**Macrophage-independent Fungicidal Action of the Pulmonary Collectins**

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Histoplasma capsulatum (Hc) is a facultative intracellular fungal pathogen that causes acute and chronic pneumonia. In this study, we investigated the role of the pulmonary collectins, surfactant proteins (SP) A and D, in the clearance of Hc yeast from the lung. Exposure of yeast to either collectin induced a dose-dependent decrease in [3H]leucine incorporation by several strains of Hc. This decrement was attributed to killing of the collectin-exposed yeast since it failed to grow on agar medium. Exposure to SP-A or -D resulted in increased yeast permeability based on a leak of protein from the organism and enhanced access of an impermeant substrate to intracellular alkaline phosphatase. Inbred and outbred SP-A null (−/−) mice were modestly more susceptible to pulmonary infection with Hc than strain and age-matched SP-A (+/+) control mice. The increase in susceptibility was associated with a decrement in the number of CD8+ cells in the lungs of SP-A−/− mice. Neither SP-A nor SP-D inhibited the growth of macrophage-internalized Hc. We conclude that the SP-A and SP-D are antimicrobial proteins that directly inhibit the growth of Hc by increasing permeability of the organism and that Hc gains asylum from collectin-mediated killing by rapid entry into pulmonary macrophages.

**Histoplasma capsulatum** (Hc) is a dimorphic fungal pathogen that causes a broad spectrum of human disease. The organism is endemic to the midwestern and southeastern United States and is the most common cause of pulmonary fungal infection in the country. Inhalation of Hc usually results in a self-limited flu-like illness that is associated with inflammatory pulmonary infiltrates. In a smaller proportion of individuals, Hc can cause a more serious pneumonia or a chronic cavitary pulmonary infection. The organism also can act as an opportunistic pathogen and can cause progressive, disseminated disease in immunosuppressed patients (1).

After inhalation into the distal airspaces, Hc is internalized into alveolar macrophages, where it persists and proliferates as a facultative intracellular pathogen. Rapid access to this intracellular niche also allows Hc to escape the immune defenses of the alveolar lining fluid, which include the antimicrobial proteins lactoferrin, lysozyme, and defensins (2).

Pulmonary surfactant lines the air-liquid interface of the lung and constitutes the first line of defense against inhaled pathogens (3). Surfactant is composed of saturated and unsaturated phospholipids and four proteins, surfactant proteins (SP), SP-A, SP-B, SP-C, and SP-D (4). SP-B and SP-C are extremely hydrophobic proteins that are thought to play critical roles in the biophysical activity of surfactant. SP-A and SP-D are hydrophilic, oligomeric glycoproteins that are highly homologous to the serum innate immune opsonin, mannose-binding protein A. Together, these three proteins form the human collectin family, so named because they each contain a collagen-like region, which is important for trimeric assembly, and a C-type lectin domain, which binds to complex carbohydrates that decorate the surface of microorganisms. The collectins have been reported to aggregate and opsonize a variety of microorganisms, to modulate macrophage oxidant and cytokine production, and to enhance the intracellular killing of mycobacteria and other microbes (5). Recently, van Rozendaal et al. (6) reported that SP-D down-regulated protein synthesis and hyphal outgrowth of Candida albicans. Although this effect was attributed to the influence of SP-D-mediated aggregation on microbial access to the radiolabeled precursor, an alternative hypothesis is that the collectins directly influence microbial physiology.

In this study, we investigated the potential of SP-A and SP-D to modulate the growth and viability of Hc in the presence and absence of alveolar macrophages. Both collectins caused a dose-dependent decrement in yeast viability, which was associated with an increase in the permeability of the yeast. Mice lacking the genes encoding SP-A manifested a modestly higher fungal burden in lungs than wild-type littermates. SP-A and SP-D failed to inhibit the growth of yeast that had been internalized by macrophages. Hence, these two collectins demonstrate potent antifungal properties, but entry of Hc into macrophages provides a haven from the inimical effects of SP-A and SP-D.

**EXPERIMENTAL PROCEDURES**

*Mice—Swiss Black SP-A−/− mice (a gift of J. Whitsett and T. Korhagen) were developed from embryonic stem cells after disruption of the mouse SP-A gene by homologous recombination and maintained by breeding with Swiss Black mice, as previously described (7). The SP-A null allele was bred into the C57Bl/6J background through nine generations using a PCR-based genotyping strategy to track the neo-locus of the gene-targeting cassette (8). All animals were housed in positively ventilated microisolator cages with automatic recircul-
ing water located in a room with laminar, high efficiency particulate-
filtered air. The animals received autoclaved food, water, and bedding. Mice used were handled in accordance with approved protocols through the Institutional Animal Care and Use Committee at University of Cincinnati School of Medicine.

Preparation of Hc Yeast and Infection of Mice—Four strains of yeast were used in these studies: G217B, 186R, and 186S, and an isolate from an immunodeficiency virus-infected patient from Panama. Yeast were grown as described (9). Briefly, they were seeded in Ham's F-12 medium supplemented with 18.2 g/liter glucose, 1 g/liter glutamic acid, 6 g/liter HEPES, and 0.084 g/liter cysteine at a concentration of 3 × 10^7/ml and grown for 60 h at 37 °C. The yeast were collected and washed three times with Heps-exchanged saline solution. Mice were inoculated with 2 × 10^6 yeast intranasally after anesthesia with isoflurane.

Synthesis and Purification of Native and Recombinant SP-A and SP-D—Surfactant was isolated from bronchoalveolar lavage of Sprague-Dawley rats that had been treated 1 month earlier with intratracheal silica to enhance the collectin yield (11). After removal of cells by centrifugation of the bronchoalveolar lavage at 300 × g, the surfactant pellet was sedimented by centrifugation at 12,000 × g and floated on NaBr gradients, as described (12). Rat SP-A was isolated and purified from the NaBr gradient pellet by delipidation with butanol and mannose-Sepharose affinity chromatography (13). Rat SP-D was purified from the bronchoalveolar lavage of silica-pretreated rats by calcium-dependent adsorption of the recalcified supernatant to mannose-Sepharose affinity columns. Human SP-A isolated from patients with pulmonary alveolar proteinosis, a lung disease associated with the accumulation of surfactant, is referred to as alveolar proteinosis protein (APP). Briefly, APP was purified by the method of Swaabe (14) from the cell-free surfactant pellet of bronchoalveolar lavage by serial sedimentation and resuspension in buffer containing 25 mM Hepes, 150 mM NaCl, and 1 mM Ca^2+ release by incubation with 2 mM EDTA, and adsorption of the recalcified supernatant to mannos-Sepharose affinity columns. In all cases, SP-A and SP-D were eluted from the carbohydrate affinity column using 2 mM EDTA. The purified proteins were dialyzed against 25 mM phosphate-buffered saline containing 10% fetal bovine serum and 10 mM HEPES and 105/200 g/ml gentamicin; 105 cells in 0.1 ml of medium were dispensed into each well of a microtiter plate in the presence of 5 × 10^6 Hc/ml in 100 μl of 5 mM Tris and stored at −20 °C. The EDTA content of all protein samples used was measured by the method of Kratochvil and White (15). The average final EDTA concentration was 5 μM and was less than 25 μM in all cases (15).

In Vitro Assays of Hc Growth—Yeast suspended in HMM were added to each well of a microtiter plate in 190 μl and mixed with 10 μl of SP-A, SP-D, or collectin filtrate. In some experiments as indicated, myelositol (Sigma) was added to SP-D-containing wells in concentrations up to 20 μM. The yeast were grown at 37 °C in 5% CO_2. After 24 h of incubation, the plates were centrifuged at 1,000 × g, and the supernatant was gently removed. Fifty μl of [3H]leucine (1 Ci/mmol, PerkinElmer Life Sciences) and 5 μl of SP-A or SP-D, respectively, and stored at −20 °C. The fungal burden was expressed as cpm ± S.E.

Assays of Hc Permeability—Protein release from Hc exposed to the collectins was measured using the thiol-specific fluorophore Thioglo (Molecular Probes) (18). Hc (5 × 10^7 organisms/ml) in 100 μl of Heps-buffered saline solution were exposed to rat SP-A or rat SP-D at various concentrations for 15 min at 37 °C and then sedimented by centrifugation. The supernatant was incubated with 10 μl Thioglo1 in the presence and absence of the protein denaturing agent SDS, and thiol-containing proteins were detected using a fluorescent plate reader with excitation and emission wavelengths of 405 and 535 nm, respectively. Thiol-containing proteins were quantified by subtracting fluorescence due to released glutathione (i.e. signal obtained in the absence of SDS) from total fluorescence. The effect of the pulmonary collectins on Hc cell wall integrity was also assessed by determining permeability to an alkaline phosphatase substrate, ELF97 (Molecular Probes) (19). The collectins were incubated with 3.5 × 10^6 Hc/ml in 100 μl of 5 mM Tris and 150 mM NaCl for 15 min at 37 °C, and 100 μl ELF97 phosphatase substrate was added. Fluorescence was measured at excitation and emission wavelengths of 355 and 535 nm, respectively, for a period of 40 min.

Statistics—Differences between groups were analyzed by the Student's t test. If the data were not normally distributed, the Wilcoxon rank sum test was employed.

RESULTS

The Pulmonary Collectins Inhibit Protein Synthesis in Hc—To determine the consequences of the interaction between collectins and Hc, we attempted to determine if purified SP-A and SP-D altered protein synthesis of Hc. Both rat SP-A and rat SP-D markedly suppressed [3H]leucine incorporation into G217B, 186R, 186S, and a clinical Hc isolate in a dose-dependent fashion (Fig. 1). At higher concentrations of each collectin, the reduction in leucine incorporation approached 95–99%.

Non-specific killing of the yeast by low molecular weight copurifying substances did not contribute to the antimicrobial effect since the 10,000 M_0 cut-off ultrafiltrate of the highest SP-A and

![Figure 1](http://www.jbc.org/journals/fig1.png)
SP-D (not shown) concentrations used did not alter [3H]leucine incorporation (Fig. 2A). Furthermore, the structural integrity of SP-A and SP-D was critically important for function since boiling of either molecule abolished its antimicrobial activity (Fig. 2A). Myo-inositol, which competes for binding of SP-D to other microbial membranes (20), had no effect on SP-D-mediated inhibition of Hc protein synthesis at carbohydrate concentrations as high as 20 mM (data not shown). To assess if the effect of the surfactant proteins was species-specific, we incubated Hc with human SP-A (APP) and determined [3H]leucine incorporation. As was demonstrated for rat SP-A, APP inhibited growth of Hc in a dose-dependent fashion (Fig. 2B).

Subsequently, we sought to ascertain if the measurements of altered protein synthesis correlated with a true decrement in CFU. Yeast from G217B were exposed to SP-A or SP-D for 24 h and then cultured on brain heart infusion/blood agar plates. The recovery of Hc on plates was markedly inhibited by both SP-A and SP-D (Fig. 3A). It is important to note that neither SP-A nor SP-D aggregated Hc, based on light microscopic inspection of Hc cultured with collectins for 1 h (Fig. 3B) or overnight (not shown). These data indicate that SP-A and SP-D exert fungicidal activity. Next, we assessed the effect of the Hc inoculum size on the antimicrobial effects of SP-A and SP-D (Fig. 4). Increasing numbers of yeast from strain G217B were exposed to 25 μg/ml SP-A or SP-D and [3H]leucine incorporation determined. SP-A and SP-D inhibited the growth of yeast ranging in numbers from 5 × 10^5 to 5 × 10^8 in vitro.

Reduced Pulmonary Clearance of Hc in SP-A Null Mouse Models—The role of SP-A in the pulmonary clearance of Hc was tested in genetically engineered mice using outbred (Swiss Black) and inbred (C3H/HeN) SP-A null (SP-A⁻/⁻) lines. Hc CFU in lung homogenates were compared 1, 2, and 3 weeks after intranasal inoculation with 2 × 10^8 yeast. The lungs of Swiss Black SP-A⁻/⁻ mice contained significantly higher numbers (p < 0.05) of CFU compared with SP-A⁺/⁺ mice at 1 and 2 weeks, whereas the spleen CFUs were higher only at week 2 (Fig. 5). Likewise, the lungs of C3H/HeN SP-A⁻/₋ exhibited increased CFU (p < 0.05) as compared with wild-type animals at 1 week (Fig. 5). Thus, the absence of SP-A impairs the efficiency of clearance of this fungus.

Is the Impaired Elimination Associated with Changes in the Inflammatory Response?—Experiments were performed to determine whether the absence of SP-A influences the pulmonary inflammatory cell profile after Hc infection, and the results are shown in Table I. At week 1, the number of CD4⁺ or CD8⁺ T cells, neutrophils, and macrophages was diminished in the SP-A⁻/₋ mice, although only the CD8⁺ cell population achieved statistical significance (p < 0.05). By 2 weeks of infection in the wild-type and null mice, there were no substantial differences in the composition of the inflammatory response.

The Pulmonary Collectins Increase Fungal Cell Wall Permeability—To explore the mechanism by which the collectins kill Hc, we assessed if the integrity of the cell wall and cell membrane was perturbed by these molecules. One approach used was to examine protein efflux from the organism upon exposure to the collectins (Fig. 6). The ThioGlo reagents are a group of maleimide naphthylpyranones that rapidly react with accessible cysteines to yield a fluororescent product with a detection limit as low as 10 nM protein thiol (18). Because neither rat SP-A and rat SP-D nor the control proteins melittin or IgG contain free cysteines, this method can be used to assess release of thiol-containing proteins from antimicrobial peptide-treated organisms. Melittin, which is known to permeabilize Gram-negative bacteria, resulted in release of protein from Hc, but a nonspecific IgG had no effect. Exposure of Hc to rat SP-A at 25 or 50 μg/ml resulted in a significant increase in the content of thiol-containing proteins in the extracellular space compared with unexposed Hc, but the ultrafiltrate from the most concentrated collectins tested did not induce protein release. The effect of collectins on permeability of Hc was also assessed using ELF97, an alkaline phosphatase substrate that does not readily cross cell membranes and which fluoresces when cleaved by the enzyme. As shown in Fig. 7A, melittin at 3 μg/ml resulted in a rapid increase in cellular permeability, as did 10 μg/ml rat SP-A, 4 μg/ml rat SP-D, and 10 μg/ml human SP-A (APP). Boiling of APP destroyed the permeabilizing effect of the protein, and the fluorescence from organisms exposed to the ultrafiltrates from the highest collectin concentrations tested was not different from Hc exposed to a nonspecific IgG or to Hc alone (no added protein). The initial rate of permeabilization by the collectins and melittin were similar and significantly different from a nonspecific IgG (Fig. 7A, inset). The effect of SP-A and SP-D on membrane integrity was calcium-dependent (Fig. 7B). These data indicate that the pulmonary collectins kill Hc by disrupting the integrity of the surface.

Internalization by Macrophages Protects Hc from the Fungicidal Effects of the Pulmonary Collectins—Hc yeast demonstrates a strong avidity for macrophages. In vitro they are rapidly internalized and initiate replication. We attempted to determine if the intracellular milieu altered the ability of SP-A and SP-D to express fungicidal activity, and the results are shown in Fig. 8. Alveolar macrophages were harvested and exposed to yeast. Extracellular organisms were removed, and [3H]leucine incorporation was assessed. As a control, we analyzed the effect of SP-A and SP-D on killing extracellularly. Both SP-A and SP-D inhibited the growth of yeast grown in the absence of macrophages. Conversely, they did not alter Hc growth within alveolar macrophages. To determine whether this effect was specific to alveolar macrophages, we performed the identical experiment in peritoneal macrophages with SP-A.
This collectin inhibited growth of Hc in the absence of macrophages, whereas this effect was not observed in Hc that had been ingested by peritoneal cells. These data indicate that the intracellular environment serves as a haven from the inimical effects of the collectins.

**DISCUSSION**

The ability of Hc yeast to cause infection within the lung environment requires evasion of the cellular and molecular mediators of protection. Host cells such as dendritic cells and macrophages and cytokines including interferon-γ, tumor necrosis factor-α, and granulocyte macrophage colony-stimulating factor are key protective factors of the immune system (21). In this study, we have demonstrated that the surfactant proteins A and D exert potent, macrophage-independent fungicidal activity against the yeast phase of Hc. Death of the yeast was measured by two assays, [3H]leucine incorporation that correlates highly with viability and the more traditional growth on agar plates. In both assays incubation with the surfactant proteins caused a marked decrement in viability. This alteration was associated with a calcium-dependent, col-

**FIG. 3. SP-A and SP-D are cidal for Hc.** Panel A, Hc was exposed to various concentrations of rat SP-A for 24 h, and serial dilutions were plated on brain heart infusion agar containing sheep erythrocytes. Colony-forming units (CFU) were counted 7 days later. Data are expressed as mean CFU ± S.E. Panel B, Hc was incubated alone or with 20 µg/ml rat SP-A, 20 µg/ml rat SP-D, or collectin filtrate (F) and examined under the light microscope for evidence of evidence of aggregation.

**FIG. 4. Effect of inoculum size on inhibition of Hc growth by SP-A and SP-D.** SP-A or SP-D (25 µg/ml) were incubated with 5 x 10^3, 5 x 10^4, or 5 x 10^5 yeast, and [3H]leucine incorporation was assessed. Data represent the results of three experiments and are expressed as mean ± S.E.

**FIG. 5. SP-A−/− mice manifest impaired clearance of Hc yeast.** Outbred (Swiss Black (SB)) and inbred (C3H/HeN (C3H)) SP-A null (SP-A−/−) mice were infected with G217B Hc intranasally. After 1, 2, and 3 weeks, lungs and spleens were harvested, homogenized and plated on agar. Data are the mean CFU ± S.E., n = 7/group. * = p < 0.05.
Collectins and Histoplasmosis

Table I

| Mouse          | Weeks of infection | Mean cell No. ± S.E. |
|---------------|--------------------|---------------------|
|               |                    | CD4⁺    | CD8⁺    | Gr-1   | Mac-3   |
| Wild type     | 1                  | 2.8 ± 0.7 | 3.6 ± 0.9 | 3.9 ± 1.0 | 2.3 ± 0.3 |
| SPA⁻/⁻        | 1                  | 1.2 ± 0.3 | 0.8 ± 0.2* | 1.5 ± 0.1 | 1.4 ± 0.2 |
| Wild type     | 2                  | 3.9 ± 0.9 | 1.0 ± 0.2  | 0.5 ± 0.1 | 1.3 ± 0.4 |
| SPA⁻/⁻        | 2                  | 3.4 ± .5  | 1.7 ± 0.5  | 0.4 ± 0.1 | 0.6 ± 0.1 |

* p < 0.05.

Among the most notable findings is that the presence of SP-A is accompanied by an in vivo effect. Estimates of the concentrations of surfactant proteins in the alveolar lining fluid range from 300 to 1800 µg/ml for SP-A and from 36 to 216 µg/ml for SP-D, manyfold higher than levels that were found to be required to kill Hc in this study. Hence, the direct antimicrobial properties of SP-A and SP-D appear to play a physiologically relevant role in innate immune defense against Hc.

The yeast phase of Hc is the element that is responsible for nearly all of the clinicopathological manifestations of histoplasmosis (1). Although microconidia are initially deposited within the distal airspaces, they rapidly convert to yeast either within alveolar macrophages or in the extracellular environment. Intracellular yeast can multiply unrestricted until macrophages are destroyed by a massive burden of fungal elements. Thus, survival of yeast forms is the critical step in the establishment of infection. Yeast that are released from lysed macrophages would be exposed to the extracellular constituents of the lung including the collectins. In this context, the host can exert partial control over the replicative capacity of the yeast cell before the onset of cellular immunity, which occurs usually between 1 to 2 weeks after infection (17).

The data indicate that SPA and SP-D inhibit the biosynthetic capacity of Hc. The collectin-mediated reduction in protein synthesis by Hc was associated with the loss of the capacity to replicate, consistent with cell death. The Hc inoculum size did not affect the fungicidal properties of the collectins through the range of Hc numbers (Fig. 4), but at lower collectin-to-Hc ratios the killing effect is titrated and submaximal (Fig. 1). Four separate strains were susceptible to killing, indicating that the cidal effect of the collectins was not merely restricted to laboratory isolates or avirulent strains. The effect of the collectins on viability of Hc is not universal for all pathogenic fungi; however, since these molecules failed to kill the yeast phase of Blastomyces dermatitidis, another pathogenic soil-based fungus (data not shown). This fungus exists in the extracellular space more commonly than Hc and, thus, has most likely evolved to combat the activity of the collectins.

The antimicrobial activity of the collectins was destroyed by boiling, indicating that conformational integrity of the proteins is required. There was no killing activity detected in the 10,000 M₀ cutoff ultrafiltrate of any of the protein reagents used. Pulmonary surfactant proteins derived from diverse sources and purified by different methods, including native collectins purified from the alveolar lavage of rats and humans, all exhibited direct antimicrobial activity. Down-regulation of synthetic function induced by agglutination of the organism did not contribute to growth inhibition in the Hc assays in this study, since neither SP-A or SP-D aggregated the organism. These findings indicate that fungicidal effects of the pulmonary collectins are an intrinsic property of the proteins.

Two separate strains of mice lacking the gene for SP-A manifested impaired clearance of Hc. However, the defect in clearance was not dramatic. One explanation for this modest effect is that SPA⁻/⁻ mice still possess SP-D, which also exhibits potent anti-Hc activity. Thus, the isolated absence of SP-A did not cause a major failure in innate immunity against Hc. A second explanation is that Hc rapidly enters host phagocytes, in particular, alveolar macrophages. The reasons for this predilection for intracellular parasitism have not been fully defined, but the results herein strongly indicate that the intracellular niche provides an escape from the lethal effects of the surfactant proteins. Exposure of Hc within alveolar macrophages to concentrations of SP-A or SP-D that killed yeast directly failed to inhibit the growth of Hc. Hc were also protected from SPA-mediated killing by internalization into mouse peritoneal macrophages, which are naive to the collectins. Although it is true that surfactant proteins enter alveolar macrophages by endocytosis, it is likely that endosomal levels are only a fraction of the concentration in the alveolar lining fluid. SP-D⁻/⁻ mice have been derived, but we did not test the course of Hc in these animals because they exhibit time-dependent accumulation of surfactant within macrophages and the alveolar spaces. We believed this altered physiology would cloud the interpretation of infection experiments spanning several weeks. The ability of Hc to invade macrophages and thrive within them permits this fungus to evade the extracellular collectin levels that would otherwise be lethal.

Although surfactant proteins are crucial for optimal gas exchange in the alveolar space, they possess several immunoregulatory properties that make them a key contributor to lung host defenses. They have been shown to exert opsonic activity, to modulate production of proinflammatory cytokines such as tumor necrosis factor-α release (22), and to down-regulate T cell function (23). In this regard, we analyzed the inflammatory response to Hc in SP-A⁻/⁻ mice to determine whether there was a correlation between the fungal burden and the inflammatory response. At 1 week of infection, the number of phagocytes and T cells in the lungs of SP-A⁻/⁻ mice was depressed compared with wild-type infected controls, but only the CD8⁺ population was statistically less than controls. Because CD8⁺ are necessary for optimal expression of protective immunity in

Fig. 6. The pulmonary collectins induce protein leak from Hc. Protein efflux from Hc was assessed after exposure to SP-A, SP-A filtrate (F), SP-D, a nonspecific IgG (Ab), or melitin (Mel) for 15 min. Fluorescence was measured after derivatization with ThioGlo in the presence and absence of SDS using a fluorimeter. Data are the mean ± S.E., n = 3. *, p < 0.05; #, p < 0.01. a.u., arbitrary units.
primary infection (24–26), the decrement in this cell subpopulation may contribute to the elevated CFUs found in the lungs.

Several lines of evidence suggest that the collectins increase the permeability of the surface of Hc, as we have recently demonstrated for some but not all strains of *Escherichia coli* (8). Incubation of yeast with both collectins increased protein leak from the organism and enhanced the access of a hydrolysia-activated fluorescent substrate to intracellular alkaline phosphatase. The membrane permeability defect occurs rapidly, with a clear increase in the slope of fluorescence within 15 min. The mechanism by which SP-A and SP-D increase permeability is unknown, but as with most collectin functions, the effect is calcium-dependent. Calcium binding produces conformational shifts in the carbohydrate recognition domain (CRD), which may expose charged or hydrophobic protein moieties capable of perturbing the cell surface. Alternatively, calcium may form the bridge for collectin binding to the membrane interface. It is interesting to note that the crystal structure of SP-D reveals large charged domains on the membrane face of the CRDs that could conceivably interact with membrane phospholipids or with other surface molecules (27) and disrupt membrane function.

The first point of contact between inhaled Hc and the distal airspaces is the alveolar lining fluid. The intimate association of SP-A and SP-D with surfactant positions the proteins at the air-liquid interface in an ideal presentation for interaction with inhaled pathogens. The affinity of SP-A and perhaps SP-D for specific phospholipid components of surfactant may also significantly alter collectin function and availability, however, and further experiments will be required to define their antifungal effects in the presence of lipids.

The Hc uses phagocytosis to gain access to its intracellular niche. Macrophages lack the ability to kill Hc through oxidant or proteolytic mechanisms (28, 29–31), and the organism has developed strategies to resist the inimical contents of phagosomes (30, 32, 33) and to acquire endosomal iron (34, 35). The ability to enter into phagocytes provides a haven from the potentially destructive extracellular environment and suggests a new strategy for host evasion by Hc.

In summary, the data in this study indicate that SP-A and SP-D are fungicidal. This newly identified collectin function provides a mechanism for control of Hc proliferation in the preinflammation phase of the host/pathogen interaction.

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