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*J Immunol* 2013; 191:2581-2588; Prepublished online 7 August 2013; doi: 10.4049/jimmunol.1300748

http://www.jimmunol.org/content/191/5/2581

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The RodA Hydrophobin on *Aspergillus fumigatus* Spores Masks Dectin-1– and Dectin-2–Dependent Responses and Enhances Fungal Survival In Vivo

Steven de Jesus Carrion,* Sixto M. Leal, Jr.,* Mahmoud A. Ghannoun,† Vishukumar Aimanianda,‡ Jean-Paul Latgé,‡ and Eric Pearlman*

*Aspergillus* and *Fusarium* species are important causes of fungal infections worldwide. Airborne spores (conidia) of these filamentous fungi express a surface protein that confers hydrophobicity (hydrophobin) and covers cell wall components that would otherwise induce a host immune cell response. Using a mutant *Aspergillus fumigatus* strain (ΔrodA) that does not express the RodA hydrophobin, and *Aspergillus* and *Fusarium* conidia from clinical isolates that were treated with hydrofluoric acid (which removes the *A. fumigatus* RodA protein), we observed increased surface exposure of β1,3-glucan and α-mannan on *Aspergillus* and *Fusarium* conidia. We also found that ΔrodA and hydrofluoric acid–treated conidia stimulate significantly higher NF-κB p65 nuclear translocation and cytokine production by macrophages from C57BL/6, but not from Dectin-1−/− or Dectin-2−/− mice. Using a murine model of *A. fumigatus* corneal infection, we showed that ΔrodA conidia induced significantly higher cytokine production, neutrophil infiltration, and more rapid fungal clearance from C57BL/6 corneas compared with the parent G10 strain, which was dependent on Dectin-1 and Dectin-2. Together, these findings identify the hydrophobin RodA as a virulence factor that masks Dectin-1 and Dectin-2 recognition of conidia, resulting in impaired neutrophil recruitment to the cornea and increased fungal survival and clinical disease. *The Journal of Immunology*, 2013, 191: 2581–2588.

Although *Aspergillus* conidia and those of other filamentous fungi are ubiquitous in the air we breathe and can reach high concentrations (>106 spores/m3 in some environments), they do not generally cause inflammatory disease following inhalation (1, 2). Fungal cell wall components such as β1,3-glucan and α-mannan have the potential to induce inflammation; however, conidia are coated by a hydrophobic rodlet layer composed of regularly arranged RodA hydrophobins, which are covalently bound to cell wall polysaccharides by GPI anchor formed of phosphodiester bonds attaching RodA to the cell wall, confers conidia recognition by human dendritic cells and murine alveolar macrophages (5). Also, mice infected with an *A. fumigatus* ΔrodA mutant caused increased lung inflammation compared with the parent strain (5). As *Aspergillus* and *Fusarium* species are also major causes of corneal infection and blindness worldwide (6), the current study examined the role of hydrophobins in a murine model of fungal keratitis (7, 8). We also identified cell wall components that are exposed in the absence of RodA and the pathogen recognition molecules that are activated.

We show that β1,3-glucan and α-mannan are exposed on the cell wall of *Aspergillus* conidia in the absence of RodA and that the C-type lectins Dectin-1 and Dectin-2 mediate the host response. Using a murine model of *Aspergillus* corneal infection, we also demonstrate that in the absence of RodA, *A. fumigatus* induces Dectin-1– and Dectin-2–dependent neutrophil recruitment to the corneal stroma and enhanced fungal killing. Together, these data represent a novel fungal adaptation to evade early recognition by Dectin-1 and Dectin-2, enabling conidia to germinate and form hyphae prior to immune recognition, which thereby enhances fungal survival during infection.

**Materials and Methods**

**Source of mice**

All animals were treated in accordance with the guidelines provided in the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Case Western Reserve University Institutional Animal Care and Use Committee. C57BL/6 mice (5–12 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Dectin-1– and Dectin-2–deficient mice were a kind gift from Yoichiro Iwakura (University of Tokyo, Tokyo, Japan).

**Fungal strains, media, and growth conditions**

Aspergillus strains used in this study were cultured in Vogel’s minimal media (VMM) with or without 4% agar ± supplementation with 10 mM uracil and 5 mM uridine at 37˚C/5% CO2 unless stated otherwise. The *A. fumigatus* parent (G10) and mutant (ΔrodA) strains were provided by Jean-Paul Latgé (Institut Pasteur, Paris, France). *A. fumigatus* Af-26 strain is a clinical isolate from a patient treated at Bascom Palmer Eye Institute (Miami, FL), provided by Dr. Darlene Miller. The *F. oxysporum* 8996 strain is a clinical isolate from a patient treated at the Cleveland Clinic Institute (Cleveland, OH) (8). The *A. flavus* TN-302 strain is a clinical isolate obtained from the Aravind Eye Hospital-Madurai (Tamil Nadu, India).
India. *Fusarium solani* B6970 was from a contact lens keratitis patient and obtained from the Centers for Disease Control and Prevention.

### RodA protein extraction method

The rodlet layer was extracted from the cell wall by incubating dry spores with 4% HF for 72 h at 4°C. The content was centrifuged (10,000 rpm, 10 min), and supernatant was discarded. Samples were fixed with 4% paraformaldehyde (PFA) for 30 min and washed three times with sterile PBS.

### Detection of surface β1,3-glucan and α-mannose

*Aspergillus* strains were cultured for 3 d in VMM plus 4% agar, and *Fusarium* strains were cultured in Sabouraud-Dextrose medium (Becton, Dickinson, and Company). Conidia were isolated from the 5-d culture and fixed in 4% PFA. Conidia were centrifuged (10,000 rpm, 5 min), incubated with 1.5% normal rabbit serum in PBS for 1 h, and then with a Dectin-1 fusion protein (9) from Dr. Chad Steele (University of Alabama at Birmingham). Conidia were then washed three times with PBS, and incubated with 1 mg/ml FITC-conjugated goat anti-mouse IgG (Invitrogen) diluted to 1 mg/ml in PBS for 1 h at 37°C. α-Mannose was detected using biotinylated Con A and DyLight 488 Streptavidin (both reagents from Aldrich) and incubated with Alexa Fluor 488–tagged rabbit-anti-rat IgG (Invitrogen) for 1 h at 37°C. The slides were washed three times in PBS plus 0.05% Tween 20 and imaged by fluorescence microscopy.

### Isolation of peritoneal and bone marrow–derived macrophages

For peritoneal macrophages, 1.5 ml 4% thioglycolate was injected into the peritoneal cavity of naive mice. Three days later, mice underwent euthanasia by CO2 asphyxiation, and macrophages were isolated from the peritoneal cavity in sterile PBS using a 10-ml syringe (BD syringes; BD Biosciences). RBCs were lysed using 1× RBC lysis buffer (eBioscience) and washed in sterile PBS. Cells were then counted and harvested.

Bone marrow–derived macrophage (BMM) isolation, mice were euthanized by CO2 asphyxiation, and femurs and tibias were removed, cleaned, and centrifuged at 6000 × g for 45 s at 4°C. Any contaminating RBCs were lysed in 5 ml RBC Lysis Buffer (eBioscience), and remaining bone marrow cells were cultured in bacteriologic-grade petri dishes in 6 ml Macrophage Growth Medium (GEM:DMEM with l-glutamine, Na-pyruvate, HEPES, 10% FBS, penicillin/streptomycin, and 30% L929 cell-conditioned medium). On day 5, and every 2 d thereafter, the cell supernatant was aspirated, and fresh Macrophage Growth Medium was added. Adherent cells were harvested between 7 and 14 d of culture and counted.

### Detection of NF-κB nuclear translocation and cell-associated conidia in BMM

Adherent cells were harvested between 7 and 14 d of culture. A total of 2.5 × 10^6 cells was cultured onto sterile 18-mm coverslips (Corning) in a six-well plate and treated with LPS (100 ng/ml; positive control), G10, or ∆rodA conidia (multiplicity of infection [MOI] 100) for 15, 30, and 60 min. Following activation, BMM were fixed with 4% PFA for 15 min at room temperature, permeabilized using 0.1% Triton X-100 in PBS for 1 min at room temperature (RT), and incubated with rabbit anti-mouse p65 (1:100; eBioscience) in PBS containing 10% goat serum for 1 h at RT. Coverslips with adherent macrophages were washed two times with PBS, and incubated with Alexa Fluor 488–labeled goat anti-rabbit IgG Ab (Molecular Probes) in PBS at RT for 1 h. The coverslips were mounted on glass slides using Vectashield mounting medium with DAPI (Vector Laboratories) and examined by fluorescence microscopy.

For cell-association studies, BMM incubated with naive or HF-treated conidia were washed and fixed in 4% PFA as described above and then incubated 5 min with Calcofluor white (Sigma-Aldrich) at a 1:1 ratio with 10% KOH. After washing two times with PBS, cells were examined by differential interference contrast (DIC) and fluorescence microscopy, and associated conidia per 100 cells and the percent cells with associated conidia were determined after direct examination of at least 50 cells/coverslip. Two coverslips were examined, and the mean and SD were calculated.

### Cytokine assays

Peritoneal macrophages were incubated with naive, HF-treated, or ∆rodA dormant conidia for 6 h, and cell-culture supernatants were obtained. LPS (10 ng/ml; Invitrogen) and curdlan (100 μg/ml; Invivogen) were used as controls for stimulations. In spleen tyrosine kinase (Syk) inhibition experiments, piceatannol (50 μM; Sigma-Aldrich) was added 30 min prior to stimulation. Half-well cytokine assays were performed using Duoset ELISA kits (R&D Systems) according to the manufacturer’s directions.

### Image analysis

To quantify the NIMP-R14–stained histological sections, we obtained the images in 12 bits on a Leica DMI 6000 B inverted microscope (Leica Microsystems) using a 40× objective connected to a Retiga EXI camera (Q-Imaging, Vancouver, British Columbia, Canada). Analysis was performed using Metamorph Imaging Software (Molecular Devices, Downington, PA). Images were stitched together to create an entire cornea. The area of the cornea was delineated and recorded. Next, the image was thresholded to recognize the NIMP-R14–staining. This resulting area within the cornea was quantified.

To quantify corneal opacity, brightfield images of mouse corneas were analyzed using Metamorph software (Molecular Devices) as described (7). Briefly, a constant circular region encompassing the cornea was defined, and the pixel intensity within this region summed to yield a numerical value that corresponds to the total amount of light reflected from the cornea (i.e., opacity). Images were taken in a Spot RT Slider KE camera using Spot Advanced Software under the same magnification, exposure, and γ parameters.

### Statistical analysis

Statistical analysis was performed for each experiment using an unpaired t test (Prism, GraphPad). A p value < 0.05 was considered significant.
Results

RodA protein masks surface β1,3-glucan and α-mannose in Aspergillus and Fusarium conidia and masks cytokine production by macrophages

Fusarium species, which cause systemic disease and corneal infections (11–13), also express hydrophobins (14). Therefore, we treated Aspergillus and Fusarium conidia from clinical isolates with HF, which unmasks the RodA hydrophobin from A. fumigatus as previously shown (5). We also examined the A. fumigatus RodA mutant and the parent G10 strain as described (5).

Using a Dectin-1 Fc fusion protein to detect β1,3-glucan (9), we found increased binding to ΔrodA compared with G10 conidia (Fig. 1A). Similarly, there was increased binding to HF-treated Aspergillus and Fusarium conidia compared with untreated conidia. Because α-mannose is also abundant in the fungal cell wall, and is a ligand for Dectin-2 (18, 19, 31), we used the lectin Con A to detect this sugar on the cell surface. Fig. 1B shows higher α-mannose staining on ΔrodA conidia compared with the G10 strain and increased binding on HF-treated Aspergillus and Fusarium clinical isolates compared with untreated conidia. β1,3-glucan and α-mannose expression was also detected on the surface of ΔrodA conidia by confocal microscopy (Supplemental Fig. 1).

As the RodA protein confers resistance to phagocytosis (15), we examined cytokine production in macrophages incubated with ΔrodA- and HF-treated conidia. Fig. 1C shows significantly higher CXCL1, CXCL2, and TNF-α production by macrophages incubated with Aspergillus ΔrodA and Fusarium HF-treated conidia compared with the G10 strain or untreated Fusarium conidia.

These data indicate that the hydrophobic RodA protein masks surface β1,3-glucan and α-mannose in Aspergillus conidia and that HF treatment removes molecules that share a similar function as RodA on Fusarium conidia.

Dectin-1 and Dectin-2 are required for conidia-induced macrophage NF-κB translocation and cytokine production in the absence of RodA

To determine if the increased cytokine production in the absence of RodA protein is related to macrophage binding or uptake of conidia, we incubated C57BL/6, Dectin-1/-/-, and Dectin-2/-/- BMM with ΔrodA conidia at an MOI of 100, and the number of conidia bound or internalized by macrophages was imaged by DIC microscopy and quantified by direct counting. As shown in Fig. 2A and 2B, the average number of associated ΔrodA conidia per macrophage was significantly higher than G10 conidia.

To assess the role of C-type lectins in conidia-induced macrophage activation in the absence of RodA, Dectin-1/-/- and Dectin-2/-/- BMM were incubated 1 h with ΔrodA conidia, and nuclear translocation of the p65 subunit of NF-κB was detected by immunofluorescence and DAPI nuclear stain. As shown in Fig. 2C and 2D, following LPS incubation, p65 was detected in the nucleus of C57BL/6, Dectin-1/-/-, and Dectin-2/-/- macrophages. In contrast, nuclear localization of p65 was detected in C57BL/6, but not Dectin-1/-/- or Dectin-2/-/- macrophages incubated with ΔrodA conidia, whereas the G10 strain failed to induce p65 translocation in C57BL/6, Dectin-1/-/-, or Dectin-2/-/- macrophages.

To determine if Dectin-1 and Dectin-2 also regulate cytokine production, macrophages were incubated for 6 h with ΔrodA- and HF-treated Fusarium conidia, and cytokine production was mea-

**FIGURE 1.** RodA protein masks surface β1,3-glucan and α-mannose in Aspergillus and Fusarium conidia and blocks cytokine production by macrophages. (A and B) Dormant conidia from Aspergillus and Fusarium clinical isolates were treated with HF to remove the rodlet layer. (A) HF-treated and ΔrodA conidia were fixed with PFA, and β1,3-glucan was detected using a Dectin-1–Fc fusion protein and FITC-conjugated goat anti-mouse IgG. (B) Con A was used to detect α-mannose, and DyLight 488 Streptavidin was used for detection. Cells were visualized by DIC and fluorescent microscopy using oil immersion. Original magnification ×100. Scale bar in upper left panel, 2 μm. (C) C57BL/6 BMM were incubated 6 h with ΔrodA- or HF-treated conidia at a ratio of 1:50 (MOI 50), and CXCL1, CXCL2, and TNF-α secretion was measured by ELISA. These experiments were repeated at least twice with similar results. **p < 0.001, ***p < 0.0001. Ctrl, Control.
FIGURE 2. The role of Dectin-1 and Dectin-2 in macrophage activation by ΔrodA- and HF-treated conidia. (A) C57BL/6, Dectin-1−/−, and Dectin-2−/− BMM were plated on coverslips and incubated 1 h with ΔrodA or G10 conidia. Original magnification ×100. Scale bar, 10 μm. (B) Mean ± SD associated conidia per macrophage. (C) p65 translocation to the nucleus of C57BL/6, Dectin-1−/−, and Dectin-2−/− BMM after 1-h incubation with ΔrodA conidia. Macrophages were fixed, permeabilized, and stained with anti-p65 primary Ab and Alexa Fluor 488–tagged anti-rabbit secondary Ab. Cells were visualized by fluorescent microscopy using oil immersion. Scale bar, 10 μm. (D) Nuclear translocation was quantified by image analysis using Metamorph software. (E) CXCL1, CXCL2, and TNF-α production by C57BL/6, Dectin-1−/−, and Dectin-2−/− BMM after 6-h incubation with ΔrodA- or HF-treated conidia at a ratio of 1:50 (MOI 50). Cytokine production was measured by ELISA. Experiments were performed at least twice with similar results. **p < 0.001, ***p < 0.0001. Ctrl, Control.

sured by ELISA. Fig. 2E shows significantly less CXCL1, CXCL2, and TNF-α production by Dectin-1−/− compared with C57BL/6 macrophages stimulated with ΔrodA, whereas Dectin-2−/− macrophages produced significantly less CXCL2 and TNF-α.
Although CXCL1 production was lower, the difference was not statistically significant). Consistent with the absence of p65 translocation, cytokines were not detected following incubation with the G10 parental strain. These results indicate that the *Aspergillus* RodA protein and HF-sensitive *Fusarium* molecules mask Dectin-1 and Dectin-2 recognition of conidia by macrophages, resulting in impaired cytokine production.

**Cytokine production induced by ΔrodA and HF conidia is dependent on Syk**

Dectin-1 and Dectin-2 signaling in macrophages and dendritic cells requires activation of Syk (16), which contains two Src homology 2 domains that allow it to bind phosphorylated tyrosines in the ITAM-like motif of Dectin-1 and in the ITAM motif of the FcR that associates with Dectin-2. Syk kinase activity then mediates downstream signaling and activation of NF-κB to activate transcription of target genes (16–19).

To examine the role of Syk in conidia-induced cytokine production, C57BL/6 BMM were incubated with LPS (which signals through TLR4), curdlan, ΔrodA, and *Fusarium* HF-treated conidia in the presence of the Syk inhibitor piceatannol. As shown in Fig. 3, LPS-induced cytokine production was not inhibited. In contrast, curdlan-induced production of CXCL1, CXCL2, and TNF-α was significantly lower in the presence of piceatannol. Cytokine production induced by ΔrodA and *Fusarium* HF-treated conidia was also significantly lower after incubation with piceatannol, indicating that the Syk-dependent pathway is essential for the increase in cytokine production. As before, cytokines were not detected following stimulation with G10 or HF-un treated conidia.

**RodA protein enhances fungal survival in vivo**

Although ΔrodA mutants were shown to induce more lung inflammation than G10 (5), the role of RodA in fungal survival and clinical disease has yet to be determined. To examine the role of RodA, we used a murine model of *A. fumigatus* corneal infection in which conidia are injected directly into the corneal stroma of immunocompetent C57BL/6 mice. Conidia germinate and hyphae grow throughout the cornea, stimulating a pronounced neutrophil infiltrate and corneal opacification (7, 20). C57BL/6 mice were therefore infected with G10 or ΔrodA conidia, and disease progression, neutrophil infiltration, and fungal viability were assessed.

As shown in Fig. 4A–C, corneas infected with the G10 parent strain developed progressively increasing corneal opacification over 72 h (quantification of corneal opacity is described in Supplemental Fig. 2), which is consistent with our earlier findings using other *A. fumigatus* strains (7, 20), and in our model of *Fusarium* keratitis (8). In contrast, corneas infected with the ΔrodA mutant exhibited significantly higher percent and total corneal opacity at 24 h, but had significantly lower corneal opacification scores at 48 and 72 h. We also found that neutrophil and macrophage infiltration into the corneal stroma was significantly higher in ΔrodA compared with G10 parent strain (Fig. 4D, 4E). Further, there was significantly less CFUs in the ΔrodA compared with G10-infected corneas at each time point. Four of the five mice given the ΔrodA strain completely cleared the infection by 96 h (Fig. 4F).

We conclude from these data that the ΔrodA strain induced more rapid cellular infiltration and fungal killing than the parent strain, with the cellular infiltration causing more corneal opacity at 24 h. Conversely, the host response to the parent G10 strain is delayed due to expression of RodA, resulting in prolonged fungal survival and corneal opacity.

**ΔrodA induces Dectin-1- and Dectin-2-dependent increased cytokine production and neutrophil recruitment to the cornea**

Results of our previous studies on *Aspergillus* and *Fusarium* keratitis suggested a sequence of events in which Dectin-1–dependent cytokine production by resident macrophages induces neutrophil recruitment to the cornea stroma and mediates hyphal killing (21). To examine if the enhanced clearance of ΔrodA conidia from infected corneas is due to a more rapid or robust cytokine response and neutrophil infiltration and to determine the role of Dectin-1 and Dectin-2, we infected corneas of Dectin-1–/– and Dectin-2–/– mice with G10 or ΔrodA conidia. After 6 h, cytokine production and neutrophil infiltration were quantified.

Fig. 5A shows that at 6 h postinfection, there were significantly higher CXCL1, CXCL2, and IL-6 levels in C57BL/6 corneas infected with ΔrodA compared with the G10 strain. Further, whereas there were no significant differences in cytokine production among Dectin-1–/–, Dectin-2–/–, and C57BL/6 corneas infected with the G10 strain, production of each cytokine was significantly lower in ΔrodA-infected corneas of Dectin-1–/– and Dectin-2–/– compared with C57BL/6 mice.

As normal corneas are avascular and have resident macrophages, but not neutrophils, we examined neutrophil infiltration using the Ly6G Ab NIMP-R14 to identify these cells in 5-μm corneal sections. Consistent with the chemokine data, the number of

**FIGURE 3.** Role of Syk in macrophage cytokine production. C57BL/6 macrophages were incubated 6 h with ΔrodA- or HF-treated conidia at a ratio of 1:50 (MOI 50) in the presence of a Syk inhibitor (piceatannol). CXCL1, CXCL2, and TNF-α were measured by ELISA. Experiments were performed twice with similar results. *p < 0.05, **p < 0.001, ***p < 0.0001. Ctrl, Control.
neutrophils in G10-infected Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> corneas at 6 h postinfection was not significantly different from C57BL/6 corneas (Fig. 5B, 5C). In contrast, ΔrodA-infected C57BL/6 corneas had a significantly higher neutrophil infiltrate compared with the G10 strain, which was significantly lower in Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> corneas. These data indicate that the early cytokine production and neutrophil recruitment induced by ΔrodA conidia are dependent on Dectin-1 and Dectin-2.

**Dectin-1 and Dectin-2 regulate corneal opacification and fungal killing following infection with ΔrodA conidia**

To determine if there is a role for Dectin-1 and Dectin-2 in fungal clearance of ΔrodA and the G10 parent strain from infected corneas, Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> mice were infected intrastromally with live conidia as before and examined after 24 h when there was a clear difference in CFUs, cellular infiltration, and corneal opacity (Fig. 5).

Fig. 6A shows that as in Fig. 5, there were significantly less CFUs in ΔrodA- compared with G10-infected corneas of C57BL/6 mice at 24 h postinfection, indicating more efficient killing of the ΔrodA strain. In contrast, CFUs recovered from Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> corneas infected with ΔrodA were significantly higher than C57BL/6 mice, indicating an essential role for Dectin-1 and Dectin-2 in fungal killing. There was no significant difference in CFUs between C57BL/6, Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> infected corneas with G10 at this time point.

Conversely, Fig. 6B–D show that corneal opacification in ΔrodA-infected C57BL/6 mice was significantly lower in Dectin-1<sup>−/−</sup> and Dectin-2<sup>−/−</sup> mice, indicating that these receptors also mediate corneal disease in the absence of RodA. Interestingly, the...
A. fumigatus strains, would have germinated and partially exposed surface β-glucan 24 h postinfection (7), has significantly less corneal opacity in Dectin-1−/− compared with C57BL/6 corneas. Consistent with these findings and with the important role for neutrophils in corneal opacification (20), there were also significantly less neutrophils in G10- and ΔrodA-infected Dectin-1−/− and Dectin-2−/− corneas compared with C57BL/6 mice (Fig. 6E). Furthermore, there was significantly more neutrophil infiltration in ΔrodA- compared with G10-infected C57BL/6 corneas, which is associated with increased corneal opacity.

Taken together, these data indicate that the RodA hydrophobin masks Dectin-1 and Dectin-2 recognition of exposed fungal cell wall β-glucan and α-mannose, impairing neutrophil recruitment to the cornea and facilitating fungal survival.

Discussion

The innate immune system recognizes microorganisms through a limited number of germline-encoded pathogen recognition receptors, which regulate the outcome of microbial infections (22). In fungal disease the C-type lectins are the largest and most diverse lectin family and comprise receptors that can bind to fungal glycan ligands in a calcium-dependent manner (23, 24). In the current study, we show that the A. fumigatus RodA hydrophobin masks surface β1,3-glucan and α-mannose in dormant A. fumigatus and A. flavus conidia in strains isolated from patients with corneal ulcers. This hydrophobic protein blocks Dectin-1 and Dectin-2 recognition of conidia, thereby evading Syk-dependent cytokine production by macrophages in addition to phagocytosis. In vivo, we demonstrate that during corneal infection with A. fumigatus, blockade of Dectin-1 and Dectin-2 signaling leads to decreased neutrophil infiltration and enhanced survival in the cornea. Consistent with our findings using the A. fumigatus ΔrodA mutant, we show that HF-treated Fusarium conidia results in exposure of surface β1,3-glucan and α-mannose, which are then recognized by Dectin-1 and Dectin-2 on macrophages and induce cytokine production. This indicates a novel role for the RodA protein in promoting fungal survival during A. fumigatus infection and implies that other pathogenic filamentous fungi that express RodA or other molecules have a similar role in evading host immune responses.

Our findings are consistent with the elevated macrophage phagocytosis of A. fumigatus laeA mutants, which have lower RodA expression than the parent strain (25). Our data also extend our understanding of the role of the rodA protein described by Latgé et al. (5), who showed that the ΔrodA mutant stimulated increased IL-6 and TNF-α production by macrophages and more severe pulmonary inflammation in mice infected with A. fumigatus ΔrodA compared with the G10 parent strain. We confirmed these observations using a model of corneal infection and increased our understanding of the mechanism by which hydrophobins prevent potentially damaging host cell responses to common airborne spores. Our in vitro and in vivo findings indicate that the RodA hydrophobin impairs macrophage recognition of spores by Dectin-1 and Dectin-2, which would otherwise induce elevated cytokine production by recognition of β1,3-glucan and α-mannose, in addition to increased phagocytosis. By blocking macrophage production of proinflammatory and chemotactic cytokines, recruitment of neutrophils to the site of infection is impaired, resulting in increased fungal growth. Masking of immunogenic cell wall components appears to be a broad strategy of fungal survival during infection, as dormant yeasts such as Candida albicans and Histoplasma capsulatum do not expose β1,3-glucan on the surface until they form bud scars during the process of germination (26–28).

Although there is a clear role for Dectin-1 in recognizing β1,3-glucan and mediating the host response to germinating A. fumi-
RoDA ENHANCES FUNGAL SURVIVAL IN VIVO


