Alterations in lung collectins in an adaptive allergic immune response

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Alterations in lung collectins in an adaptive allergic immune response. Am J Physiol Lung Cell Mol Physiol 282: L573–L584, 2002; 10.1152/ajplung.00117.2001.—Although surfactant apoproteins are known to be mediators of innate responses, their relationship to adaptive responses has not been examined extensively. We investigated possible links between surfactant apoproteins and responses to allergens by studying alterations in surfactant apoproteins A, B, and D in a murine model of allergic pulmonary inflammation. Three murine strains (BALB/c, C57BL/6, and 129J) demonstrated increased immunoactivity of surfactant apoproteins A and D in nonciliated epithelial cells of noncartilaginous airways after aerosolized challenge. In contrast, surfactant apoprotein B immunostaining was unchanged. Immunoblotting demonstrated increased surfactant A in bronchoalveolar lavage fluid after allergen sensitization and challenge. Surfactant apoprotein A and D induction required T and/or B lymphocyte responses to allergen, since the induction was absent in recombinase-activating gene-deficient mice. Moreover, SP-A expression is important to both innate immunity and adaptive immune responses to allergens.

THE ADAPTIVE AND INNATE IMMUNE responses complement each other in their roles to promote host defenses. Adaptive immunity involves antigen-specific receptors and requires hours to days for differentiation of effector cells. In contrast, innate immunity involves pattern recognition receptors, and the effector stage occurs within minutes to hours of exposure. Although adaptive antigenic responses are known to play essential roles in allergic asthma, the role of innate immunity is less well characterized.

Innate responses involve many cell types and mediators, including surfactant apoproteins (8, 9, 14, 38, 40, 55). The best known function of surfactant and the surfactant apoproteins is to decrease the surface tension in the alveoli. However, surfactant apoproteins also modulate pulmonary inflammatory responses (8, 14, 40, 55).

Two of the surfactant apoproteins, surfactant A (SP-A) and surfactant D (SP-D), are collectins. The collectins are a family of proteins characterized by a collagen-like region and a C-type lectin at their carboxy terminus (20), which also includes mannose-binding protein (18–20, 38). The collectins are important mediators of innate immunity, with their functions including opsonizing bacteria and promoting phagocytosis; mannose-binding protein has also been demonstrated to activate complement (8, 20).

SP-A and SP-D can modulate innate immune responses in the lung. Both SP-A and SP-D can bind and opsonize bacteria (28, 32, 48) and fungi (1, 9, 17, 34, 37, 39, 42, 44, 46, 47, 54). In addition, SP-A can bind viruses such as influenza A and herpes simplex (35, 49, 50) as well as Pseudomonas carinii (57) and is a chemoattractant for macrophages (56). Mice deficient in SP-A are more susceptible to infections resulting from Pseudomonas (30) and Streptococcus (31) compared with wild-type mice. Moreover, SP-A expression is induced during lung inflammation after exposures that include lipopolysaccharide (LPS), bleomycin, and silica (51).

Although allergic pulmonary responses are clearly dependent on adaptive immune responses, the relationship of innate mediators to these responses has not been characterized extensively. Given the potential role of surfactant apoproteins in innate and adaptive in vivo responses, we asked whether the surfactant apoproteins are involved in the pulmonary response to allergen. To investigate pulmonary adaptive responses, we analyzed an in vivo murine model of asthma, elicited by systemic sensitization to the allergen ovalbumin (OVA) followed by aerosolized challenge, resulting in eosinophilic pulmonary infiltrates and airway hyperresponsiveness (AHR; see Refs. 11, 26, 27, 36). We hypothesized that immune responses...
observed in this allergic model involve innate immunity, specifically the collectin surfactant apoproteins SP-A and SP-D. To test our hypothesis, we compared the expression of collectins SP-A and SP-D with that of a noncollectin hydrophobic surfactant apoprotein (SP-B) after allergic sensitization and challenge. In our murine model, increased AHR was associated with changes in SP-A and SP-D expression. Expression of SP-A and SP-D within the nonciliated epithelial cells of the noncartilaginous airways (bronchiolar cells) in-

Fig. 1. Histological analysis of surfactant apoprotein expression after allergen [ovalbumin (OVA)] sensitization and challenge at time points day 0 (naive) and day 21 (after seventh aerosolized OVA challenge, as described in METHODS). Hematoxylin and eosin stains of representative lung sections are presented in A (day 0) and B (day 21). Representative immunostaining for surfactant protein (SP)-A is presented in C (day 0, n = 3) and D (day 21, n = 4). Representative immunostaining for SP-D is presented in E (day 0, n = 3) and F (day 21, n = 4). Representative immunostaining for SP-B is presented in G (day 0, n = 3) and H (day 21, n = 4). L, airway lumen; E, airway epithelium. Magnification, ×200.
creases after allergen sensitization and challenge, suggesting that the bronchiolar cells may participate in the pulmonary immune response to allergens. These results are also consistent with a role for SP-A and SP-D in pulmonary adaptive allergic responses.

METHODS

Mice. Eight-week-old male BALB/c, C57BL/6, 129J, and recombinase-activating gene-1 (RAG1)-deficient mice on a C57BL/6 background were purchased from Jackson Laboratories (Bar Harbor, ME) and stored in a sterile environment. Mice that overexpress a mutated form of the inhibitor of κB (IkBα, C57BL/6 background) and mice deficient in c-Rel (C57BL/6 background; generous gift of H. C. Liou), a constituent of nuclear factor (NF)-κB, were generated as previously described (2, 11, 33). Mice were fed rodent diet 5001 (Harlan, Madison, WI). The mice were maintained according to the guidelines of the Committee on Animals of the Harvard Medical School and the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Protocol for allergen sensitization and challenge. Mice were sensitized and challenged with OVA as previously described (26). Briefly, mice were sensitized by intraperitoneal injection of 10 μg chicken OVA and 1 mg Al(OH)3 (Alum) on days 0 and 7. On days 14–20, mice received daily aerosolized OVA challenges with 6% OVA for 25 min/day. OVA was dissolved in 0.5× PBS. Control mice received 1 mg Alum in PBS ip on days 0 and 7 and were nebulized with PBS on days 14–20. An ultrasonic nebulizer (model 5000; DeVilbiss, Somerset, PA) was used for nebulizations in a chamber.

Determination of AHR. After the last aerosol challenge (24 h), AHR was assessed using whole body plethysmography (Buxco, Troy, NY), as has been described previously (16). For the kinetic studies, AHR was assessed at time 0 (naive mice), day 15 (after the first aerosolized challenge), day 17 (after the third challenge), day 19 (after the fifth challenge), and day 21 (after the seventh challenge). Mice were placed in individual chambers. Methacholine was nebulized in the chambers via an inlet at a concentration of 100 mg/ml for 3 min, since this dose of methacholine produces significantly increased airway resistance compared with baseline measurements in mice sensitized and challenged with OVA, but not in PBS-exposed mice (13). Readings were averaged over 10 min from the beginning of the nebulization. The whole body plethysmography system measures changes in box pressure during expiration and inspiration, allowing the calculation of enhanced pause (P enh), which directly correlates with airway resistance (13, 16). Baseline airway resistance was determined by measuring P enh after aerosolized PBS.

Western (immunoblotting) analysis. For Western analysis, 1 ml of bronchoalveolar lavage (BAL) fluid was centrifuged at 7,900 g for 10 min to remove cells and then at 11,900 g for 40 min. The supernatant was removed, and the pellet (containing SP-A) was resuspended in 100 μl of running buffer. Samples were loaded on the gel, subjected to electrophoresis, and electroblotted on nitran. The blot was blocked in 5% milk in PBS with 0.1% Tween 20 (PBST; Fisher Scientific) for 1 h at room temperature. Rabbit anti-SP-A antisera (generous gift of E. Ingenito and R. Mora) was diluted 1:500 in PBST and applied to the blot for 1 h at room temperature. The blot was washed in PBST, and peroxidase-labeled goat anti-rabbit secondary antibody (Pierce Chemical, Rockford, IL) diluted 1:5,000 in 5% milk in PBST was applied for 30 min at room temperature. The blot was washed in 5% milk in PBST, then in PBST, and finally in PBS. The location of SP-A (monomer molecular mass 29 kDa) was identified by Renaissance Western Chemiluminescence (NEN Life Sciences, Boston, MA), following kit instructions. The densities of the individual bands were compared using densitometry ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Immunohistochemical analysis. For immunohistochemical analysis, the lung tissue samples were removed from the thoracic cavity and snap-frozen in liquid nitrogen. Lung tissue samples were obtained from each time point. Frozen sections 5 μm thick were cut, and the tissues were screened using hematoxylin and eosin to determine that alveolar tissue and at least three nonciliated bronchioles were visible in the section. Tissue sections meeting these standards were stained using a modified avidin-biotin technique (15). The time points analyzed were controls (naive and OVA-sensitized, aerosolized PBS challenge), day 2 (after first OVA ip injection), day 8 (after second OVA ip injection), day 13 (day before aerosol challenge), day 15 (after the first aerosolized OVA challenge); day 17 (after the third aerosolized OVA challenge), day 19 (after the fifth aerosolized OVA challenge), and day 21 (after the seventh aerosolized OVA challenge). For each time point, n = 3–5 determinations. The primary antibodies included an IgG fraction of rabbit polyclonal anti-serum against bovine SP-A used at 1:1,000, a whole rabbit polyclonal anti-serum against bovine SP-B used at 1:1,000 (generous gifts of R. Mora and E. P. Ingenito), and a whole rabbit polyclonal anti-serum against rat SP-D (generous gift of E. Crouch) used at 1:750. The cross-reactivity of these antisera to murine apoproteins and the specificity of these antisera for their respective apoproteins have been shown previously (22, 41). In addition, the immunostaining patterns were replicated using a second set of whole rabbit antisera against murine SP-D, human SP-A, and bovine SP-B (Chemicon International, Temecula, CA). The SP-B immunostaining was enhanced by tyramide amplification using the TSA Biotin System kit, following the manufacturer’s instructions (NEN). Negative controls consisted of substituting PBS and purified rabbit IgG for the primary antibody. An experienced reader (Haley) scored the slides in a blinded fashion. All of the nonciliated bronchioles and alveolar tissue from at least one lobe were examined and scored for each animal. The intensity of immunostaining was assessed by a semiquantitative method as follows: 0 designating no immunostaining, 1+ designating faint intensity of immunostaining, 2+ designating moderate immunostaining, and 3+ designating intense immunostaining.

Statistical analysis. Data analysis was performed using Sigma Stat for airway measurements. Parametric data were analyzed with the Tukey-Kramer test, and nonparametric

Table 1. Adaptive allergic responses and surfactant

<table>
<thead>
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<th>Surfactant Apoprotein</th>
<th>Naive Mice</th>
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Semiquantitative comparison of immunostaining intensity among three murine strains (BALB/c, C57BL/6, and 129J) after ovalbumin (OVA) sensitization and challenge. SP-A, surfactant apoprotein A; SP-D, surfactant apoprotein D; SP-B, surfactant apoprotein B; D19, day 19 of the OVA protocol (after the fifth aerosolized challenge); D21, day 21 of the OVA protocol (after the seventh aerosolized challenge); +, faint intensity of immunostaining; ++, moderate immunostaining; ++++, intense immunostaining.
data were analyzed by the Wilcoxon-Kruskal-Wallace rank-sum test. Data are reported as means ± SE. Statistical significance was defined as $P < 0.05$.

**RESULTS**

Surfactant apoprotein expression after OVA sensitization and challenge. Three murine strains (BALB/c, C57BL/6, and 129J) sensitized and challenged with the antigen OVA developed peribronchial and perivascular eosinophilic and lymphocytic infiltrates, similar to previous studies by our laboratory analyzing the BALB/c strain (26). Histological examination of all three murine strains on day 21 (after the seventh aerosolized OVA challenge) confirmed the development of abundant inflammatory infiltrates (Fig. 1), concomitant with AHR, increased

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**Fig. 2.** Kinetics of SP-A, SP-D, and SP-B in BALB/c mice. Representative immunostaining is presented for SP-A on day 0 (naive; A), day 19 (after the fifth aerosolized OVA challenge; B), and day 21 (after the seventh aerosolized OVA challenge; C). Representative immunostaining for SP-D is presented on day 0 (D), day 19 (E), and day 21 (F). Representative immunostaining for SP-B is presented on day 0 (G), day 19 (H), and day 21 (I). Arrows designate alveolar cells immunopositive for SP-B, demonstrating that SP-B was detected in these lung tissue samples. Magnification, ×200.
serum IgE, and eosinophilia, similar to our previous analyses of this model (11, 13, 26, 27, 36).

We initially focused on the BALB/c strain, which we have found to have a greater degree of AHR relative to other strains in this allergic model (26). BALB/c mice demonstrated increased expression of SP-A and SP-D by bronchiolar cells, but not the alveolar epithelial cells, after OVA sensitization and challenge (Fig. 1, C–F). In contrast, control naive mice had low levels of SP-A and SP-D expression in bronchiolar cells. Both OVA-treated and control mice had constitutive expression of SP-A and SP-D in cells located in the alveolar epithelial cells. No immunostaining was appreciated when PBS or purified rabbit IgG was substituted for the primary antibody in either the PBS or OVA-treated animals (see Fig. 3, insets). The increased expression of SP-A and SP-D in the bronchiolar cells after allergen sensitization and challenge corresponded to the development of abundant peribronchial and perivascular infiltrates (Fig. 1, A and B). Furthermore, there were no changes after allergen sensitization and challenge in the expression of the noncollectin surfactant apoprotein SP-B (Fig. 1, G and H). The patterns of immunostaining for SP-A, SP-D, and SP-B were the same for primary antisera for these apoproteins obtained from multiple independent sources. A semiquantitative comparison of the intensity of immunostaining for SP-A, SP-D, and SP-D is shown in Table 1. The kinetics of allergen-induced SP-A and SP-D expression were examined (Fig. 2). SP-A and SP-D immunostaining remained at baseline expression after OVA sensitization and the first three aerosolized challenges (days 15 and 17; data not shown). After the fifth challenge (day 19) with aerosolized OVA, the bronchiolar cells showed increased expression of SP-A and SP-D (Fig. 2), which persisted for at least 48 h (day 21).

To determine whether the changes in SP-A and SP-D expression were strain dependent, we examined two additional murine strains, C57BL/6 and 129J, after OVA sensitization and challenge (Fig. 3 and data not shown). These two strains exhibit pulmonary allergic inflammation and increased AHR after OVA sensitization and challenge (6, 11, 36). These two strains also demonstrated increased SP-A and SP-D immunostaining in nonciliated bronchiolar cells after allergen sensitization and challenge. The kinetics of increased
SP-A and SP-D expression after OVA sensitization and challenge were similar for the BALB/c (Fig. 2) and the C57BL/6 (Fig. 3) strains. Analysis by immunoblotting demonstrated increased amounts of SP-A in murine BAL fluid after OVA sensitization and challenge compared with PBS controls (Fig. 4). As was observed for the BALB/c strain, there were no differences in the expression of SP-B after allergen sensitization and challenge (data not shown). Although there were some kinetic differences, the 129J mice also showed enhanced SP-A and SP-D immunostaining in the bronchiolar cells after OVA sensitization and challenge. In 129J mice, increased SP-A immunoreactivity was observed after the third challenge (day 17), with increased SP-D immunoreactivity noted after the fifth challenge (day 21; data not shown). Similarly to the BALB/c and C57BL/6 strains, the 129J mice did not demonstrate any differences in the expression of SP-B after allergen sensitization and challenge (data not shown).

The kinetics of AHR after OVA sensitization and challenge were analyzed using whole body plethysmography. The development of AHR correlated with the increased expression of SP-A and SP-D (Fig. 5). Specifically, at the times of greatest AHR (within the first 6 days of aerosolized challenge), SP-A and SP-D expression was also maximal.

Dependence on intact lymphocytic functioning. To determine whether the increased expression of SP-A and SP-D was a nonspecific reaction to the presence of pulmonary inflammation or a specific response requiring intact B and/or T lymphocyte functioning, we examined mice with defects in their responses to OVA sensitization and aerosolized challenge. We first examined the responses of mice deficient in c-Rel, a lymphoid-predominant NF-κB component, after OVA sensitization and aerosolized challenge. Prior reports indicated that these mice do not develop significant pulmonary infiltrates after OVA sensitization and aerosolized challenge (11). Compared with wild-type C57BL/6 controls, the c-Rel-deficient mice demonstrated attenuated IgE levels after OVA sensitization and aerosolized challenge (11). However, T cells from mice deficient in c-Rel express normal amounts of the costimulatory molecules CD28 and CD40 ligand after stimulation with anti-CD3 (33). These findings indicate that mice deficient in c-Rel have diminished, but not absent, T cell responses. We used immunostaining to evaluate the expression of SPA in c-Rel-deficient mice after OVA sensitization and aerosolized challenge. Naive c-Rel-deficient mice demonstrate constitutive SP-A expression similar to that of their background strain, C57BL/6 (Fig. 6A). After OVA sensitization and aerosolized challenge, c-Rel-deficient mice demonstrate increased bronchiolar immunostaining for SP-A, without changes in alveolar epithelial cell staining (Fig. 6B). As was observed in the wild-type C57BL/6 mice, this increased expression of SP-A persisted for at least 48 h (Fig. 6C). Thus the increased expression of SP-A after OVA sensitization and aerosolized challenge does not require allergic pulmonary inflammation but may depend on aspects of lymphocyte activation that remain active in c-Rel-deficient mice.

To evaluate whether altered surfactant apoprotein immunoreactivity was associated with activated T lymphocytes, we analyzed transgenic mice that overexpress a mutated form of the inhibitor of IkBα, an inhibitory component of NF-κB, selectively in T cells. This mutated form of IkBα, designated as IkBα(ΔN), resists degradation and therefore inhibits NF-κB acti-

![Fig. 4. Immunoblotting of bronchoalveolar lavage (BAL) fluid for SP-A. BAL fluid was prepared from PBS control (n = 2) and OVA-sensitized and -challenged (n = 3) C57BL/6 mice (day 21) and analyzed by immunoblotting for SP-A (monomer size 29 kDa), as described in METHODS. A representative immunoblot demonstrating increased amounts of SP-A in BAL after OVA sensitization and challenge is shown. Relative density is average of densitometry of OVA samples (4.178) divided by average of PBS samples (2.755). Densitometry values were determined by ImageQuant software. Molecular mass markers are presented at the side of the immunoblot, with approximate masses in kDa indicated.](http://ajplung.physiology.org/ Downloaded from)

![Fig. 5. Kinetics of airway hyperresponsiveness (AHR) BALB/c mice. Mice were sensitized and challenged with OVA as described in METHODS. AHR was evaluated on day 0 (naive), day 15 (after the first aerosolized OVA challenge), day 17 (after the third OVA challenge), day 19 (after the fifth OVA challenge), and day 21 (after the seventh OVA challenge; n = 3–5 at each time point). Baseline airway resistance was determined at each time point by measuring enhanced pause (P<sub>aw</sub>) after aerosolized PBS. Results of in vivo plethysmography are reported as adjusted P<sub>aw</sub>(a value that directly correlates with airway resistance), which is the average of the P<sub>aw</sub> responses to methacholine divided by the average of the P<sub>aw</sub> responses to PBS. *Significantly different from naive (P < 0.05).](http://ajplung.physiology.org/ Downloaded from)
After OVA sensitization, these mice demonstrate decreased AHR, pulmonary infiltrates, and bronchoalveolar eosinophils but show preserved IgE responses compared with control mice (2). Inhibitor mice were evaluated for changes in surfactant apoprotein expression after OVA sensitization and aerosolized challenge by examining immunostaining for SP-A. At baseline, these transgenic mice have constitutive expression of SP-A similar to that of their background strain, C57BL/6 (Fig. 6D). After OVA sensitization and aerosolized challenge, the IkBa(ΔN) transgenic mice demonstrated increased SP-A immunostaining in the bronchiolar cells without any substantial increases in the amount of alveolar epithelial cell staining (Fig. 6E). The kinetics of maximal SP-A expression were similar in IkBa(ΔN) and wild-type C57BL/6 mice. However, unlike wild-type C57BL/6 mice, the IkBa(ΔN) animals did not sustain increased immunostaining for SP-A in that expression of SP-A returned to baseline by day 21 (Fig. 6F).

Previously, we have found that OVA-induced allergic inflammation and AHR are dependent on T lymphocyte activation, as evidenced by increased expression of the IL-2 receptor and dependence on T cell accumulation in this model (26, 27, 36). To examine whether T and/or B lymphocytic activation was required for the increased expression of surfactant apoproteins, we analyzed mice deficient for RAG1, which have no functioning T or B lymphocytes. The RAG1-deficient mice are incapable of mounting adaptive immune responses, but innate responses of nonlymphoid cells would be expected to be preserved. In contrast to wild-type mice, the RAG1-deficient mice did not develop AHR after OVA sensitization and challenge (Fig. 7). Moreover, the RAG1-
Fig. 7. Lack of allergen-induced AHR in recombinase-activating gene-1 (RAG1)-deficient (RAG1 KO) mice, as demonstrated by comparison of adjusted P_{muc} in RAG1-deficient and wild-type C57BL/6 mice. Both RAG1-deficient and wild-type C57BL/6 mice were sensitized and subjected to aerosolized OVA challenges as described in METHODS. PBS controls received ip PBS and Al(OH)\(_3\) followed by aerosolized PBS. Both groups of mice were examined on day 21 (after the seventh aerosolized challenge). *OVA-treated C57BL/6 wild-type mice significantly different from PBS-treated control mice (P < 0.05).

deficient mice did not develop any peribronchial or perivascular infiltrates after OVA sensitization and aerosolized challenge (Fig. 8, A and B). Importantly, the expression of SP-A or SP-D was no longer induced in the bronchiolar cells, whereas the constitutive alveolar expression was maintained (Fig. 8, C–F). As was noted in three wild-type strains, BALB/c, C57BL/6, and 129J, SP-B expression did not change in the bronchiolar cells after OVA sensitization and challenge in the RAG1-deficient mice, and constitutive alveolar expression was maintained (data not shown). Thus there was no allergen-induced increase of bronchiolar SP-A and SP-D expression in the absence of functioning B or T lymphocytes.

DISCUSSION

This study demonstrates that expression of collectins SP-A and SP-D is altered in the airways after allergen sensitization and challenge in a murine model of asthma. Three murine strains, BALB/c, C57BL/6, and 129J, were analyzed. All three strains demonstrated increased expression of SP-A and SP-D in the bronchiolar cells. Analysis of BAL fluid by immunoblotting demonstrated increased SP-A protein after OVA sensitization and challenge, suggesting that the increased immunostaining was the result of increased protein expression. In contrast, there were no changes in the bronchiolar cell expression of SP-B, a noncollectin surfactant apoprotein that is critical in reducing surface tension in the lung. Thus far, there have not been reports of SP-B modulating the function of leukocytes (40).

The increased surfactant apoprotein expression was also observed in c-Rel-deficient and IκBα(ΔN) transgenic mice, both of which demonstrate attenuated responses to OVA sensitization and challenge. This finding suggests that the increased surfactant apoprotein expression does not require the development of parenchymal pulmonary infiltrates. Furthermore, our data also show that the increased expression of SP-A and SP-D is not seen in mice deficient in RAG1, which lack any functioning T and/or B cells. Together, these data suggest that some degree of lymphocyte activity is required for the increased SP-A and SP-D expression after OVA sensitization and aerosolized challenge. Indeed, the presence of increased surfactant apoprotein expression in the context of diminished B and/or T cell function, and the absence in the context of ablated B and/or T cell function, suggests that collectins, important mediators of innate responses, are also relevant to pulmonary adaptive allergic responses.

The increase in SP-A and SP-D was observed in the bronchiolar cells, which are critical effector cells in pulmonary injury and repair. In mice, the majority of the bronchiolar cells express Clara cell-specific protein/CC10, the major secretory product of Clara cells (12). Arsalane and colleagues (3) showed decreased expression of Clara cell-specific protein after intratracheal LPS. In addition to models of acute lung injury, alterations in bronchiolar cell function have also been demonstrated in asthma, with decreased Clara cell-specific protein demonstrated in asthmatic vs. control subjects (45). Bronchiolar cells may have anti-inflammatory properties, since Clara cell-specific protein decreases expression of interferon-γ in cultured peripheral blood mononuclear cells (10) and inhibits cytosolic phospholipase A\(_2\)-mediated fibroblast chemotaxis (29). Our data suggest that bronchiolar cells may have a role in adaptive allergic responses.

Investigations in nonallergic models have demonstrated that SP-A and SP-D modulate adaptive pulmonary immune responses. SP-A and SP-D decrease some in vitro responses (4, 5, 7, 53) but augment others (23–25). SP-A and SP-D also decrease proliferation of human peripheral blood lymphocytes stimulated with anti-CD3 or phytohemagglutinin (4, 5). Additionally, SP-A decreases cytokine expression in human pulmonary macrophages stimulated with Candida albicans (43) and in human eosinophils stimulated with 12-o-tetradecanoylphorbol-13-acetate (12). In contrast, SP-A increases proliferation and the release of both IgA and IgG from cultured rat splenocytes stimulated with concanavalin A (24) and augments the expression of tumor necrosis factor-α, IL-1β, and IL-6 by both the monocyte-like cell line THP-1 (25) and unstimulated human peripheral blood mononuclear cells (23). Our findings differ from previous investigations of the roles of surfactant apoproteins in immunological responses in that the current study focuses on an allergic adaptive immune response.

Previous in vitro analyses also suggest a role for SP-A and SP-D in asthma. The carbohydrate recognition domain of both SP-A and SP-D can bind the most common allergen associated with the dust mite, Der p 1 (52). Both SP-A and SP-D decreased lymphocyte
Fig. 8. Histological analysis of representative lung sections from RAG1-deficient mice. Hematoxylin and eosin staining for day 0 (A) and day 21 (B) demonstrating lack of typical pulmonary infiltrates after OVA sensitization and aerosolized challenge. Representative immunostaining for SP-A on day 0 (naive; C) and day 21 (after the seventh aerosolized challenge; D) demonstrating unchanged expression of SP-A after OVA sensitization and challenge. Representative immunostaining for SP-D on day 0 (naive; E) and day 21 (after the seventh aerosolized challenge; F) demonstrating unchanged expression of SP-D after OVA sensitization and challenge. Magnification, ×200.
proliferation after exposure to Der p 1 from asthmatic children (53). The effects of SP-A and SP-D were dose dependent and were also observed with pretreating the cultures with the surfactant apoproteins. Both SP-A and SP-D decrease lymphocyte proliferation and cytokine production in asthmatic patients (53). These studies, together with our in vivo analyses indicating an upregulation of SP-A and SP-D in bronchiolar cells concomitant with increased AHR, support the notion that SP-A and SP-D may play a role in allergic asthma.

Our proposal that the increased expression of SP-A and SP-D after OVA sensitization and aerosolized challenge involves a T cell-dependent mechanism is supported by recent studies. Ikegami and colleagues (21) demonstrated that mice that overexpress IL-4 in Clara cells have increased expression of both SP-A and SP-D. Moreover, the expression of SP-D was significantly greater than that predicted by the increase in lung-saturated phosphatidylcholine, consistent with regulation of SP-D by IL-4 (21).

Increased surfactant apoprotein expression could modulate the pulmonary response to allergens in several possible ways. One possible explanation for the increased SP-A and SP-D expression is a compensatory increase in surfactant production to offset the dysfunction caused by the influx of serum proteins that occurs in the alveolar space after an allergic challenge. However, although this mechanism is possible, it is not the most likely explanation of our results, since the expression of the hydrophobic surfactant apoprotein B did not change after OVA sensitization and aerosolized challenge. Moreover, the location of the increased SP-A and SP-D expression in our immunohistochemical studies was the bronchiolar epithelial cells. In our experiments, the alveolar epithelial cells, which would be expected to have greater impact on the alveolar space than the bronchiolar cells, did not change their expression of surfactant apoproteins after OVA sensitization and challenge.

Increased expression of SP-A and SP-D may be an integral part of the pulmonary adaptive allergic response. Thus our findings imply a novel function for the surfactant apoproteins. We speculate that the increased expression of SP-A and SP-D in our model depends on lymphocyte-associated cytokines.

Support for the proposal that alterations in the expression of SP-A and SP-D are an integral part of the pulmonary response to allergens, as opposed to a non-specific response to pulmonary inflammation, is found in our in vivo demonstration that SP-A and SP-D, but not SP-B, are upregulated after allergen sensitization concomitant with the kinetics of allergen-induced AHR. Additional support for a role for SP-A and SP-D in adaptive allergic responses is found in our analysis of models in which the adaptive responses are disrupted. In RAG1-deficient mice, the inability to mount an adaptive allergic response after OVA sensitization is paralleled by a loss of the increased SP-A and SP-D expression in bronchiolar cells. However, increased SP-A and SP-D expression is preserved in the c-Rel mice despite diminished pulmonary infiltrates after OVA sensitization and challenge. Together, these data suggest that it is unlikely that the alterations in SP-A and SP-D expression are nonspecific responses to the presence of pulmonary inflammation but rather are responses to B and/or T cell activation. Interestingly, after LPS administration, both SP-A and SP-B expression decrease, which is a different pattern of surfactant apoprotein expression than that observed in the current study (22).

In summary, our data demonstrate that SP-A and SP-D, but not SP-B, are altered after OVA sensitization and challenge in a murine model. The changes in the expression of these apoproteins were conserved among three murine strains, BALB/c, C57BL/6, and 129J, and thus were not strain-specific responses. The innate immunity mediators SP-A and SP-D may play a role in the pulmonary inflammatory responses to allergen, since allergen-induced expression of SP-A and SP-D in bronchiolar cells is dependent on functioning T and/or B cells. SP-A and SP-D may be important links between the innate and adaptive immune systems in the pulmonary response to allergen.

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