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Surfactant Protein D Inhibition of Human Macrophage Uptake of \textit{Mycobacterium tuberculosis} Is Independent of Bacterial Agglutination\textsuperscript{1}

J. Scott Ferguson,\textsuperscript{2*} Dennis R. Voelker,\textsuperscript{2†} Jennifer A. Ufnar,\textsuperscript{2*†} Amanda J. Dawson,\textsuperscript{†} and Larry S. Schlesinger\textsuperscript{2†}

The innate immune system in the lung is essential for controlling infections due to inhaled pathogens. \textit{Mycobacterium tuberculosis} (\textit{M.tub}) encounters components of the innate immune system when inhaled into the lung, but the outcomes of these interactions are poorly understood. Surfactant protein D (SP-D) binds to and agglutinates \textit{M.tb} bacilli, and reduces the uptake of the bacteria by human macrophages. In the current studies, we utilized a recombinant SP-D variant (CDM) that lacks the collagen domain to further characterize the interaction of SP-D with \textit{M.tb}, and determine the effects of agglutination on bacterial uptake by human monocyte-derived macrophages. These studies demonstrate that the binding of SP-D and CDM to \textit{M.tb} is saturable and inhibited by carbohydrate competition and Ca\textsuperscript{2+} chelation, implicating the carbohydrate recognition domain in the interaction. Fluorescence microscopy reveals that dodecameric SP-D leads to agglutination of the bacilli, whereas the trimeric CDM does not, demonstrating that the multivalent nature of SP-D is essential for agglutination of \textit{M.tb}. However, preincubation of \textit{M.tb} with increasing concentrations of SP-D or CDM leads to a concentration-dependent reduction in the uptake of the bacteria by macrophages, indicating that agglutination does not play a direct role in this observation. Finally, the reduced uptake of \textit{M.tb} by SP-D is associated with reduced growth of \textit{M.tb} in monocyte-derived macrophages. These studies provide direct evidence that the inhibition of phagocytosis of \textit{M.tb} effected by SP-D occurs independently of the aggregation process. \textit{The Journal of Immunology}, 2002, 168: 1309–1314.

\textbf{Mycobacterium tuberculosis} (\textit{M.tb}),\textsuperscript{3} the causative bacterium of pulmonary tuberculosis, is a highly host-adapted intracellular pathogen of mononuclear phagocytes that has the ability to evade multiple mechanisms of host defense. Evidence from several studies supports a role for pulmonary surfactant and its collectin proteins, surfactant proteins A and D (SP-A and SP-D), in the innate immune response of the lung against multiple pathogens through direct effects on host cells, agglutination of microorganisms, and modulation of the adaptive immune response (1, 2). Mice with homozygous null alleles for the SP-A gene are more susceptible to infection by several microorganisms than wild-type animals (3–7). In addition, mice with homozygous null alleles for SP-D have increased inflammation in the lungs when challenged with group B \textit{Streptococcus} or \textit{Hemophilus influenzae} (8). As \textit{M.tb} bacilli are inhaled into the lung, the bacteria will encounter pulmonary surfactant before and during uptake into the alveolar macrophage. It is likely that this interaction between the bacterium, surfactant, and the alveolar macrophage will have significant effects on the uptake and subsequent intracellular fate of \textit{M.tb}.

Work from our laboratory demonstrates that SP-A increases the phagocytosis of \textit{M.tb} by alveolar macrophages through a direct interaction with the macrophage, potentially by up-regulating macrophage mannose receptor activity (9). In contrast to SP-A, we have recently demonstrated that SP-D reduces the phagocytosis of \textit{M.tb} due in part to an interaction between SP-D and the bacterium (10). These data provided evidence for distinct immune functions of SP-A and SP-D in the lung. SP-D binds to \textit{M.tb} in a manner consistent with an interaction between the carbohydrate recognition domain (CRD) of the protein and the surface of the bacterium. SP-D binds to the terminal mannose units (mannose caps) of the \textit{M.tb} surface lipoglycan, lipoarabinomannan (LAM).

The effects of microbial agglutination in vitro on the pathogenesis of diseases due to respiratory pathogens are largely unknown. It is possible that agglutination could serve to enhance mucociliary clearance of bacteria and viruses, alter host cell function such as cytokine release or reactive oxygen species formation, or modulate the phagocytic interactions between host cell and microbe. The concentration of SP-D in the airspace is estimated to be ≈36–212 μg/ml in the rat (2), which is much higher than concentrations required for \textit{M.tb} aggregation in vitro (10). The purpose of this study was to determine the relationship between agglutination of \textit{M.tb} and the subsequent uptake of bacteria...
into macrophages. We specifically tested the role of SP-D valency on mycobacterial agglutination and the subsequent effects of altered valency on modulation of macrophage uptake. Our findings reveal that collagen domain-dependent loss of valency abolishes microbial aggregation, but does not alter the inhibitory effect of SP-D on macrophage uptake of the pathogen.

Materials and Methods

Reagents

RPMI 1640 medium with l-glutamine (RPMI) was purchased from Life Technologies (Grand Island, NY). Middlebrook 7H9 broth was purchased from DIFCO. Materials and Methods (BBL Microbiology Systems (BD Biosciences, Mountain View, CA). 7H11 agar was prepared with Bacto Middlebrook 7H10 agar (Difco Laboratories, Detroit, MI), oleic acid-albumin-dextrose-catalase enrichment medium (Difco), 0.1% casein enzymatic hydrolysate (Sigma, Aldrich, St. Louis, MO), and glycerol (Difco), as described (11), and dispersed into 100 × 15-mm bacteriological petri dishes. Auramine-rhodamine stain (Difco) was purchased. Formaldehyde solution 37% w/w and potassium Hausser chamber. Bacteria prepared in this fashion are (13). A rabbit anti-SP-D antiserum was prepared as described (10).

Growth and preparation of bacteria

Lyophilized M.tb Erdman strain (ATCC 35801) was obtained from American Tissue Culture Collection (Manassas, VA), reconstituted, and used as described (11). Briefly, for each experiment, aliquots of frozen stock in 7H9 broth were thawed. The bacteria were then cultured for 9–11 days on 7H11 agar, scraped from the culture plate into microfuge tubes containing buffer or medium and two 4-mm glass beads, pulse-vortexed briefly (1–2 s), and allowed to settle over 30 min. The upper bacterial suspension was then removed to a second tube, and allowed to settle 10 min. An aliquot from the second tube was then transferred to a third tube and allowed to settle for an additional 10 min. The top portion of this aliquot was used in the experiments. The concentration of bacteria (1–2 × 10^6 bacteria/ml) and the degree of clumping (≤10%) were determined by counting in a Petroff-Haussner chamber. Bacteria prepared in this manner are ≥90% viable by CFU assay.

Isolation and preparation of human macrophages

Monolayers of monocyte-derived macrophages (MDM) were prepared from healthy purified protein derivative-negative human volunteers using a protocol that has been approved by the Internal Review Board, College of Medicine, University of Iowa (Iowa City, IA; 9709753) as described (14). Briefly, mononuclear cells were isolated from heparinized blood on Ficoll, infected protein derivative-negative human volunteers using a microplate spectrophotometer (Benchmark; Bio-Rad). The mean ± SD of duplicate wells for each concentration of collectin was calculated in each experiment. The background (≤0.15 absorbance units) for each concentration of collectin was defined as the absorbance of wells incubated without collectin in the absence of M.tb, and was subtracted out in each case. The absorbance of wells containing M.tb that were incubated with primary and/or secondary Abs, but not collectin was below background levels (≤0.05 absorbance units). In experiments to determine the calcium-dependence or carbohydrate-inhibition of SP-D or CDM binding to M.tb, collectins were incubated at a fixed concentration in triplicate microtiter wells, in the presence or absence of 2 mM CaCl2, 100 mM maltose, 100 mM N-acetyl-d-galactosamine, or 10 mM EDTA, and then processed as described above.

Agglutination assay

To determine whether increasing concentrations of SP-D or CDM caused agglutination of M.tb, we used fluorescence microscopy combined with ordinate scale counting. Bacterial suspensions (1 × 10^7/ml in TBS) were incubated in the presence of 2 mM CaCl2, with increasing concentrations of SP-D or CDM, and then allowed to dry on glass coverslips. After fixing with 10% formalin, the bacteria were stained with auramine-rhodamine, and examined under fluorescence microscopy at ×1250. At least 300 sequential single bacteria and clumps (two or more bacteria in direct contact) were counted per coverslip. The ratio of percentage clumps in the experimental condition (SP-D or CDM) to the percentage clumps in the control condition, human serum albumin (HSA), was used as the agglutination index.

M.tb uptake by macrophages

M.tb was incubated in RPMI containing 20 mM HEPES, and 1 mg/ml HSA (pH 7.2) in the presence or absence of increasing concentrations of SP-D or CDM for 1 h at 4°C. Following the incubation period, the M.tb/cell mixture was added to MDM previously incubated for 2 h at 37°C in 5% CO2 and air. The multiplicity of infection was 10 bacteria/MDM. After nonadherent bacteria were washed away, the coverslips were fixed in 10% formalin, and bacteria were stained with auramine-rhodamine. The macrophages were counterstained using 5% potassium permanganate. The mean number (±SD) of cell-associated bacteria per macrophage on three coverslips per condition was determined by counting ≥100 consecutive macrophages per coverslip using phase-contrast and fluorescence microscopy at ×1250 magnification (14). Under these conditions for Erdman M.tb, uptake by the macrophage is equivalent to phagocytosis as assessed by transmission electron microscopy (10).

Bacterial ELISA

In experiments designed to determine the saturable binding of SP-D or CDM to M.tb, bacteria were suspended (1.5 × 10^7 bacteria/ml) in 50 mM Tris-HCl containing 150 mM NaCl, pH 7.5 (TBS). Aliquots (100 μl) of the bacterial suspension or buffer control were then dried in the wells of a microtiter plate (Immulon 1; Dynatech Laboratories, Chantilly, VA) (10, 1, 1), followed by exposure to UV light for 2 h to kill viable bacteria. After blocking with TBS containing 2 mM CaCl2, 3% BSA, and 0.2% Triton X-100 overnight at 4°C, the wells were then incubated with increasing concentrations of SP-D or CDM in blocking buffer for 60 min at room temperature. After washing away unbound collectin, the wells were incubated with a 1/10,000 dilution of anti-SP-D antiserum in blocking buffer for 2 h at room temperature, washed, and then incubated with a 1/3,000 dilution of HRP-conjugated goat anti-rabbit IgG for 2 h at room temperature. After washing, ABTS substrate (Bio-Rad, Hercules, CA) was added. The substrate development was stopped after 20 min by 1% oxalic acid, and the absorbance of individual wells was determined at 405 nm (A405) in a microplate spectrophotometer (Benchmark; Bio-Rad). The mean ± SD of triplicate wells for each concentration of collectin was calculated in each experiment. The background (≤0.15 absorbance units) for each concentration of collectin was defined as the absorbance of wells incubated without collectin in the absence of M.tb, and was subtracted out in each case. The absorbance of wells containing M.tb that were incubated with primary and/or secondary Abs, but not collectin was below background levels (≤0.05 absorbance units). In experiments to determine the calcium-dependence or carbohydrate-inhibition of SP-D or CDM binding to M.tb, collectins were incubated at a fixed concentration in triplicate microtiter wells, in the presence or absence of 2 mM CaCl2, 100 mM maltose, 100 mM N-acetyl-d-galactosamine, or 10 mM EDTA, and then processed as described above.

Collectin proteins and Abs

Recombinant rat SP-D (SP-D) and the collagen domain deletion mutant of SP-D (CDM) were produced in Chinese hamster ovary cells (12, 13). Briefly, the cDNA for rat SP-D or the CDM mutant were ligated into the pEE14 vector using HindIII and SacI and/or secondary Abs, but not collectin was below background levels (≤0.05 absorbance units). In experiments to determine the calcium-dependence or carbohydrate-inhibition of SP-D or CDM binding to M.tb, collectins were incubated at a fixed concentration in triplicate microtiter wells, in the presence or absence of 2 mM CaCl2, 100 mM maltose, 100 mM N-acetyl-d-galactosamine, or 10 mM EDTA, and then processed as described above.

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M.tb intracellular growth assays in macrophages

Intracellular growth assays using CFU were performed as described (15). Briefly, 12-day-old MDMs were washed with RPMI, then incubated with M.tb at the presence or absence of 20% autologous serum. The cells were then removed from the Teflon wells and washed, then resuspended in RPMI containing 10% autologous serum. For M.tb uptake experiments, the MDM were then plated onto Chromerge-cleaned glass coverslips in 24-well tissue culture plates (Falcon; BD Labware, Mountain View, CA) for 2 h at 37°C (1 × 10^6 MDM/coverslip). The wells were washed with RPMI and then used in the experiments. For M.tb intracellular growth assays, the MDM were plated in the wells of 24-well tissue culture plates for 2 h, washed with RPMI, then incubated with RPMI containing 20% serum for an additional 7 days at 37°C.

Statistical analysis

For M.tb uptake and intracellular growth assays, the absolute level of uptake or growth by macrophages in each experiment varies among donors, although the pattern of experimental results is the same from donor to donor.
donor. To account for this variability, we normalized data to the internal control for each experiment. A ratio of experimental results to control (expressed as percent decrease) was obtained, and the mean ratio was then tested for a significant difference from one using $t$ statistics. Statistical significance was defined as $p < 0.05$. This method has been verified by the Statistical Support Center, University of Iowa.

**Results**

**Characterization of anti-SP-D antiserum against SP-D and CDM**

To determine whether a rabbit anti-rat SP-D antiserum recognized SP-D and CDM, Western blotting was performed (Fig. 1). By densitometry (not shown), the anti-SP-D antiserum recognition of recombinant rat SP-D and CDM was comparable on a weight for weight basis. For a given weight, more molecules of CDM are present because the m.w. of the two proteins differ ($\approx$512 kDa for SP-D, $\approx$66 kDa for CDM; Ref. 13).

*The binding of SP-D and CDM to M.tb*

The binding of SP-D to M.tb is carbohydrate inhibitable and calcium dependent, consistent with CRD-mediated binding of the protein to carbohydrate structures on the surface of the bacteria (10). However, the importance of the collagen-like region of SP-D and its associated N-linked sugar in this interaction is unknown. To determine whether the binding of SP-D to M.tb is modulated by the collagen domain or N-linked sugar, we used a previously characterized mutant SP-D protein (CDM) that is deficient in these domains. The collagen domain is necessary for complete oligomer (dodecamer) formation of SP-D, but is not necessary for trimerization. Thus, the CDM protein forms trimers but does not form dodecamers, and contains the identical CRD of SP-D (13). Using the solid-phase bacterial ELISA (Fig. 2), both CDM and SP-D bind to M.tb in a saturable fashion, although the $A_{405}$ for a given concentration is less for the CDM protein. Double reciprocal plot analyses of these data reveal that the approximate dissociation constant of SP-D binding to M.tb is $\approx$10-fold less than that for CDM ($7.1 \times 10^{-10}$ M for SP-D, $7.4 \times 10^{-9}$ M for CDM), indicating that the avidity of SP-D binding to M.tb is greater than its mutant counterpart.

Because the CRDs of CDM and SP-D are identical, the binding of CDM to M.tb should also be carbohydrate inhibitable and calcium-dependent. To investigate this, CDM and SP-D binding to M.tb was compared in the presence of competing carbohydrates or EDTA. By ELISA (Fig. 3), maltose and EDTA inhibited the binding of CDM to M.tb, whereas the binding in the presence of N-acetyl-d-galactosamine was similar to control. These data indicate that the binding of CDM to M.tb is carbohydrate-inhibitable and calcium-dependent, consistent with the known carbohydrate-binding properties of the CRD of SP-D.
These data combined with our earlier studies provide strong evidence that the CRD of SP-D binds to M.tb. The avidity of this interaction is enhanced by the structure imparted by the collagen domain, likely due to the multivalent nature of SP-D.

Agglutination of M.tb by SP-D and CDM
The innate immune properties of SP-D are believed to be multiple. One of these properties is agglutination of bacteria and viruses (1). Previously, we determined that SP-D at concentrations of ≥5 μg/ml agglutinated M.tb, whereas concentrations <1 μg/ml did not (10). The concentration of SP-D in pulmonary surfactant is estimated to be ~36–212 μg/ml (2), a concentration that would be expected to cause agglutination of the bacilli based on our previous data. We hypothesize that the ability of SP-D to agglutinate M.tb is in part determined by the multivalent binding properties of the protein. To investigate this, we performed fluorescence microscopy experiments comparing M.tb agglutination due to SP-D or CDM (Fig. 4, A and B). Because of the hydrophobic nature of the M.tb surface, this bacterium has some degree of inherent clumping that is observed in the control condition (Fig. 4Aa). However, SP-D at concentrations ≥1 μg/ml caused agglutination of M.tb bacilli that was visibly apparent compared with the control condition (Fig. 4Ab). In contrast, no agglutination above control was measured using CDM at any concentration.

Uptake of M.tb by human macrophages in the presence of SP-D or CDM
Nonagglutinating concentrations of SP-D reduce the phagocytosis of M.tb by human macrophages due to an interaction between the bacteria and the protein (10). However, phagocytosis of M.tb in the presence of agglutinating concentrations of SP-D, such as those likely to exist in the lung, could have different effects. SP-D could reduce phagocytosis simply by masking phagocytic receptor ligands on the surface of the bacteria, or if agglutination is present, could reduce phagocytosis by presentation of large, poorly phagocytosed particles. Alternatively, it is conceivable that agglutination of the bacteria may serve to paradoxically enhance the net uptake of M.tb by uptake of clumps of bacteria, albeit possibly with fewer phagocytic events. We performed experiments to determine whether agglutination of M.tb due to SP-D alters the reduction in bacterial uptake by MDM (Fig. 5). SP-D reduced the uptake of M.tb by MDMs over a range of concentrations including those that do and do not lead to agglutination of bacteria. These data suggest that agglutination of the bacilli by SP-D does not lead to enhanced uptake by the macrophage. The CDM mutant is trimeric rather than dodecameric like wild-type SP-D, and does not agglutinate M.tb (Fig. 4). Despite this, the CDM protein showed an equivalent reduction in uptake of bacteria by MDMs when used at much higher molar concentrations than the concentration of SP-D. Thus, agglutination of M.tb by SP-D does not play a significant role in the reduction of uptake of M.tb bacilli. These data, along with the data in our previous study, provide evidence that SP-D reduces the phagocytosis of M.tb by binding to ligands on the surface of the bacteria, and thereby modulating the bacteria-macrophage interaction.

Intracellular growth of M.tb in macrophages in the presence of SP-D
Successful infection of the human host by M.tb requires multiplication of the bacilli within macrophages. Because SP-D reduces the phagocytosis of M.tb by macrophages, we hypothesized that SP-D would also reduce the intracellular growth of M.tb. To investigate this, we performed CFU assays for intracellular growth of M.tb in MDMs in the presence and absence of SP-D (Table I).
We demonstrate that agglutination of the bacilli per se is not responsible for the reduced uptake of \( M.\text{tb} \) by macrophages. Finally, our data provide evidence that SP-D reduces the intracellular growth of \( M.\text{tb} \).

Because the CRDs of SP-D and CDM are identical, these data demonstrate that the binding between SP-D and \( M.\text{tb} \) occurs via the CRD of the protein and carbohydrates on the surface of the bacteria. The CRD of SP-D is relatively specific in its binding to carbohydrates, with the highest affinity binding occurring to di- and trisaccharides, and low-affinity binding to amino sugars (16). The surface of \( M.\text{tb} \) is composed of complex and simple carbohydrates, lipopolysaccharides, glycolipids, and glycoproteins (17). LAM, the major lipoglycan on the surface of \( M.\text{tb} \), has terminal dimannosyl units that are acceptor molecules for SP-D binding (10, 18).

The estimated dissociation constants of SP-D and CDM binding to \( M.\text{tb} \) differ by \( \approx 10\)-fold. This result is of interest because the CRDs of the proteins are identical. The most likely explanation for this result is that SP-D in its native dodecameric form is multivalent with respect to trimeric CRDs. Each trimeric CRD is functionally univalent with respect to carbohydrate binding, but in dodecameric SP-D, four trimeric CRDs linked by the rigid collagen-like region, are available for binding to carbohydrates (19–21). In the cruciform configuration it is likely that two trimeric CRDs can interact with a single bacterial surface, thereby amplifying the affinity of a single CRD.

The presence of the collagen domain is necessary for SP-D to agglutinate \( M.\text{tb} \) bacilli, because the CDM protein does not have agglutinating properties, even at very high concentrations. Therefore, it is likely that in addition to imparting multivalency to SP-D, the long, rigid structure of the collagen domain also allows for greater bridging interactions between the bacteria (20, 21). These data are supported by other studies that indicate that the aggregating properties of collectins is determined by valency, carbohydrate affinity, and the spatial array of the CRDs (13, 22–24).

Our previous and current data also demonstrate that SP-D does not enhance the interaction between \( M.\text{tb} \) and macrophages, but serves to reduce this interaction (10). Agglutination of bacteria does not appear to play a role in this observation, because the CDM protein does not agglutinate \( M.\text{tb} \), but reduces the uptake of \( M.\text{tb} \) by human macrophages. Because SP-D binds to the terminal dimannosyl units of LAM that are ligands for the mannose receptor, these data suggest that SP-D reduces the uptake of \( M.\text{tb} \) by human macrophages by binding to the surface-exposed LAM and blocking its interaction with the mannose receptor.

Although agglutination of \( M.\text{tb} \) by SP-D was not required for the altered bacterial phagocytosis by the macrophage in this study, the possibility remains that agglutination may be an important host-defense mechanism. For instance, mucociliary clearance is important in several pulmonary infections (25). Agglutination of bacteria may enhance mucociliary clearance. In addition, postphagocytic events may be altered by aggregation of bacilli (26).

Phagocytosis of an organism is the net result of multiple interactions between the microorganism and host cell. These interactions depend upon ligation of phagocytic receptors by microbial ligands, or suitable opsonins. A microorganism that has many “natural” receptors on the surface of the host cell, may in fact enter the cell without the aid of opsonins, whereas other microorganisms that have few natural receptors may require binding by opsonins to engage phagocytic receptors. There is convincing evidence that \( M.\text{tb} \) is phagocytosed by macrophages via complement receptors and the macrophage mannose receptor in the absence of opsonins in vitro (11, 14, 27, 28). Therefore, SP-D may modulate the uptake of \( M.\text{tb} \) by altering the usual interaction between the ligands on the bacterial surface and receptors on the macrophage. The relative affinities of phagocytic receptors, opsonins, opsonin acceptor sites, and native microbial surface ligands for one another likely influence the ultimate outcome of pathogen interactions with the host. In support of these hypotheses, accumulating data indicate that the effects of the collectins, SP-A and SP-D, will be unique for different pathogens. SP-D enhances the phagocytosis of Aspergillus fumigatus conidia, and certain strains of Escherichia coli, Strep.

tococcus pneumoniae, and Staphylococcus aureus by neutrophils (22, 29). SP-D is an opsonin for Pseudomonas aeruginosa and Klebsiella pneumoniae by alveolar macrophages (30, 31). Thus, SP-D can either inhibit or enhance interactions between microbes and host cells, and as such, is not simply a nonspecific host defense protein.

Beyond cell association, the intracellular fate of organisms that are phagocytosed by macrophages will likely be influenced by surfactant proteins (7, 29, 32). Our data provide evidence that reduced uptake of \( M.\text{tb} \) is associated with reduced intracellular growth in macrophages.

Table I. SP-D reduces the intracellular growth of \( M.\text{tb} \) in MDM

<table>
<thead>
<tr>
<th>SP-D (µg/ml)</th>
<th>0.5</th>
<th>5.0</th>
</tr>
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<tbody>
<tr>
<td>Growth (% of control CFU)</td>
<td>50.72 ± 8.1</td>
<td>29.94 ± 3.9</td>
</tr>
<tr>
<td>p value</td>
<td>0.013</td>
<td>0.018</td>
</tr>
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</table>

* \( M.\text{tb} (1.6 \times 10^3) \) was incubated with SP-D as described for uptake assays, then incubated with MDM for 2 h at 37°C. After washing away nonadherent bacteria, the monolayers were fixed in 10% formalin. The bacteria were stained with auramine-rhodamine, and visualized under phase-contrast and fluorescence microscopy. The number of bacteria per MDM was enumerated by counting ≥100 consecutive MDM per coverslip. The percent of control uptake was calculated by dividing the mean number of bacteria per MDM in the presence of collectin by the control value (mean number of CFU in the absence of SP-D for each experiment). The percent of control uptake was calculated by dividing the mean number of bacteria per MDM in the presence of collectin by the control value (mean number of CFU in the absence of SP-D for each experiment) × 100. Shown is the mean ± SEM of triplicate determinations from two (5.0 µg/ml SP-D), or three (0.5 µg/ml SP-D) independent experiments, and the p value as determined by \( t \) statistics.
Although there are similarities between SP-A and SP-D, these collectins appear to have unique host defense effects (33). The role that these proteins play in the defense of the host when both are available in the airspace is not known. It is likely that the precise role for each protein will be influenced by host-specific and microbe-specific factors. It is interesting to note that recent data suggest an association among several SP-A and SP-D alleles and the susceptibility to tuberculosis (34).

The human alveolar macrophage is a natural niche for virulent M.tuberculosis, whereas for many other pathogens, entry into this host cell is fatal for the pathogen. Those factors that enhance uptake of M.tuberculosis into macrophages, such as SP-A, are likely to be detrimental to the host. We speculate that reducing the alveolar macrophage uptake and growth of M.tuberculosis will be protective of the human host, and therefore that SP-D serves as a host defense protein against tuberculosis infection.

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