Surfactant protein A inhibits T cell proliferation via its collagen-like tail and a 210-kDa receptor

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Departments of 1Medicine and 2Biochemistry, The Lawson Research Institute, St. Joseph’s Health Center, The University of Western Ontario, London, Ontario, Canada N6A 4V2; 3Department of Pulmonary and Critical Care Medicine, Children’s Hospital Medical Center, Cincinnati 45229-3039; 4Department of Pulmonary Biology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267-0564; 5Department of Anesthesiology, University of Alabama at Birmingham, Birmingham, Alabama 35233; 6Department of Cell Biology, Duke University, Durham, North Carolina 27710; and 7Department of Biochemistry, Vanderbilt University School of Medicine, Veterans Affairs Medical Center, Nashville, Tennessee 37212-2637

Borron, Paul, Francis X. McCormack, Baher M. Elhalwagi, Zissis C. Chroneos, James F. Lewis, Sha Zhu, Jo Rae Wright, Virginia L. Shepard, Fred Possmayer, Kevin Inchley, and Laurence J. Fraher. Surfactant protein A inhibits T cell proliferation via its collagen-like tail and a 210-kDa receptor. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L679–L686, 1998.—Investigation of possible mechanisms to describe the hyporesponsiveness of pulmonary leukocytes has led to the study of pulmonary surfactant and its constituents as immune suppressive agents. Pulmonary surfactant is a phospholipid-protein mixture that reduces surface tension in the lung and prevents collapse of the alveoli. The most abundant protein in this mixture is a hydrophilic molecule termed surfactant-associated protein A (SP-A). Previously, we showed that bovine (b) SP-A can inhibit human T lymphocyte proliferation and interleukin-2 production in vitro. Results presented in this investigation showed that different sources of human SP-A and bSP-A as well as recombinant rat SP-A inhibited human T lymphocyte proliferation in a dose-dependent manner. A structurally similar collagenous protein, C1q, did not block the in vitro inhibitory action of SP-A. The addition of large concentrations of mannan to SP-A-treated cultures also did not disrupt inhibition, suggesting that the effect is not mediated by the carbohydrate recognition domain of SP-A. Use of recombinant mutant SP-As revealed that a 36-amino acid Arg-Gly-Asp (RGD) motif-containing span of the collagen-like domain was responsible for the inhibition of T cell proliferation. A polyclonal antisera directed against an SP-A receptor (SP-R210) completely blocked the inhibition of T cell proliferation by SP-A. These results emphasize a potential role for SP-A in dampening lymphocyte responses to exogenous stimuli. The data also provide further support for the concept that SP-A maintains a balance between the clearance of inhaled pathogens and protection against collateral immune-mediated damage.

THE HYPORESPONSIVE STATE of pulmonary leukocytes compared with peripheral blood leukocytes suggests an organ-specific regulation of immune function (4, 5, 35). It has been suggested that pulmonary surfactant plays a significant role in the induction and maintenance of this hyporesponsiveness (3, 2, 35, 47). Pulmonary surfactant is a phospholipid-protein mixture that prevents collapse of alveoli at the end of expiration. There are four known surfactant-associated proteins (SPs), termed SP-A, SP-B, SP-C, and SP-D, which have been isolated from lung lavage. SP-B and SP-C are hydrophobic molecules, whereas SP-A and SP-D are hydrophilic (32). SP-A is the most abundant of these proteins, representing 3% of the total mass of pulmonary surfactant in humans (13). Studies by a variety of researchers have reported that lymphocyte hyporesponsiveness can be induced in vitro by the addition of cell-free lung wash (3, 35, 47) or pulmonary surfactant (2, 47) to stimulated cells. These observations led to the hypothesis that pulmonary surfactant protects the lung from immune-mediated damage initiated by inhaled antigens and particles, in part by influencing pulmonary leukocyte responses (33, 47). We have previously shown that SP-A inhibited T cell proliferation induced by either phytohemagglutinin (PHA) or anti-CD-3 and also inhibited interleukin-2 (IL-2) secretion from these cells in a dose-dependent manner (7). We also showed that SP-A inhibited T cell proliferation up to 24 h after the lymphocytes had been treated with mitogen (7).

SP-A belongs to a subgroup of molecules termed collectins (25), which are composed of a collagen-like region and a carbohydrate recognition domain (20). More specifically, this molecule is organized into the following four domains: a short NH2-terminal segment; a collagen-like domain possessing reiterated Gly-X-Y triplets, a neck region that includes a span of hydrophobic amino acids, followed by the COOH-terminal carbohydrate recognition domain that binds mannose (6). Examples of other collectins are mannose-binding protein, conglutinin, CL-43, and SP-D, which differ in their carbohydrate-binding specificity as well as their oligomeric structure (20). In the case of SP-A, trimeric subunits associate by the folding of the collagen-like

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region into triple helices. Fully assembled SP-A is composed of six trimeric units that are laterally associated in the first part of the collagen domain and stabilized by disulfide bridges at the NH₂ terminus. The overall structural organization is very similar to C₁q (10, 12), such that the molecules appear indistinguishable by electron microscopy (19). It has also been shown that unlabeled C₁q can displace fluorescein 5-isothiocyanate-conjugated SP-A binding to human peripheral blood monocytes, suggesting that a common receptor(s) may exist on the surface of these cells (15). Monocytes that were plated on tissue culture wells coated with C₁q or anti-C₁qR monoclonal antibody were less responsive to the opsonic effects of SP-A (15).

In contrast, it has been shown that C₁q or soluble C₁q receptor only mildly inhibited SP-A binding to U-937 cells (25). Tenner et al. (40) used human proteins and demonstrated that C₁q only mildly displaced SP-A binding to rat type II cells and that an anti-C₁q antibody that inhibits C₁q-mediated phagocytosis lacked the same activity with SP-A-mediated phagocytosis. Together, these studies suggest that SP-A and C₁q may share certain common receptors on some cells. SP-A and C₁q may also possess receptors that do not bind these molecules interchangeably. One such receptor has been characterized on the surface of macrophages and type II cells and found to bind SP-A even in the presence of a 100-fold excess of mannan (11). Polyclonal antiserum raised against this 210-kDa receptor (SP-R210) was also able to prevent SP-A from inhibiting phosphatidylcholine secretion by phorbol ester-treated type II cells and to inhibit the SP-A-dependent uptake of bacillus Calmette-Guerin by macrophages (45).

The objective of the current study was to 1) determine if the various sources and species of SP-A inhibit T cell proliferation to confirm that the effect is conserved across species and compare these results to a structurally similar molecule C₁q, 2) determine the structural domains of SP-A that mediate inhibition of T cell proliferation using a panel of mutant recombinant SP-A proteins, and 3) determine the contribution of a recently characterized SP-A receptor expressed on leukocytes in mediating the suppression of [³H]thymidine incorporation by T cells.

MATERIALS AND METHODS

Isolation of SP-A

Bovine SP-A. Bovine SP-A (bSP-A) was prepared as previously described (12). Briefly, delipidated SPs were solubilized in 5 mM HEPES solution with 0.1 mM EDTA, pH 7.4. The solubilized protein was applied to an immobilized β-mannose affinity column in 5 mM HEPES solution with 0.1 mM EDTA, pH 7.4. The calcium-precipitated pellet was solubilized with mercaptoethanol, urea, and Nonidet P-40 (NP-40) and was used for preparative IEC with a Bio-Rad Rotofor apparatus. The resulting white precipitate that formed was dialyzed for several days against H₂O and finally dialyzed against polyvinylagarose to reduce endotoxin to <1.2 pg lipopolysaccharide/µg SP-A (22). This preparation initially exists as large insoluble granules in tissue culture media at room temperature, which we assume becomes more soluble with time at 37°C.

Native rat SP-A. Native rat SP-A was obtained from the bronchoalveolar lavage of adult rats after intratracheal instillation of 40 mg/kg of silica instilled 4 wk before lavage. Butanol extraction of the cell-free bronchoalveolar lavage was undertaken followed by application of the crude protein pellet to an immobilized mannan affinity column in the presence of calcium. After thoroughly washing unbound protein through the column, SP-A was eluted with an EDTA-containing buffer (17).

Recombinant SP-A. Recombinant rat SP-A was generated using insect cell lines and baculovirus vectors. Briefly, the 1.6-kb cDNA for rat SP-A (37) was ligated into the pVL1392 recombination vector, which was then cotransfected into Spodoptera frugiperda (sf 9) cells with a modified Autographa californica virus. The recombinant viruses, generated in situ by homologous recombination, were then plaque purified and used to infect Trichoplusia ni cells (29). Recombinant SP-A was isolated from the serum-free culture media by affinity chromatography on immobilized mannanose. This SP-A has been shown to have measurable functional properties that are similar to native SP-A (27). The final preparation was dialyzed against 5 mM Tris (pH 7.4) and stored at −20°C (27).

Recombinant SP-A mutants. To further specify the region of SP-A affecting T cell proliferation, recombinant SP-A mutants with deletions and point mutations in the major structural domains were developed by site-directed mutagenesis. We used mutagenic oligonucleotides and the polymerase chain reaction to generate telescoping deletions in the cDNA for SP-A by overlap extension (21), as previously described (14). The nucleotides encoding Asn₁–Ala₇ were deleted, and the signal sequence was directly juxtaposed to downstream sequences encoding Gly₄—Phe²⁸. Additional truncated mutant cDNAs were generated in a similar fashion by deletion of the nucleotides encoding Asn₁—Gly₄₄ and Asn₁—Pro³⁰ and ligation of the native signal sequence to downstream nucleotide sequences encoding Gly²⁵—Phe²² and Ala₄₁—Phe²³, respec-
tively. The truncated proteins were denoted N1–A7, N1–G44, and N1–P80. A mutant SP-A was developed in which the collagen-like region was deleted as a cassette, directly joining the intermolecular disulfide-containing NH2-terminal fragment and the neck region of the protein (30). This protein was denoted G8–P80. Finally, mutant SP-As containing mutations that block carbohydrate binding (E195A) or alter carbohydrate-binding specificity (E195Q, R197D) were produced as previously reported (28, 29). The coding region for all mutant cDNAs was sequenced by the dideoxy method of Sanger et al. (36) to confirm the intended deletions and exclude spurious mutations. The production of mutant recombinant SP-A in the baculovirus system was performed as for the wild-type recombinant protein (27, 29).

Cell Isolation and Culture

Lymphocytes and monocytes were obtained from the peripheral blood of healthy volunteers by buoyant density centrifugation using Lymphoprep resolving medium (Nycomed, Oslo, Norway). The peripheral blood mononuclear cells (PBMC) were then washed three times in cold tissue culture media, RPMI-1640 (GIBCO BRL, Burlington, ON, Canada) containing penicillin (100 μg/ml; GIBCO BRL), streptomycin (100 μg/ml; GIBCO BRL), amphotericin B (2.5 μg/ml; GIBCO BRL), β-mercaptoethanol (5.5 × 10−5 M; GIBCO BRL), and gentamycin (0.1 μg/ml; GIBCO BRL). Before culture, tissue culture media were supplemented with 10% (vol/vol) newborn calf serum (GIBCO BRL). Cells were cultured at a concentration of 2 × 106 cells/well, 200 μl/well, in flat-bottomed 96-well sterile plates (Corning). Unstimulated PBMC from four separate donors were also cultured for 24 h in 48-well tissue culture plates to readily differentiate monocytes/macrophages from lymphocytes. All cells were recovered with multiple incubations and washes with a cold PBS-2 mM EDTA solution. PBMC were then mounted on glass slides by use of a Cytospin 2 apparatus (Cytospin, Shandon, PA) and stained with a Hemacolor stain kit (EM Diagnostic Systems). Differential cell counts of these samples demonstrated that ~89.5% of the cells were small resting lymphocytes, whereas the remaining cells (10.5%) were much larger, vacuolated macrophages.

T Cell Proliferation Assays

[3H]thymidine incorporation assay was used as a bioassay for lymphocyte function. Two different T cell mitogens were used: 1) PHA-P (Sigma, St. Louis, MO) and 2) anti-CD-3 (UCHT1; ID Laboratories, London, ON, Canada). Several different reagents were used in the T cell proliferation assays, including human C1q (Sigma), different preparations of SP-A, including SP-Ahyp and a panel of mutants, the mannose including human C1q (Sigma), different preparations of different reagents were used in the T cell proliferation assays, (UCHT1; ID Laboratories, London, ON, Canada). Several used:

1 µCi/well of [3H]thymidine was added (specific activity 6.7 Ci/mmol; Amersham International, Oakville, ON, Canada). Cells were subsequently harvested with a semiautomated Skatron cell harvester, which bound labeled DNA to glass filter papers via the cell harvester vacuum manifold. Filter papers were dried, and the amount of [3H]thymidine incorporated into DNA was measured via liquid scintillation spectrophotometry. Data are expressed as the means ± SE of the percentage of [3H]thymidine incorporation compared with cultures treated with mitogen only.

Western Blot Analysis of PBMC and Nonplastic Adherent PBMC Lysates for Presence of the SP-A Receptor SPR-210

PBMC were isolated as described previously and resuspended at a final concentration of 2 × 106 cells/ml. A 5-ml aliquot was then added to a T-25 flask (Corning) and incubated under standard tissue culture conditions for 90 min with occasional rocking. Nonadherent PBMC were then gently aspirated from the flask and washed two times in serum-free media. Aliquots were added to 1.5-ml Eppendorf tubes (2 × 105 cells/tube) and centrifuged for 10 s in a bench top microfuge. Cell pellets were resuspended in 25 μl of distilled H2O, 12 μl of 5 × Laemlli sample buffer, and 25 μl of a lysing buffer containing 1% NP-40 and 2 mM EDTA.

To examine whether treating PBMC with the T cell mitogen anti-CD-3 monoclonal antibody for 24 h had an effect on expression of SPR-210, PBMC cultures from three separate donors (5 × 105 cells/well of a 48-well plate) were cultured alone or activated with 50 ng/ml of anti-CD-3 for 24 h under standard culture conditions. Adherent and nonadherent cells were combined with 150 μl of a 5 × protease inhibitor cocktail (Sigma) and assayed for protein content before equal protein concentrations of resting and activated cell lysates were resolved by SDS-PAGE. As a negative control, Chinese hamster ovary cells (CHO-K1) were scraped off a culture flask and treated in a similar fashion to resting and activated PBMC. Samples were lysed by freezing and thawing one time, then vortexed and briefly centrifuged before the collection of lysates. Samples were heated at 95°C for 5 min in 5 × Laemlli sample buffer containing dithiothreitol before separation on a 7.5% SDS-polyacrylamide gel. Separated proteins were then electrophoretically transferred to a nitrocellulose membrane. The nitrocellulose membrane was blocked with 0.5% instant nonfat milk in Tris-buffered saline (TBS) for 1 h at room temperature and washed three times with TBS. A 1:3,000 dilution of rabbit anti-rat SPR-210 (11) in TBS was then added to the membrane and incubated for 1 h at room temperature on a gently shaking platform. The properties of the anti-SPR-210 antibody have been described in detail previously (11). After three washes with TBS, a secondary goat anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1:20,000 and incubated with the membrane for 1 h followed by three TBS washes. The enhanced chemiluminescence Western blotting detection kit (Amersham Life Sciences) was used to visualize the SPR-210 protein. An EPSON ES-1200C image scanner was used in conjunction with the National Institutes of Health Image computer software to analyze the density of the 210-kDa SPR-210 and its two primary degradation products to quantify differences in the amount of cross-reactive protein detected.

Statistics

All results shown are expressed as the means of a minimum of three separate experiments. Statistical significance of data was determined by a one-way analysis of variance followed by comparison of experimental results between the experimental groups using the Student-Newman-Keuls test. P values < 0.05 were considered significant.
RESULTS
Comparison of the In Vitro Inhibitory Action of SP-A From a Variety of Sources

Previously, it was reported that SP-A from alveolar proteinosis patients enhanced concanavalin A-induced proliferation of rat splenocytes (17). To verify our initial observations using the T cell mitogen PHA, we compared SP-A from different species and methods of isolation. In the first of these experiments, native and recombinant (SP-Ahyp) rat SP-As were added in increasing amounts to PBMC activated with PHA-P (1 µg/ml). Figure 1A demonstrates that both preparations can inhibit T cell proliferation in a dose-dependent fashion. Figure 1B shows the results obtained from experiments in which PHA-P (1 µg/ml)-activated PBMC were treated with increasing amounts of bSP-A and two preparations of human alveolar proteinosis SP-A purified via different methods. All three preparations of SP-A inhibited T cell proliferation in a dose-dependent manner. The ability of IEF isolated human SP-A to inhibit T lymphocyte proliferation is consistent with our previous results (7).

Evaluation of Which Region of SP-A Inhibits Proliferation of Human PBMC

The effect of mannan, a mannose homopolysaccharide, on the ability of SP-A to inhibit PHA-driven T cell proliferation was tested (Fig. 2). T cell proliferation was compared between cultures treated with bSP-A and mannan with cultures treated with bSP-A alone. No significant difference in [3H]thymidine incorporation was observed at either dose of mannan combined with bSP-A compared with stimulated cultures treated with PHA and SP-A.

To determine if a structurally similar collagenous protein had the capacity to block the suppressive activity of bSP-A on activated T cells, increasing amounts of C1q were added to PHA (1 µg/ml)-stimulated cells in the presence of 12.5 µg/ml of bSP-A (Fig. 3). The ratios of C1q to SP-A tested (0.16, 0.31, 0.63, 1.25, 2.5, and 5)
did not result in any statistically significant difference from bSP-A-inhibited cultures.

To more clearly define the region of SP-A that affects T cell proliferation, recombinant rat SP-As with deletions and point mutations in the major structural domains were developed by site-directed mutagenesis. The proteins expressed in the insect cell system have been shown to have functional activities that are comparable to native SP-A despite incomplete proline hydroxylation (27). Results in Fig. 4 show that native rat SP-A inhibited PHA-stimulated T cell proliferation. The wild-type recombinant SP-A (SP-A
\textsuperscript{hyp}) also inhibited T cell proliferation, albeit with a lower magnitude than native SP-A. Deletion of the first seven amino acids of the globular NH\textsubscript{2} terminus (SP-A
\textsuperscript{hyp,N1-A7}) did not greatly alter the inhibitory action of the preparation in vitro nor did deletion of amino acids 1–44 (SP-A
\textsuperscript{hyp,N1-G44}). These proteins have recently been shown to be a mixture of alternatively processed isoforms that form disulfide-dependent dimers via an NH\textsubscript{2}-terminal cysteine residue (14). SP-A-induced suppression was blocked with two separate deletion mutants shown in Fig. 4. Deletion of amino acids 1–80 (SP-A
\textsuperscript{N1-P80}) abolished the inhibitory activity of SP-A
\textsuperscript{hyp}, but SP-A
\textsuperscript{N1-P80} does not form disulfide-dependent dimers. The loss of inhibitory activity in SP-A
\textsuperscript{hyp,N1-P80} was not due to loss of intermolecular disulfide bond formation, since SP-A
\textsuperscript{hyp,G8-P80}, which contains the disulfide-forming NH\textsubscript{2}-terminal segment, was also inactive. In contrast, the mutant SP-A, created by an inactivating E195A with a substitution of Glu\textsuperscript{195} by Ala, inhibited PHA-stimulated T cell proliferation in a comparable fashion to SP-A
\textsuperscript{hyp}. Similarly, mutant E195Q/R197D with substitutions of Glu\textsuperscript{195} by Gln and Arg\textsuperscript{197} by Asp that altered the carbohydrate-binding specificity of the molecule to favor galactose over mannose binding (29) did not result in a disruption of antiproliferative activity. Thus the carbohydrate-binding activity of SP-A is not involved in inhibition of T lymphocyte proliferation, since mutations that inhibit or alter carbohydrate binding do not influence the activity of SP-A in our assays. These data suggest that the domain of SP-A that mediates the inhibition of T cell proliferation is a 36-amino acid portion of the collagen-like domain between amino acids 45 and 80. This region contains several motifs with alternating positive and negative charges including an RGD sequence.

Expression of an SP-A Receptor on Human PBMC

Recently, Chronos et al. (11) characterized an SP-A receptor expressed on the surface of type II pneumocytes, rat bone marrow-derived macrophages, alveolar macrophages, and U-937 cells. Because this receptor was shown not to bind C1q and does not interact with the carbohydrate-binding site of SP-A, it was of interest to determine whether SP-A mediates its inhibitory effect on T cell proliferation through this receptor. Initially, we tested whether human PBMC and monocyte-depleted nonadherent PBMC (PBMC NA) express this receptor. Shown in Fig. 5A is a Western blot of cell lysates from 24-h cultured unstimulated PBMC (lanes 2, 4, and 7) and similar cells treated with T cell mitogen anti-CD-3 antibody (50 ng/ml; lanes 1, 3, and 6). Lanes 1 and 2, 3 and 4, and 6 and 7 contain equal amounts of protein (within donor groups) from 3 separate blood donors. Treatment of PBMC from these donors with anti-CD-3 resulted in 1.9-, 2.4- and 4.4-fold increases, respectively, in the amount of cross-reactive material detected by densitometry. Lane 5 had CHO-K1 cell lysates added equivalent to protein in lanes 6 and 7. MW markers, molecular-mass markers.

![Western Blot](Image)

**Fig. 5.** A: detection of a 210-kDa SP-A receptor (SPR-210) in cell lysates of PBMC and plastic nonadherent PBMC (PBMC NA) using Western blot analysis. Lysates from cell pellets containing 2 x 10\textsuperscript{6} cells were prepared with a detergent-containing buffer and Laemmli SDS-PAGE buffer. Samples were heated and electrophoresed on 7.5% gels before transfer to nitrocellulose and treatment with a 1:3,000 dilution of rabbit anti-rat SPR-210 polyclonal antisera. B: detection of SPR-210 in cell lysates from 24-h cultured unstimulated PBMC (lanes 2, 4, and 7) and similar cells treated with T cell mitogen anti-CD-3 antibody (50 ng/ml; lanes 1, 3, and 6). Lanes 1 and 2, 3 and 4, and 6 and 7 contain equal amounts of protein (within donor groups) from 3 separate blood donors. Treatment of PBMC from these donors with anti-CD-3 resulted in 1.9-, 2.4-, and 4.4-fold increases, respectively, in the amount of cross-reactive material detected by densitometry.

![Treatment](Image)

**Fig. 4.** Treatment of human PBMC with PHA (1.0 µg/ml) and native rat SP-A and a panel of SP-A
\textsuperscript{hyp} (25 µg/ml) mutants. Each bar is the mean ± SE of 3 separate experiments, replicates of 4. *P < 0.05 compared with cells treated with PHA alone.
mitogen-treated cultures do express more protein that cross-reacts with the anti-SPR-210 antiserum than unstimulated PBMC cultures. This was also determined by use of densitometric analysis of the 210-kDa band and two faster-migrating products of degradation. The mean increase in band densities from T cell mitogen-treated PBMC was over twofold (1.9-, 2.4-, and 4.4-fold increases). Shown in lane 5 of Fig. 5B and confirmed using densitometric analysis is a lack of specific cross-reactivity to CHO-K1 lysates.

Polyclonal Antisera to an SP-A Receptor Blocks the Inhibitory Action of SP-A on Human PBMC

The mechanism of SP-A-mediated inhibition of T cell proliferation was then explored with the same polyclonal antisera used to detect the SP-A receptor on PBMC. We found that the suppressive action of SP-A on T cell proliferation was blocked by anti-SP-R210 and PBMC. We found that the suppressive action of SP-A on clonal antiserum used to detect the SP-A receptor on proliferation was then explored with the same poly-chromatography (48). These activities constitute an important contribution of both infection and inflammation.

DISCUSSION

Reviews of the literature have suggested that SP-A and other collectins function as opsonins for defense against pathogens entering the lung (8, 20, 26, 41, 42). The proposed mechanism for this protective role was thought to be via their ability to recognize cell surface carbohydrate residues that distinguish pathogens from the host’s own cells. Further evidence for this protective role is that collectins interact with immune cell surface receptors via their collagen-like region and facilitate microbial phagocytosis and intracellular killing of bacteria (20, 25, 38, 43, 47). Other functions of collectins include stimulation of superoxide production as well as chemotaxis (48). These activities constitute an important first line defense for controlling the growth and spread of pathogens before production of significant quantities of neutralizing antibodies. We have previously shown that SP-A reduces the proliferative response of T lymphocytes to mitogens, suggesting that the protein maintains a state of hyporesponsiveness to prevent flooding of the air spaces with inflammatory cells. Our results combined with these previously published antimicrobial studies suggest that SP-A maintains immunological homeostasis in the lung, attenuating both infection and inflammation.

The current study has demonstrated that SP-A from different species and SP-A obtained by different means of isolation also inhibited T cell proliferation. These data indicate that the inhibitory effect is not species specific. Interestingly, preparations of SP-A obtained from patients with alveolar proteinosis were significantly less active in inhibiting T cell proliferation compared with native bSP-A and rat SP-A molecules. There are several potential explanations for this. A significant portion of alveolar proteinosis SP-A is composed of subunits that form nonreducible dimers (44). Under nonreducing conditions, proteinosis SP-A exists as multimers consisting of more than one octadecamer (16). These alterations in the secondary and tertiary structure of the human proteinosis SP-A may render the region of the molecule responsible for in vitro inhibitory activity less accessible to surface receptors on lymphocytes and macrophages. It is also possible that posttranslational or enzymatic modifications of both native and recombinant SP-A may influence the binding affinity of the different SP-A proteins. Species differences may contribute to different binding affinities for different cell types.

The domains of SP-A that mediate inhibition of T cell proliferation were also explored using mutant recombinant proteins and specific inhibitors. The role of the carbohydrate domain was examined. Mannan did not reverse the inhibitory effect at higher molar concentrations than SP-A. Recombinant rat SP-A containing inactivating mutations (E195A) or substitutions that alter carbohydrate-binding specificity (E195A, R197Q) were as active as SP-Ahyp in inhibition of T cell proliferation. Collectively, these data indicated that inhibition of T cell proliferation by SP-A is not mediated by the carbohydrate recognition domain.

The collagen-like region of SP-A was also studied to determine what effect it may have on the observed anti-proliferative effect. We tested whether C1q, a structurally homologous protein to SP-A, could disrupt the suppressive effect of SP-A by coincubation with 12.5 µg/ml of bSP-A and varying amounts of C1q. The results in Fig. 3 demonstrated that molar ratios of C1q to SP-A as high as 5:1 did not significantly reverse SP-A-mediated inhibition of PHA-induced T cell proliferation. An explanation for the appearance of more proliferation at higher concentrations of C1q is most probably due to SP-A binding C1q in vitro, which sterically hinders SP-A-PBMC interactions (31). Of note, in a previous study, we found that the addition of C1q did not result in a dose-dependent increase or
decrease in T cell proliferation (9). In these series of experiments, it was assumed that the SP-A and C1q preparations utilized had comparable half-lives in culture. The demonstration that C1q did not interfere with the in vitro action of SP-A on T lymphocyte proliferation at the majority of ratios tested suggests that SP-A may interact with SPR-210, which binds SP-A but not C1q. These results indicate that a property of bSP-A not shared with C1q mediates suppression of T cell proliferation. Consistent with our interpretation, Tenner et al. (40) also showed that a 100-fold excess of C1q (by mass) did not inhibit SP-A binding to type II pneumocytes.

Mutant recombinant SP-As with telescoping deletions from the NH2 terminus were used to find regions that interacted with PBMC. The inhibitory effect of SP-A was retained despite deletions through Ala7 and Gly44 but was lost with deletions of residues through P80. Because the disulfide-dependent assembly of SP-A is partially preserved in N1–A7 and N1–G44, but lost in N1–P80, we evaluated the activity of an additional collagen region deletion, mutant protein G8–P80, which also exists as a monomer (30). This protein is identical to the N1–P80 except that it contains the disulfide bond forming the NH2 terminus of SP-A. The G8–P80 was also inactive as an inhibitor of T cell proliferation, indicating that the sequence of SP-A from G45 to P80 contained an important motif for T cell inactivation. Examination of this region revealed structural domains with alternating positive and negative charges, including an RGD motif at residues 65–67. We cannot however, exclude the possibility that the deletions created changes in the oligomeric structure of SP-A that may also affect inhibition.

Western blot and densitometric analysis of lysates from adherent or nonplastic adherent PBMC as well as from resting and T cell mitogen-treated PBMC suggest that this protein is expressed on lymphocytes in addition to macrophages. Furthermore, these data suggest that T cells may increase the expression of SPR-210 upon activation. Together, the Western blots and results obtained with anti-SPR-210 antiserum in vitro suggest that the antisera blocked the inhibitory activity of bSP-A by disrupting an SP-A-receptor interaction. Further binding studies with radiolabeled SP-A, isolated PBMC, and the F(ab')2 of the anti-SPR-210 antibody will clarify this interpretation.

A number of receptors expressed on PBMC that recognize the RGD sequence to activate T cells and macrophages (1, 18, 34). More specifically, it has been shown that immobilized anti-CD-3 antibody and fibronectin act synergistically to stimulate CD-4 cell proliferation and that this proliferative effect can be inhibited with antibody directed against the common β-subunit of the very late antigen subfamily of integrins (26). Cardarelli et al. (10) also demonstrated that fibronectin increased IL-2 receptor (CD-25) expression on CD-3-activated PBMC in a synergistic fashion. It is therefore possible that SP-A inhibits T cell proliferation through sequences that block costimulatory signals.

In summary, this study shows that SP-A inhibited T cell proliferation in vitro and suggests that this effect is mediated by a specific receptor, which is not recognized by C1q. The domain responsible is a segment of the collagen-like domain, perhaps an RGD motif. The carbohydrate recognition domain does not play a role. These data support the hypothesis that SP-A contributes to the inhibition of in vivo T cell proliferation and that this effect helps maintain the overall hyporesponsive state of pulmonary leukocytes.

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