II lung epithelial cells following ficolin-A opsonization.

Interestingly, ficolin-A itself was observed to lead to some IL-8 production. The exact mechanisms of this interaction need to be elucidated, as much is unknown with regard to the immunomodulatory role of ficolin-A on host cells. However, it has been observed that murine ficolin-A possesses a functionally relevant signal peptide on its N-terminal and that this could prove useful in the production of secretory proteins (52). It could therefore be postulated that the early recognition of conidia by ficolin-A promotes a proinflammatory environment in anticipation of production of hyphae and the need for polymorph nuclear cell recruitment via IL-8 production.

We conclude that ficolin-A may play an important role in the innate defense against *Aspergillus* by opsonizing conidia and immobilizing this fungus through enhanced adherence to epithelial cells and production of proinflammatory cytokines. In addition, it appears that other immune pattern recognition molecules, i.e., those of the collectin MBL-C, are involved in the *Aspergillus*-lectin complement pathway activation rather than ficolin-A. Nevertheless, the utilization of specific complement-deficient transgenic animal models will be useful to verify the role of ficolin-A and the lectin pathway in the defense against *Aspergillus* infections *in vivo*.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council, grant reference number G0801952.

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