Complement-mediated host defense in the lung

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Complement-mediated host defense in the lung. Am J Physiol Lung Cell Mol Physiol 279: L790–L798, 2000.—Complement is a system of plasma proteins that aids in the elimination of pathogens from the body. We hypothesized that there is a functional complement system present in the lung that aids in the removal of pathogens. Western blot analysis revealed complement proteins of the alternative and classical pathways of complement in bronchoalveolar lavage fluids (BALF) from healthy volunteers. Functional classical pathway activity was detected in human BALF, but there was no significant alternative pathway activity in lavage fluid, a finding that correlates with the low level of the alternative pathway protein, factor B, in these samples. Although the classical pathway of complement was functional in lavage fluid, the level of the classical pathway activator C1q was very low. We tested the ability of the lung-specific surfactant proteins, surfactant protein A (SP-A) and surfactant protein D (SP-D), to substitute for C1q in classical pathway activation, since they have structural homology to C1q. However, neither SP-A nor SP-D restored classical pathway activity to C1q-depleted serum. These data suggest that the classical pathway of complement is functionally active in the lung where it may play a role in the recognition and clearance of bacteria.

bronchoalveolar lavage fluid; surfactant protein A; C1q; collectin

COMPLEMENT IS A SYSTEM OF plasma and cell-associated proteins that aids in local inflammatory responses, recruitment and activation of immune cells, opsonization and clearance of immune complexes, and lysis of some types of bacteria. The liver is the major site of synthesis of complement proteins (4), but extrahepatic tissues also synthesize and secrete complement proteins locally. Tissue-specific complement synthesis is thought to provide an immediate, localized source of complement components to tissues at the onset of infection, but in most cases these tissue-specific complement pools are poorly characterized.

The epithelium of the lung is a very thin barrier that promotes rapid gas exchange between the external environment and the pulmonary blood. This delicate structure is vulnerable to damage caused by inhaled microorganisms and other noxious particles. A local source of complement along the alveolar epithelium may provide an important early clearance mechanism for foreign particles before immune cells are recruited and systemic complement can reach the lung.

A few studies have provided evidence for the presence of complement components at the alveolar epithelium where inhaled airborne particles and microorganisms are deposited. Isolated human alveolar macrophages, the resident phagocytic cells in the lung, and alveolar type II epithelial cells synthesize and secrete specific components of the classical (C2, C3, C4, and C5) (1, 9, 29) and alternative pathways (factor B) (9, 29) in culture. In addition, low levels of some complement proteins have been detected in alveolar surfacet lining fluid obtained by lung lavage of humans (26, 27), baboons (19), rats (10), and rabbits (12).

Although some complement components have been detected in lavage fluid, only indirect evidence suggests that this local complement pool functions in lung host defense. Increased susceptibility to infections of the respiratory system has been reported in individuals with complement deficiencies (2, 3, 11). The common occurrence of pulmonary infections in these patients suggests that complement may play a role in mucosal immunity of the lung. Also, the effect of complement depletion on lung bacterial clearance was previously investigated using a mouse model of complement deficiency induced by intraperitoneal injection of cobra venom factor, which stabilizes the alternative pathway C3 convertase so that C3 and C5 sources are exhausted. Complement-depleted animals were unable to clear certain types of bacteria (Streptococcus pneumoniae and Pseudomonas aeruginosa) from their lungs as efficiently as saline-treated control animals (13). These results suggest a role for complement in the clearance of pathogens from the lung, but they do not exclude the possibility that the decreased clearance of bacteria from the lung may be due to systemic complement depletion rather than depletion of components present locally in the lung. Therefore, the aim of our study was to analyze the presence and functional significance of complement components in the lung.

MATERIALS AND METHODS

Human serum and lavage samples. Normal human serum was prepared from blood drawn from healthy volunteers.

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Aliquots of serum were frozen at −80°C until used. Healthy human nonsmokers between the ages of 18 and 35 were used for voluntary lung lavage. Subsegments of both the lingula and right middle lobes of the lung were each lavaged with 270 ml of sterile saline, and 80% of this volume was routinely recovered. Cells were removed from whole lavage by centrifugation at 228 g for 10 min. Several aliquots of each uncentrifuged lavage sample were saved at −80°C for protein quantification and Western blot analysis. Because such a large volume of dilute human lavage fluid was obtained, samples were concentrated ~200-fold by centrifugation at 4°C using Centriprep 10 concentrators (Amicon, Beverly, MA) with a molecular mass cutoff size of 10,000 Da in the presence of 10 mM EDTA to minimize complement activation during the concentration process. Aliquots of concentrated bronchoalveolar lavage fluids (BALF) were stored at −80°C until used. Calcium and magnesium were added back to samples just before their use in the functional assays described below. For classical pathway assays, 2.6 mM calcium and 17.4 mM magnesium were added to samples before use. For alternative pathway assays, 20 mM magnesium (and no calcium) was added to samples before use.

**Protein concentration.** The total protein concentration of lavage and serum samples was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL).

**Western blot analysis.** Equal amounts of total protein (1.2 μg) from serum or dilute lavage samples (before concentration) were reduced with 50 mM dithiothreitol and subjected to SDS-PAGE on 7.5% gels using the Hoefer minigel system (Hoefer Scientific Instruments, San Francisco, CA) at 30 mA for 1 h. Proteins were transferred to nitrocellulose membranes for 2 h at 250 mA or overnight at 30 V. Membranes were blocked for at least 1 h at 25°C in Tris-buffered saline (TBS) containing 5% nonfat powdered milk and probed for complement components using polyclonal antibodies directed against various complement proteins. Goat anti-human C3 and goat anti-human C4 were purchased from Chemicon (Temecula, CA); goat anti-human Clq, goat anti-human factor B, and goat anti-human C1 inhibitor were purchased from Calbiochem (La Jolla, CA). The primary antibody was heat inactivated at 56°C for 30 min and added to TBS with 5% nonfat powdered milk at concentrations from 1:500 to 1:2,500 according to the manufacturer’s suggestions for each antibody. In all cases, a secondary antibody labeled with horseradish peroxidase (rabbit anti-goat IgG-HRP; Pierce) was used at a concentration of 1:5,000, and immunoreactive bands were detected using the enhanced chemiluminescence assay (Amersham Life Science, Buckinghamshire, UK). The relative intensities of immunoreactive bands detected by Western blot analysis in serum and lavage samples were quantified by densitometry using National Institutes of Health Image software. The apparent molecular masses of the bands were also compared.

**Hemolytic assay.** The functional activity of the classical pathway in serum and lavage samples was determined by a standard hemolytic assay (18). Two hundred microliters of antibody-coated sheep erythrocytes (EA; 1 × 10⁸; obtained from Advanced Research Technologies, San Diego, CA) were added to 300 μl of GVB++ (veronal-buffered saline containing 0.1% gelatin, 0.15 mM CaCl₂, and 1 mM MgCl₂; pH 7.4–7.5) containing 240 μg of either serum or lavage total protein as a complement source. This 500-μl volume was incubated for 30 min at 37°C with gentle shaking. After the reaction, 1 ml of cold, sterile saline was added to each tube and unlysed EA were pelleted by centrifugation at 2,000 g for 5 min at 4°C. The optical density at 412 nm (OD412) of the supernatants was used to determine the amount of hemoglobin released by the red blood cells (RBC), and, therefore, the amount of lysis that took place. For each hemolytic assay, a 0% lysis (spontaneous lysis) tube was included, which contained EA but no complement source. A 100% lysis tube was also included, which contained EA without a complement source but which was lysed by the addition of 1 ml of water rather than saline. The percent lysis for each condition was calculated by comparison to the 100% lysis tube and 0% lysis tube as follows:

\[
\text{Percent Lysis} = \frac{\text{OD}_{\text{supernatant from test sample}} - \text{OD}_{\text{supernatant from 0% lysis tube}}}{\text{OD}_{\text{supernatant from 100% lysis tube}} - \text{OD}_{\text{supernatant from 0% lysis tube}}} \times 100\%
\]

A negative value indicated that the test sample resulted in less lysis than the 0% control tube. BALF activity is expressed as the percent of activity in a serum sample with equivalent protein concentration.

The functional activity of the alternative pathway in serum and lavage samples was determined in an alternative pathway hemolytic assay using rabbit RBC, which activate the alternative pathway of complement spontaneously. One hundred microliters of GVB-MgEGTA (20% veronal-buffered saline, 0.02% gelatin, 10 mM EGTA, 7 mM MgCl₂, and 3.1% d-glucose) containing 10⁷ rabbit erythrocytes (obtained from Advanced Research Technologies) were added to 100 μl of GVB-MgEGTA containing 160 μg of total protein from either serum or lavage as a complement source. This 200-μl volume was incubated for 30 min at 37°C with gentle shaking. After the reaction, 2 ml of saline were added to each tube, and unlysed erythrocytes were pelleted by centrifugation at 2,000 g for 5 min at 4°C. The OD₄₁₂ of the supernatants was used to determine the amount of lysis that took place. A 0% lysis tube and a 100% lysis tube were also included as in the classical pathway assay above, and the percent lysis was calculated for each sample.

**Bacteria.** Group B streptococcus (GBS) used in this study was obtained as a clinical pulmonary isolate from a patient at the University of North Carolina at Chapel Hill Medical Center and was a generous gift of Dr. Roy Hopfer. Further typing of the bacteria has not been done. GBS were cultured overnight (13–18 h) on trypticase soy agar plates supplemented with 5% defibrinated sheep blood (Becton Dickinson, Cockeysville, MD). Bacterial cultures were scraped from plates and resuspended in GVB++. Bacteria were washed twice with GVB++ buffer and resuspended in 1 ml GVB++. The optical density of the suspension at 660 nm, along with a previously determined extinction coefficient, was used to calculate the bacterial concentration of the suspension.

**C3 deposition assay.** A modification of an assay for functional activity of complement described by China and co-workers (8) was used to show whether the components present in lavage samples are functionally as active as those in serum samples. Briefly, complement activation was assessed by the ability of the complement source to deposit C3 fragments onto a bacterial target. GBS (10⁹ colony-forming units) were incubated with either serum or concentrated BALF (250 μg total protein) in a total volume of 500 μl for 1 h at 37°C in GVB++. The reaction was stopped by chilling the samples on ice for 5 min. Bacteria were pelleted by centrifugation, and the supernatant was removed. The bacteria were resuspended in Dulbecco’s phosphate-buffered saline without calcium or magnesium (DPBS; pH 7.4; GIBCO BRL, Rockville, MD) and stained for analysis by fluorescence-activated cell sorting (FACS). For each sample, 5 × 10⁷ bacteria were stained with an FITC-labeled antibody specific for C3 (goat anti-human C3; ICN Pharmaceuticals, Aurora, OH) or an isotype control antibody (FITC-labeled nonim-
mune goat IgG; ICN Pharmaceuticals) for 30 min on ice in the dark. Bacteria were then washed twice with DPBS to remove free C3 and/or free antibody. The bacteria were fixed with DPBS containing 1% formaldehyde and stored at 4°C in the dark until analysis by FACS. The percentage of C3-positive cells for each sample was determined by subtracting the percent of cells positive for the isotype control (background) from the percent of cells positive for C3. In some cases, data are expressed as percent of activity in a serum sample.

In both C3 deposition experiments and hemolytic assays, 10 mM EDTA, 10 mM EGTA, C1q-depleted serum (Advanced Research Technologies), or 5 μl of goat anti-human C1q (or 5 μl nonimmune goat serum) were added to some samples as a negative control or to help determine which complement pathway was activated. EDTA prevents complement activation through either the classical or alternative pathway. EGTA prevents classical pathway activation only.

Statistical analysis. Data were compared by the one-tailed Student's t-test with unequal variances. In all cases except Fig. 5, samples were unpaired. In all figures, error bars represent SE.

RESULTS

Normal human BALF ranged in total protein concentration from 37 to 104 μg/ml (Table 1) which is consistent with previously published reports (14, 16). Depending on the volume of lavage recovered, samples were concentrated up to 270-fold by volume, which resulted in concentrated lavage samples with total protein concentrations of up to 15 mg/ml.

We characterized complement components present in unconcentrated BALF using Western blot analysis. To determine whether complement components are present at physiological concentrations in lavage samples, aliquots of dilute, unconcentrated BALF samples were compared with serum samples at the same total protein concentration by Western blot analysis (Fig. 1). Samples were normalized to total protein because it would be inappropriate to normalize to volume, since the lavage process unavoidably dilutes lavage samples. Classical pathway components C3 and C4 were both present in lavage fluid obtained from healthy human volunteers at the same apparent molecular weights as in normal human serum. Also, C3 and C4 were present in similar amounts in serum and lavage samples that were normalized to total protein (Table 2). Classical pathway recognition unit, C1q, was present in lavage samples, but at much lower levels than in serum samples. Alternative pathway component factor B was also present in lavage samples at much lower levels than in serum samples.
The functional state of the complement components in lung lavage fluid was tested using standard hemolytic assays for total classical pathway activity and total alternative pathway activity. We detected significant functional classical pathway activity in BALF using antibody-coated sheep RBC. Concentrated BALF samples consistently induced 29 ± 3% lysis of target RBC, whereas serum samples at the same total protein concentration caused lysis of 81 ± 9% of the RBC (data not shown). The level of classical pathway activity in BALF was ~39% of serum activity (Fig. 2A). The lysis of RBC by BALF was determined to be complement mediated since 10 mM EDTA, 10 mM EGTA, or anti-C1q antibodies all prevented lysis by BALF (Fig. 2B). Functional alternative pathway activity was investigated using uncoated rabbit RBC as the target. Whereas serum resulted in 41 ± 6% lysis of the RBC, there was no detectable alternative pathway activity in BALF at the same total protein concentration (Fig. 2A).

In addition to hemolytic assays, we tested the functional activity of complement in lavage fluid in a more physiological assay. An important consequence of complement activation is the covalent attachment of C3 fragments to activator surfaces. These fragments aid in the recognition of foreign particles by immune cells. A C3 deposition assay has been developed previously (8) that distinguishes C3-positive from C3-negative bacteria by FACS. A similar C3 deposition assay was used to assess the functional complement activity in BALF with a clinical pulmonary isolate of GBS. The lavage samples were able to support significant complement activation, assessed by C3 deposition, but less efficiently than serum samples at the same total protein concentration (Fig. 3A). During incubation with serum, 57 ± 3% of the bacteria became labeled with C3 activation products, whereas 10 ± 1% of the bacteria were labeled during an incubation with concentrated lavage fluid. Furthermore, C3 deposition by both serum and lavage was determined to be calcium and C1q dependent, since deposition could be inhibited by the addition of EDTA or EGTA, by antibodies against C1q (Figs. 3B and 4), or by using C1q-depleted serum rather than whole serum (Fig. 7). Therefore, complement activation by GBS proceeds through the classical pathway, which requires both C1q and calcium for activity. Because BALF has less complement activity than serum samples at the same total protein concentration, we tested whether a lavage fluid-specific factor was inhibiting complement activation by BALF in this assay. However, when serum and BALF were added to GBS together, there was an additive (rather than inhibitory) effect on C3 deposition (Fig. 5). C3 deposition was equivalent to the sum of C3 deposition by BALF alone and serum alone. The low BALF activity also could not be attributed to higher levels of C1 inhibitor (C1INH) in lavage samples, since C1INH is less abundant in lavage samples than in serum samples (Fig. 6).

Because C1q concentrations in BALF are very low compared with other classical pathway complement proteins by Western blot analysis, we investigated the hypothesis that SP-A or SP-D, lung surfactant proteins with structures very similar to the complement activa-

**Table 2. Densitometric analysis of proteins in Fig. 1**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Protein Chain</th>
<th>Pathway</th>
<th>BALF Signal, %serum signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>A, B, C</td>
<td>CP</td>
<td>29</td>
</tr>
<tr>
<td>C1INH</td>
<td>a</td>
<td>CP</td>
<td>51</td>
</tr>
<tr>
<td>C4</td>
<td>a</td>
<td>CP</td>
<td>102</td>
</tr>
<tr>
<td>C3</td>
<td>a</td>
<td>CP, AP</td>
<td>115</td>
</tr>
<tr>
<td>Factor B</td>
<td></td>
<td>AP</td>
<td>16</td>
</tr>
</tbody>
</table>

Immunoreactivity of bands in BALF is expressed as the percent of signal in the corresponding serum band. CP, classical pathway; AP, alternative pathway; ND, not determined.
tors C1q and mannose-binding protein (MBP), could substitute for C1q in complement activation in the lung. Either SP-A, SP-D, or C1q was added to C1q-depleted serum in the C3 deposition assay. Although C1q was able to restore significant activity to C1q-depleted serum at 0.5 μg/ml (Fig. 7), neither SP-A (Fig. 7) nor SP-D (data not shown) was able to reconstitute complement activity at any concentration tested, which ranged between 0.5 and 25 μg/ml for SP-A and 100 ng/ml and 5 μg/ml for SP-D.

**DISCUSSION**

The involvement of the complement system in lung host defense mechanisms has been suggested by studies of human patients who are genetically deficient in specific complement components. Patients with a deficiency for an early classical pathway component (C1, C2, C4) or C3 frequently develop infections in the respiratory tract (2, 3, 11). This is probably due to a defect in opsonization, since all of these proteins aid in C3 deposition onto bacteria. Therefore, opsonization by
complement may be a major mechanism for mucosal immunity in the lung. Although previous in vivo studies investigating the role of complement in host defense have suggested that local complement pools may suppress pulmonary infection, they do not exclude the possibility that a defect in systemic complement activation is involved. Therefore, we set out to determine whether lung lavage fluid contains a functional complement system.

Western blot analysis of complement components in human BALF confirmed that many complement components are present in the lung, including C1q, C3, C4, and factor B. Classical pathway components C3 and C4 are present in lavage fluid at concentrations that are similar to their serum concentrations when samples are normalized to total protein. The classical pathway recognition unit C1q is present in low abundance in BALF compared with the other classical pathway components tested. Alternative pathway component factor B is also present in lavage fluid at much lower levels than in serum. The different relative concentrations of the components in lavage fluid vs. serum suggest that these components are not just serum contaminants leaking into the lung as a result of the lavage process. For example, factor B (93 kDa) and C1INH (110 kDa) are much smaller than C3 (185 kDa) and C4 (205 kDa) and should diffuse into the lung more readily than C3 and C4. Therefore, factor B and C1INH should equilibrate between serum and lavage fluid more readily than C3 and C4 if serum components are simply diffusing into the lung. Because this is not the case, it is likely that certain complement components are produced in the lung, at least in part, or are preferentially transported into the lung.

Several previous studies have used albumin concentrations in lavage fluid vs. serum as a measure of the permeability of (or damage to) the alveolar epithelium. By this logic, many, but not all, of the complement proteins in the lung could be accounted for by serum permeability of the alveolar epithelium (10, 26). However, Bignon and co-workers (7) have demonstrated that albumin is not only a normal constituent of alveolar lining fluid, but it also appears to be transported across the epithelium of the lung and into the air spaces by type I epithelial cells. Because of the normal presence of albumin in alveolar fluid, albumin concentrations do not necessarily represent serum contamination of the BALF and were not used in this study.

Complement components in lavage fluid probably originate from multiple sources, including lung-specific production as well as serum complement components that may transverse the alveolar epithelium during an infection. In support of lung-specific complement sources, more recent studies have shown that primary cultures of lung-specific cell types, including alveolar macrophages and type II epithelial cells, synthesize and secrete components of both the classical and alternative complement pathways (1, 9, 29). Strunk and co-workers (29) found that human alveolar macrophages and type II cells produce C3, C4, and factor B in culture, which correlates with our Western blot analysis of BALF, although their results suggest a higher level of expression of factor B compared with C3 than we detect in BALF.

We have shown that complement components in concentrated lavage fluid were functionally active in a C3 deposition assay with GBS and in a hemolytic assay using antibody-coated RBC. The magnitude of activation by lavage was 16–39% of the magnitude of activation by serum samples at the same total protein concentration in the C3 deposition assay and hemolytic assay, respectively. The variability in activity is probably a consequence of the different sensitivities of the two assays. The hemolytic assay theoretically requires the formation of a single membrane attack complex to observe the functional consequence, i.e., lysis of the erythrocyte. The C3 deposition assay does not provide this level of sensitivity, but it is probably a more physiological test. Although BALF has less complement activity than serum, it is capable of supporting

Fig. 6. Western blot analysis of C1 inhibitor in BALF. Equal amounts of total protein (1.1 μg) from lavage samples (L) and control serum samples (S) were analyzed by Western blot. Arrow indicates full-length C1 inhibitor. Molecular mass standards are given in kDa. A densitometric comparison of the amount of C1 inhibitor in BALF vs. serum is included in Table 2.

Fig. 7. Effect of SP-A on complement activation by C1q-depleted serum. C1q-depleted serum supplemented with SP-A or C1q at either 0.5 or 2.4 μg/ml was assayed for functional activity in the C3 deposition assay as in Figs. 3 and 4. Activity in C1q-depleted samples is presented as the percent of activity in a corresponding sample of whole serum. Complement activity was not detectable (n.d.; mean, −1.3 ± 1.6% of whole serum activity) in C1q-depleted serum with no protein added. Data represent 3 or more experiments. Error bars represent SE. Statistical significance was determined by a one-tailed Student’s t-test with unequal variances. *P < 0.001.
significant complement deposition onto bacteria. Lavage fluid complement activation in this assay was inhibited by EGTA or anti-C1q antibodies, suggesting that GBS activates lavage fluid complement via the classical pathway. GBS has previously been shown to activate the classical pathway of serum complement as well (17, 28).

Alternative pathway activity was also investigated using a hemolytic assay but was undetectable in BALF. This low activity correlates with the near absence of factor B in BALF by Western blot analysis. Because factor B cleavage products were detected in lavage fluid by Western blot, we cannot exclude the possibility that significant cleavage of the protein had occurred. Extensive cleavage of factor B could explain both the inability to detect alternative pathway activity and the very low level of factor B in BALF, given that it is among the most highly expressed complement components by human alveolar type II epithelial cells and alveolar macrophages in vitro (29). In addition, BALF caused rabbit RBC to agglutinate during the alternative pathway hemolytic assays even in the absence of calcium. The agglutinated RBC may have been less susceptible to lysis by BALF because complement components in BALF did not have free access to complement binding sites on the RBC, which may explain the apparent low level of alternative pathway activity. Activation by the alternative pathway is generally regarded as less specific than activation by the classical pathway, which uses specific activator recognition units, C1q or MBP. Because potentially dangerous inflammatory mediators are produced by complement activation, the apparent functional absence of the alternative pathway in the lung may also represent negative regulation of this pathway along the delicate alveolar epithelium.

BALF was less active than serum in both functional assays tested. The lower activity may be an experimental consequence of the extensive concentration process the lavage samples have undergone compared with the serum samples. Alternatively, there may be an inhibitor present in BALF that can restrict complement activation in the lung as was previously reported for rabbit BALF (12). A lavage-specific complement inhibitor could be a mechanism for preventing complement activation along the delicate epithelium of the lung. Because human BALF did not inhibit serum complement activity in the C3 deposition assay, the decreased complement activity of BALF compared with serum is not likely due to an inhibitory factor in BALF. However, a lavage complement inhibitor may not affect serum and lavage complement activation similarly and cannot be excluded as an explanation for the lower activity in lavage fluid compared with serum.

The total protein concentration of the BALF used in functional assays is believed to approximate the protein concentration of the alveolar lining fluid in vivo. It has been estimated that the area weighted thickness of the alveolar hypophase is ∼0.2 μm (6). If a 75-kg person has an alveolar surface area of 750,000 cm², then the extrapolated volume of alveolar lining fluid would be 15 ml, which correlates well with an estimate of 20 ml by Macklin (22). This would average 3 ml of alveolar lining fluid per lobe of the lung, assuming an even distribution between compartments. Estimating 50% recovery of protein by lung lavage, lavage fluid from one lobe of the lung would have to be concentrated to 1.5 ml to approximate the protein concentration in the lining fluid in vivo. Lavage fluid used in this study was concentrated to 1.0–1.5 ml, and therefore should approximate lavage protein concentrations in vivo.

One initial hypothesis was that lung-specific surfactant proteins (SP-A and SP-D) that have structural similarity to C1q and MBP may be able to substitute for C1q in lung complement activation in an environment where the C1q concentration is very low. SP-A (24, 31), SP-D (25), MBP (15), and C1q (5) have all been shown to function in innate immunity as opsonins. Like C1q, SP-A has been shown to act as an activation ligand by enhancing the uptake of particles opsonized by IgG (30). There is even evidence that SP-A, MBP, and C1q all bind to the same C1q receptor (23), further demonstrating the functional similarities of these proteins.

Tenner and colleagues (30) investigated the ability of SP-A to substitute for C1q in a hemolytic complement activation assay in which the activator was IgG-coated sheep erythrocytes. They reported that SP-A was not able to activate complement, a finding that may have been due to an inappropriate target for SP-A-mediated complement activation. SP-A recognizes foreign particles by binding directly to carbohydrates that are abundant on bacterial surfaces through its lectin domain, without the need for specific antibodies. Although one study has shown that SP-A binds to IgG (20), the functional significance of this binding is not clear, and SP-A has not been shown to bind directly to pathogens through IgG on their surfaces. Therefore, IgG-coated erythrocytes may not be recognized well by SP-A, and this may account for the negative result in the complement activation assay.

We used the GBS C3 deposition assay to test whether SP-A could substitute for C1q in complement activation, since SP-A has previously been shown to bind to GBS and to enhance phagocytosis of GBS by alveolar macrophages (21). Although the addition of either SP-A or SP-D to C1q-depleted serum did not restore C3 deposition onto GBS, the addition of C1q did restore the ability of C1q-depleted serum to deposit C3 onto GBS. It is possible that lavage-specific factors may be required by SP-A for complement activation. For example, just as MBP can use its own set of serine proteases, the MBP-associated serine proteases, in place of C1r and C1s, SP-A may require a different set of proteins.

We also showed that anti-C1q antibodies inhibited C3 deposition onto GBS by concentrated lavage, suggesting that the low level of C1q in lavage fluid is necessary and sufficient for complement activation. The anti-C1q antibodies were also found to be specific for C1q, since they did not cross-react with SP-A by Western blot analysis (data not shown). The lower level
of complement activity in BALF compared with serum at the same total protein concentration could not be attributed to the low amount of C1q present in lavage samples alone, since the addition of C1q to BALF did not enhance BALF activity any further (data not shown). Therefore, other complement components involved in classical pathway activation (such as C2) are possibly altered in BALF as well. Even though C1q levels are low in lavage fluid compared with other classical pathway components, there is enough C1q present to give ∼40% of the signal provided by serum at the same total protein concentration in the classical pathway hemolytic assay, which is likely a significant level functionally. The other complement activator, MBP, has a broader reactivity than C1q and might function in the lung where C1q is limited. However, Western blots of rat lavage fluid and rat serum have demonstrated that although MBP is easily detectable in serum, there is no detectable MBP in the rat lavage fluid at the same total protein (data not shown). Rat lavage fluid was used because the antibody was created against rat MBP and did not react with human MBP. Local complement sources contribute relatively little to the total complement pool (4) but likely play important roles in the very early stages of infection by initiating local inflammatory responses that recruit immune cells and serum complement. We have shown that there are complement components of both the classical and alternative pathways present in human lung lavage fluid and that the classical pathway is functionally active. This local complement pool may provide immediate protection against infection along the delicate epithelium of the lung.

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