

Susceptibility of Mice Genetically Deficient in the Surfactant Protein (SP)-A or SP-D Gene to Pulmonary Hypersensitivity Induced by Antigens and Allergens of *Aspergillus fumigatus*¹

Taruna Madan,* Kenneth B. M. Reid,[†] Mamta Singh,* P. Usha Sarma,^{2*} and Uday Kishore[‡]

Lung surfactant protein A (SP-A) and D (SP-D) are innate immune molecules which are known to interact with allergens and immune cells and modulate cytokine and chemokine profiles during host hypersensitivity response. We have previously shown therapeutic effects of SP-A and SP-D using a murine model of lung hypersensitivity to *Aspergillus fumigatus* (*Afu*) allergens. In this study, we have examined the susceptibility of SP-A (AKO) or SP-D gene-deficient (DKO) mice to the *Afu* allergen challenge, as compared with the wild-type mice. Both AKO and DKO mice exhibited intrinsic hypereosinophilia and several-fold increase in levels of IL-5 and IL-13, and lowering of IFN- γ to IL-4 ratio in the lungs, suggesting a Th2 bias of immune response. This Th2 bias was reversible by treating AKO or DKO mice with SP-A or SP-D, respectively. The AKO and DKO mice showed distinct immune responses to *Afu* sensitization. DKO mice were found more susceptible than wild-type mice to pulmonary hypersensitivity induced by *Afu* allergens. AKO mice were found to be nearly resistant to *Afu* sensitization. Intranasal treatment with SP-D or rhSP-D (a recombinant fragment of human SP-D containing trimeric C-type lectin domains) was effective in rescuing the *Afu*-sensitized DKO mice, while SP-A-treated *Afu*-sensitized AKO mice showed several-fold elevated levels of IL-13 and IL-5, resulting in increased pulmonary eosinophilia and damaged lung tissue. These data reaffirm an important role for SP-A and SP-D in offering resistance to pulmonary allergenic challenge. *The Journal of Immunology*, 2005, 174: 6943–6954.

Two of the hydrophilic lung surfactant proteins (SP),³ SP-A and SP-D, are considered carbohydrate pattern recognition molecules of innate immunity which have been shown to interact with a range of pathogens, allergens, and apoptotic cells (1, 2). This interaction effects recruitment and activation of a host of immune cells, leading to differential pulmonary cytokine and chemokine profiles as a part of host response (3). The primary structure of SP-A and SP-D is organized into four regions: an N-terminal region involved in the formation of interchain disulphide bonds, a collagen region composed of Gly-X-Y repeats, a neck peptide, and a C-terminal C-type lectin domain. They are large oligomeric structures, each assembled from multiple copies of a single polypeptide chain (human SP-A has two closely-related chains). The lectin domains are spaced, in a trimeric orientation, at the end of triple-helical collagen stalks (4). Six of these trimeric

subunits make up the overall structure of SP-A, while SP-D is composed of a cruciform-like structure, with four arms of equal length.

The lectin domains are usually the ligand recognition domain which are known to interact with carbohydrate structures on the surfaces of a wide range of pathogens, such as viruses, bacteria, and fungi. SP-A and SP-D are also known to interact with phagocytic cells and enhance their chemotactic, phagocytic, and oxidative properties (1, 5). Therefore, the recognition of non-self via lectin domain and subsequent engagement of collagen region with immune cells via the collectin receptor enhances killing by activated phagocytic cells (6). The interaction between the collagen region of SP-A and SP-D (when bound to ligand via lectin domain) with immune cells is generally considered to be mediated via a common collectin receptor, calreticulin/CD91 complex (7). This interaction has been shown to enhance p38 MAPK activation, NF- κ B activity, and production of proinflammatory cytokines/chemokines in macrophages (7). SP-A and SP-D also mediate another independent signal transduction pathway, which appears anti-inflammatory and results from direct interactions of trimeric lectin domains with specific cell surface glycoproteins (7).

SP-A and SP-D have also been shown to be involved in the modulation of pulmonary inflammatory responses and resistance to allergen-induced airway hypersensitivity (2, 8–10). Abnormal levels of SP-A and SP-D in bronchoalveolar lavage (BAL) have been reported in hypersensitivity lung diseases and asthmatics show increased amounts of SP-A and SP-D in BAL as compared with those in controls (11, 12). Serum SP-D levels for two allergic patients have been found elevated at diagnosis which decreased following corticosteroid therapy (13). The patients of birch pollen allergy and pulmonary alveolar proteinosis (PAP) showed a shift toward lower oligomeric forms of SP-A, in comparison to healthy volunteers with a possible loss or alteration of biological function (14).

SP-A and SP-D can bind via their lectin domains to allergenic extracts derived from pollens, the house dust mite, and *Aspergillus*

*Institute of Genomics and Integrative Biology, Council for Scientific and Industrial Research, Delhi, India; [†]Medical Research Council Immunochimistry Unit, Department of Biochemistry, University of Oxford, and [‡]Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

Received for publication July 23, 2004. Accepted for publication March 18, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Council for Scientific and Industrial Research (to T.M., M.S., and P.U.S.), the Medical Research Council (to K.B.M.R.) and the European Commission (to K.B.M.R. and U.K.).

² Address correspondence and reprint requests to Dr. P. Usha Sarma, Institute of Genomics and Integrative Biology, Delhi University Campus, Mall Road, Delhi-110007, India. E-mail address: u_sarma@hotmail.com

³ Abbreviations used in this paper: SP-A, human surfactant protein A; SP-D, human surfactant protein D; BAL, bronchoalveolar lavage; PAP, pulmonary alveolar proteinosis; *Afu*, *Aspergillus fumigatus*; AKO, SP-A gene deficient; DKO, SP-D gene deficient; MMP, matrix metalloproteinase; rhSP-D, a recombinant fragment of human surfactant protein D, composed of homotrimeric neck and C-type lectin domains; WT, wild type; 3wcf, three week culture filtrate; ABPA, allergic bronchopulmonary aspergillosis; EPO, eosinophil peroxidase; ROS, reactive oxygen species; IAV, influenza A virus.

fumigatus (*Afu*) inhibit specific IgE binding to allergens, and block allergen-induced histamine release from sensitized basophils (15–17). SP-A and SP-D can reduce the proliferation of PBMC isolated from mite-sensitive asthmatic children (18), and SP-D, in particular, has a suppressive effect on the secretions of IL-2 by PBMC (19). Using murine models of pulmonary hypersensitivity induced by allergens derived from *Afu* (8), the house dust mite (20), and OVA (21), it has been shown that therapeutic treatment of sensitized mice with SP-A or SP-D can reverse hypersensitivity response which involves lowering of specific IgE levels and blood and pulmonary eosinophilia, and a shift in cytokine profile from Th2 to Th1 type.

The experiments conducted using the transgenic mice, genetically deficient in SP-A or SP-D, have also emphasized a key role played by SP-A and SP-D in pulmonary immune response. The SP-A gene-deficient (AKO) mice are less effective in clearing lung pathogens (22). Concentrations of TNF- α , IL-6, and IL-1 α are increased in BAL fluid of AKO mice, which can increase further on adenoviral administration. Coadministration of adenovirus and purified human SP-A can ameliorate adenoviral-induced lung inflammation in AKO mice (23). Mice genetically deficient in SP-D (DKO) show chronic inflammation, foamy alveolar macrophages secreting 10-fold higher levels of hydrogen peroxide, increased activity of matrix metalloproteinases (MMP), emphysema, and fibrosis in the lungs (24).

The present study was undertaken to comparatively evaluate the effect of deficiency of SP-A or SP-D genes on eosinophilia and Th2 cytokines in view of their role in the pathogenesis of allergy and asthma. We observed that both AKO and DKO mice showed intrinsic hypereosinophilia and several-fold increase in the levels of IL-5 and IL-13, and lowering of IFN- γ to IL-4 ratio, suggesting a shift to a Th2 type of response in comparison to the wild-type (WT) mice. Gene expression and exogenous administration of SP-A and SP-D has been able to complement some of the defects of AKO and DKO mice (25–29). Therefore, we examined whether intranasal administration of native human SP-A to AKO, and SP-D or a recombinant fragment of SP-D (rhSP-D) to the DKO mice may reverse hypereosinophilia and Th2 predominance. Because both SP-A and SP-D play a role in *Afu*-mediated hypersensitivity, we have also examined whether AKO and DKO mice were more susceptible to *Afu* sensitization than WT mice and whether intranasal administration of native human SP-A, SP-D, and rhSP-D can rescue the *Afu*-sensitized KO mice.

AKO and DKO mice showed a distinct immune response to *Afu* sensitization. Although DKO showed a cytokine profile similar to that of WT mice on *Afu* sensitization, the magnitude of the effect was higher suggesting that the DKO mice are more susceptible than the WT mice. AKO mice showed a different trend in the cytokines in comparison to WT mice on *Afu* sensitization. However, the magnitude of change was not significant suggesting that AKO may be resistant to *Afu* sensitization. SP-D and rhSP-D were effective in rescuing the *Afu*-sensitized DKO mice while SP-A administered *Afu*-sensitized AKO mice showed manyfold elevated levels of IL-5 and IL-13, resulting in severe pulmonary eosinophilia and damaged lung tissue.

Materials and Methods

Mice

The generation of AKO (30, 31) and DKO (32) mice, by backcrossing in the C57BL/6 background, has been reported. Specific-pathogen-free, 6–8 wk old, male and female C57BL/6 mice of the two strains used for generating AKO mice (termed as WT (AKO type) and DKO mice (termed as WT (DKO type) were obtained from Harlan-OLAC, Shaw's Farm. Mice were housed in the animal care facility at the Department of Biochemistry,

University of Oxford (Oxford, U.K.). They received Purina chow and acidified water ad libitum. Both AKO and DKO mice were pathogen-free and repeated attempts to culture bacterial and fungal organisms from the lungs of these mice were negative. Mice were randomized before experiments. All mice were kept in isolator cages with sterile beddings in a barrier facility for the duration of this study. The beddings were changed daily and four to five animals were housed in each cage.

Antigens

Three-week culture filtrate (3wcf; protein-enriched antigenic fraction, 27 mg/ml) of *Afu* (strain 285, isolated from sputum of an allergic bronchopulmonary aspergillosis (ABPA) patient visiting the V. P. Chest Institute, Delhi, India) were used to sensitize the mice. Its preparation and characterization have been described previously (8).

Preparation of native human SP-A and SP-D

Native human SP-A and SP-D were purified from human BAL collected of patients suffering from PAP, as described earlier (33). Both protein preparations were judged to be pure by SDS-PAGE, Western blot, and amino acid composition. SP-A preparation was free of any SP-D contamination and vice versa. Gel filtration confirmed that ~92% of SP-A preparation is octadecamer and 95% of SP-D preparation is dodecamer oligomers. SP-A and SP-D preparations were further evaluated for endotoxin levels by the QCL-1000 *Limulus* amoebocyte lysate system (BioWhittaker). The amount of endotoxin present in purified SP-A was observed to be 16 pg/ μ g SP-A and for purified SP-D, it was found to be 56 pg/ μ g SP-D.

Expression and purification of rhSP-D

A recombinant fragment, composed of the trimeric α -helical coiled-coil neck region and three C-type lectin domains of human SP-D (rhSP-D), was expressed in *Escherichia coli* and purified to heterogeneity, as recently described (20). The rhSP-D preparation was functionally characterized (34) and its crystallographic structure complexed with maltose in the carbohydrate-binding pockets is available (35). The amount of endotoxin present in the rhSP-D preparations was estimated, as described above, and found to be 4 pg/ μ g rhSP-D.

Immunization of mice

A murine model of ABPA was prepared as previously described (8). Briefly, AKO, DKO, and WT mice (all in C57BL/6 background) were lightly anesthetized with ether, and 50 μ l (100 μ g) of the Ag mixture per mouse was slowly applied to the nostrils using a micropipette with a sterile disposable tip. Mice were then held upright for a few minutes until Ag solution applied to the nostril was completely inhaled. These mice also received 100 μ l (200 μ g) of the same Ag mixture per mouse i.p. Intranasal instillation and i.p. injections were given twice a week to each mouse for four weeks. The last immunization with Ag was conducted on 28th day (named as "0" day for the treatment study) followed by treatment with SP-A, SP-D, rhSP-D, or BSA (as a control protein therapeutic) for the next 3 days (days 1–3 of the treatment study). Mice in the control groups were immunized in the same manner with sterile PBS. A brief description of various mice groups is given in Table I (study design).

Administration of SP-A, SP-D, and rhSP-D

Groups of untreated ABPA mice and untreated control mice of WT, AKO, and DKO mice were intranasally administered 50 μ l of PBS on days 1–3. Groups of mice receiving treatment were named after respective proteins being administered. Human SP-A (3 μ g in 50 μ l of PBS per mouse) was intranasally administered to "SP-A-treated ABPA mice" and "SP-A-treated control mice" on days 1–3. Human SP-D (1 μ g in 50 μ l of PBS per mouse) was intranasally administered to the "SP-D-treated ABPA mice" and "SP-D-treated control mice" on days 1–3. The rhSP-D (1 μ g in 50 μ l of PBS per mouse) was intranasally administered to the groups of "rhSP-D-treated ABPA mice" and "rhSP-D-treated control mice" on days 1–3. BSA (3 μ g in 50 μ l of PBS per mouse) was intranasally administered to "BSA-treated ABPA mice" and "BSA-treated control mice" groups on days 1–3. The selected dose of SP-A and SP-D was based on the physiological concentrations of these proteins reported in rodent BAL, the SP-A concentration in the rat BAL was 7.3 ± 0.8 μ g/ml and the SP-D concentration in the BAL from C57BL/6 mice 6–8 wk of age was observed to be 552 ng/ml. For human BAL, the SP-A concentration ranges from 1 to 10 μ g/ml and

Table I. Study design

Group		I.N. and I.P. 3wcf twice a week for 4 wk	I.N. Proteins on Days 1–3	Protein concentration (μ g) in 50 μ l of PBS
1	WT (AKO type) (test group) (WT-Ag (AKO)-BSA)	Ag	BSA	3.0
2	WT (AKO type) (control group) (WT-C (AKO)-BSA)	PBS	BSA	3.0
3	WT (DKO type) (test group) (WT-Ag (DKO)-BSA)	Ag	BSA	3.0
4	WT (DKO type) (control group) (WT-C (DKO)-BSA)	PBS	BSA	3.0
5	WT (AKO type) (test group) (WT-Ag (AKO)-SP-A)	Ag	SP-A	3.0
6	WT (AKO type) (control group) (WT-C (AKO)-SP-A)	PBS	SP-A	3.0
7	WT (DKO type) (test group) (WT-Ag (DKO)-SP-D)	Ag	SP-D	1.0
8	WT (DKO type) (control group) (WT-C (DKO)-SP-D)	PBS	SP-D	1.0
9	WT (DKO type) (test group) (WT-Ag (DKO)-rhSP-D)	Ag	rhSP-D	1.0
10	WT (DKO type) (control group) (WT-C (DKO)-rhSP-D)	PBS	rhSP-D	1.0
11	AKO (test group) (AKO-Ag-BSA)	Ag	BSA	3.0
12	AKO (control group) (AKO-C-BSA)	PBS	BSA	3.0
13	AKO (test group) (AKO-Ag-SP-A)	Ag	SP-A	3.0
14	AKO (control group) (AKO-C-SP-A)	PBS	SP-A	3.0
15	DKO (test group) (DKO-Ag-BSA)	Ag	BSA	3.0
16	DKO (control group) (DKO-C-BSA)	PBS	BSA	3.0
17	DKO (test group) (DKO-Ag-SP-D)	Ag	SP-D	1.0
18	DKO (control group) (DKO-C-SP-D)	PBS	SP-D	1.0
19	DKO (test group) (DKO-Ag-rhSP-D)	Ag	rhSP-D	1.0
20	DKO (control group) (DKO-C-rhSP-D)	PBS	rhSP-D	1.0

the SP-D concentration varies between 300 ng and 600 ng/ml. Furthermore, similar conditions have previously been applied to examine the therapeutic effects of SP-A, SP-D, and rhSP-D in a murine model of ABPA using the BALB/c strain (8).

Afu-IgG and Afu-IgE Abs in mice

The *Afu*-IgG and *Afu*-IgE levels in the serum were measured by ELISA (8). The serum dilutions used for IgG and IgE estimation were 1/50 (v/v) and 1/25 (v/v), respectively. Protein A-HRP (for IgG) and anti-mouse IgE-HRP (for IgE) conjugates were used at 1/1000 (v/v) dilutions.

Anti-BSA IgE and IgG Abs in mice

Levels of anti-BSA IgE and IgG Abs in mice were determined in BSA-treated groups by an indirect ELISA using similar conditions as described above for *Afu* Abs. BSA (1 μ g in 100 μ l per well)-coated plates were incubated with mouse sera diluted 1/100 (for IgG) and 1/50 (IgE). Protein A-HRP (for IgG) and anti-mouse IgE HRP (for IgE) conjugates were used at 1/1000 (v/v) dilutions.

Peripheral eosinophil count

The eosinophils were estimated using heparinized whole blood (1 μ l). Eosinophils were stained with Dunger's reagent, an aqueous solution containing eosin (0.1% w/v), acetone (10% v/v) and Na_2CO_3 (0.1% w/v). The volume of blood was made up to 10 μ l with the reagent before counting using a hemocytometer.

Preparation of single cell suspension from lungs

Lungs were isolated from the mice and homogenized in RPMI 1640 medium containing 10% (v/v) bovine serum at a concentration of 5×10^5 cells/ml.

Eosinophil peroxidase (EPO) assay

For the EPO assay, a lung cell suspension (200 μ l/well) was plated in a 96-well tissue culture plate and incubated in a humidified CO_2 incubator at 37°C for 48 h. The medium was aspirated and *o*-phenylenediamine (OPD) was added (100 μ l of 1 mM solution was prepared using sterile PBS containing Triton X-100 (0.1% v/v) and H_2O_2 (0.0125% v/v)). After a 30-min incubation at room temperature, the color reaction was terminated by addition of 50 μ l of 4 N H_2SO_4 and the A_{490} was measured.

Cytokine levels in lung suspension and spleen culture

Spleen and lung from animals sacrificed at different time intervals were collected aseptically. Organs were minced, cells were suspended in culture medium (2×10^6 cells/well), and allowed to proliferate in RPMI 1640 medium with 10% (v/v) bovine serum and 10 μ g/ml gentamicin for 72 h. The supernatants from lung suspension and spleen cell culture were as-

sayed for IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, TNF- α , and IFN- γ , according to the manufacturer's instructions (Endogen).

Histological examination of the lung sections

Lungs removed from the sacrificed animals were trimmed of extraneous tissue and fixed in 10% (v/v) formaldehyde and stored at 4°C. The tissue sections, made using a microtome and stained with H&E, were examined at magnifications of $\times 40$ and $\times 400$. The histopathology sections have been prepared from three different lobes of both the lungs of an animal. Each picture is a representative of six sections (three each from two animals of each group).

Statistical analysis

All data were expressed as mean $\pm$ SD and compared using the one-population ANOVA test using the MicroCal Origin version 3.0 statistical package (MicroCal Software). Cytokine data were compared using unpaired two-tailed Mann-Whitney (nonparametric) test. The *p* values were considered statistically significant if they were <0.05 .

Results

Comparative evaluation of eosinophilia and cytokine profile of WT, AKO, and DKO control mice on day 0

AKO mice showed elevated peripheral eosinophilia (1.85-fold) and EPO activity (1.29-fold) than WT mice (Table II), consistent with increased eosinophil infiltrations seen in the lung sections (Fig. 1). AKO mice showed an increase in IL-13 (13.1-fold), IL-5 (3.93-fold), and IL-2 (3.43-fold) and a 1.92-fold decrease in IFN- γ than WT mice (Fig. 2; Table III). The ratio of IFN- γ to IL-4 was 1.525-fold less in AKO than WT mice, suggesting that AKO mice have a Th2 bias, as opposed to the predominantly Th1 profile of the WT C57BL/6 mice (Table II).

DKO mice also showed elevated peripheral eosinophil count (2.02-fold) than WT mice (Table II). Increased eosinophil infiltration was seen around perivascular areas in the lung sections of DKO mice (Fig. 1). DKO mice showed a more pronounced Th2 bias, as evident by a 3.67-fold decrease in IFN- γ and increase in IL-13 (11.6-fold), IL-5 (4.681-fold), and IL-2 (2.84-fold) (Fig. 3; Table III). The ratio of IFN- γ to IL-4 was 3.717-fold less in DKO than WT, further supporting the notion that DKO mice have a Th2 bias (Table I). However, IL-4, IL-10, IL-12, and TNF- α levels in both AKO and DKO mice did not show a significant change in comparison to WT mice (Table III).

Table II. Comparison of levels of *Afu* IgE and *Afu* IgG Abs^a

	<i>Afu</i> IgE (A ₄₉₀) (Ratio to Control Group)	<i>Afu</i> IgG (A ₄₉₀) (Ratio to Control Group)	Peripheral Eosinophil Count × 10 ⁷ /ml	EPO Activity (A ₄₉₀)	IFN-γ pg/ml of the Lung Suspension	IL-4 pg/ml of the Lung Suspension	IFN-γ/IL-4
WT-Ag	0.1530 (1.04)	1.838 (8.27)	18.75	4.221	337.5	51.74	6.522
WT-C	0.1465	0.2220	6.75	5.302	422.5	85.23	4.957
WT-naive	0.1398	0.1876	6.5	4.98	412.7	86.12	4.792
AKO-Ag	0.1864 (1.31)	1.720 (11.94)	25	6.445	360	145.3	2.476
AKO-C	0.1422	0.1441	12.5	6.84	220	90.6	2.428
AKO-naive	0.1286	0.1322	12.82	7.02	243	84.7	2.868
DKO-Ag	0.0853 (0.69)	1.689 (10.11)	24.66	3.796	42.3	18.40	2.298
DKO-C	0.1231	0.1671	13.66	5.832	156.3	87.66	1.783
DKO-naive	0.1356	0.1524	14.1	5.231	142.7	78.45	1.818

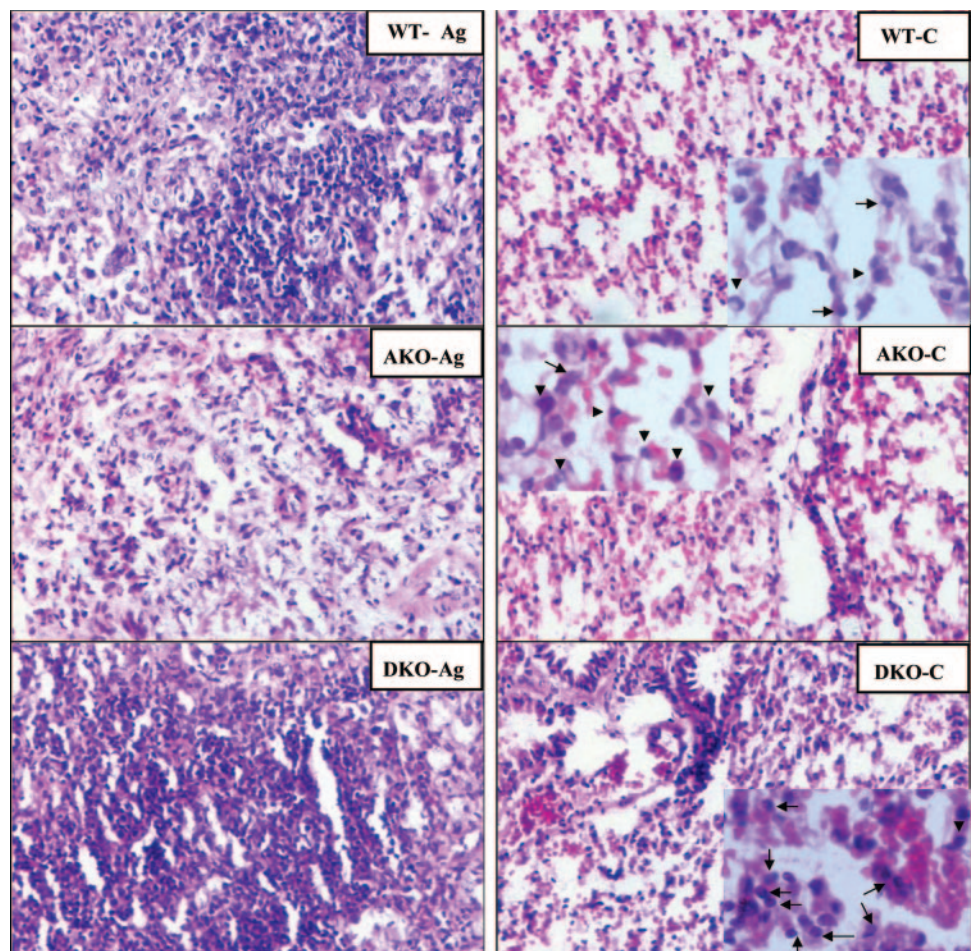
^a Comparison of levels of *Afu* IgE and *Afu* IgG Abs, peripheral eosinophil count, eosinophil peroxidase activity, and IFN-γ/IL-4 ratio of *Afu*-sensitized WT, AKO, and DKO mice with their respective control groups on zero day. Each value represents a mean of nine readings (triplicate values from three animals of each group). The deviations were calculated for each mean value and were within ±5%. The values for WT mice are pooled from WT (AKO type) and WT (DKO type).

Distinct immune response to BSA by WT-C, AKO-C, and DKO-C

Administration of BSA, as a control therapeutic protein, led to a significant increase in peripheral eosinophil count in WT mice (1.62-fold) and AKO mice (1.41-fold) (Table IV). Lung histopathology showed increased infiltration of eosinophils in WT, AKO, and DKO mice. No significant elevation in anti-BSA IgE and IgG Abs were observed in the sera of BSA-treated WT mice. Administration of BSA to AKO or DKO mice led to a significant increase in anti-BSA IgE (1.31-fold in DKO and 1.45-fold in AKO) and anti-BSA IgG (12.84-fold in DKO and 12.25-fold in AKO) (Table

IV). WT mice showed an increase in levels of IL-13 (2.28-fold on day 4 and 6.28-fold on day 10), while both AKO (2.46) and DKO mice (2.25-fold) showed a decrease in IL-13 levels. A decrease in IL-2 was observed in all three groups of mice (WT: 2- and 4-fold on days 4 and 10, respectively; AKO: 4.9-fold; DKO: 2.61-fold). IFN-γ levels decreased in WT (2.5-fold on day 4 and 3.46 on day 10) and DKO mice (3.83-fold). The ratio of IFN-γ to IL-4 decreased (1.65-fold on day 4 and 1.9-fold on day 10) in BSA-treated WT mice and AKO mice (1.6-fold) (Table IV). DKO mice also showed a decrease in IL-4 (4.66-fold) and IL-10 (1.97-fold), however, the ratio of IFN-γ to IL-4 did not change significantly (Table

FIGURE 1. Histopathological examination of the lung sections stained with H&E observed at ×40 magnification, from the wild-type mice (WT), SP-A gene-deficient (AKO) mice, and SP-D gene-deficient (DKO) mice sensitized with allergens/Ags of *A. fumigatus* (Ag) and their respective control groups on day 0 of the treatment study. The insets are at ×400 magnification to show the presence of eosinophils in the infiltrated cells. The arrows indicate eosinophils in the section. Each picture is a representative of six sections (three each from two animals of each group).



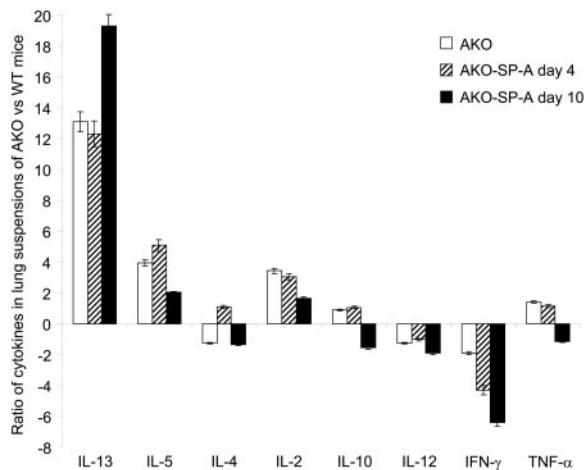


FIGURE 2. Ratio of the levels of various cytokines in lung suspensions of untreated and SP-A-treated AKO mice to the respective groups of WT mice. □, Untreated AKO mice on day 0; ▨, SP-A-treated AKO mice on day 4; ■, SP-A-treated AKO mice on day 10. Each value represents a mean of nine readings (triplicate values from three animals of each group).

IV). A summary of ratio of cytokine profiles observed in various groups of mice on fourth day with respect to their levels on day 0 is provided in Table V.

Administration of SP-A, SP-D, and rhSP-D leads to an increase in IL-5 and EPO activity in WT mice

WT mice showed an increase in IL-5 in mice treated with SP-A (3.08-fold), SP-D (3.86 on day 4 and 1.56 on day 10), and rhSP-D (3.38-fold). Increase in EPO activity was observed on administration of SP-A (1.8-fold) and SP-D (1.94-fold) but a decrease was observed on administration of rhSP-D (0.7-fold). Peripheral eosinophil count increased in SP-D (2.5-fold) and rhSP-D (2-fold)-treated mice (Table IV). However, lung histopathology did not show significant changes in WT mice on treatment with SP-A, SP-D, and rhSP-D. A decrease in IL-4 levels was observed in SP-A (3.5-fold), SP-D (8.5-fold), and rhSP-D (2.87-fold)-treated mice. A transient effect was observed on IL-13 levels in SP-A (4.66-fold decrease on day 4 followed by 3.21-fold increase on day 10) and SP-D (2.9-fold increase on day 4 and a 3.1-fold decrease on day 10)-treated mice while rhSP-D (2.63-fold)-treated mice showed a decrease. The ratio of IFN- γ to IL-4 increased significantly on day 4 (2.76-fold) followed by a decrease on day 10 (1.59-fold) in SP-A-treated mice (Table IV). IFN- γ (5.28-fold) decreased significantly on administration of SP-D. The ratio of IFN- γ to IL-4 initially decreased on day 4 (1.3-fold) but significantly increased on day 10 (1.41-fold increase) in SP-D-treated mice (Ta-

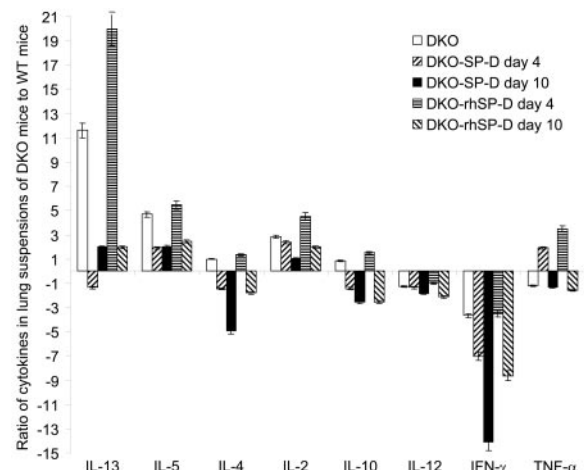


FIGURE 3. Ratio of cytokine levels in lung suspensions of untreated, SP-D, or rhSP-D DKO mice to the respective groups of WT mice. □, Untreated DKO on zero day; ▨, SP-D-treated DKO on day 4; ■, SP-D-treated DKO mice on day 10; ▤, rhSP-D-treated DKO mice on day 4; ▥, rhSP-D-treated DKO mice on day 10. Each value represents a mean of nine readings (triplicate values from three animals of each group).

ble IV). The ratio of IFN- γ to IL-4 did not change significantly in rhSP-D-treated mice (Table IV).

Administration of SP-D or rhSP-D led to decrease in levels of IL-13, IL-5 and eosinophilia in DKO mice

Administration of SP-D or rhSP-D to DKO-C mice led to decrease in peripheral eosinophil count (2.5- and 1.7-fold, respectively) and EPO activity (1.69- and 1.25-fold, respectively) with respect to the WT mice (Table IV). SP-D administration to DKO-C led to a decrease in IL-13 (16.36-fold) and IL-5 (2.29-fold), while an increase in TNF- α (1.96-fold), on day 4 followed by decrease in IL-13 (6-fold), IL-5 (2.18), IL-4 (5.83-fold), IL-2 (3.5-fold), IFN- γ (2.88-fold) and IL-10 (2.61-fold) on day 10 (Fig. 3). rhSP-D administration to DKO-C led to an increase in all the cytokines with most significant increase in TNF- α (5.0-fold) and IFN- γ (3.4-fold) on day 4. On day 10, however, all the cytokines showed a decrease (IL-13: 5.8-fold, IL-5: 2.2-fold, and IL-10: 2.38-fold) (Fig. 3). The IFN- γ to IL-4 ratio did not change significantly in SP-D-treated DKO mice while it increased in rhSP-D-treated mice (Table IV). Infiltration of eosinophils was significantly reduced in SP-D-treated DKO mice on day 10 and in rhSP-D-treated mice on day 4 as well as day 10 (Fig. 4).

Table III. Ratio of cytokine levels of lung suspensions of Afu-sensitized and control AKO and DKO mice groups to their respective groups of WT mice on zero day^a

	IL-13	IL-5	IL-2	IL-4	IL-10	IL-12	IFN- γ	TNF- α
AKO-Ag	27.2	8.177	16.93	2.8	2.68	1.94	1.06	3.94
AKO-C	13.1	3.93	3.43	1	1.13	-1.24	-1.92	1.41
DKO-Ag	7.7	6.697	4.46	-2.8	1	-1.29	-7.97	-1.3
DKO-C	11.6	4.681	2.84	1	1.01	-1.26	-2.7	-1.23

^a Each value represents a mean of nine readings (triplicate values from three animals of each group). The deviations were calculated for each mean value and were within $\pm 5\%$. The negative sign indicates a decrease in the level of cytokine in the KO mice group with respect to the respective WT mice group. The values for WT mice are pooled from WT (AKO type) and WT (DKO type). Actual cytokine levels (picograms per milliliter) of lung suspension of various groups are given below. WT-C: IL-13 (3.0), IL-5 (136.7), IL-2 (15.2), IL-4 (85.23), IL-10 (660), IL-12 (35.5), IFN- γ (422.5), TNF- α (54.7); AKO-C: IL-13 (39.5), IL-5 (537), IL-2 (51.5), IL-4 (84.7), IL-10 (752), IL-12 (28.5), IFN- γ (220), TNF- α (77); DKO-C: IL-13 (35), IL-5 (640), IL-2 (42.66), IL-4 (87.66), IL-10 (670), IL-12 (28.0), IFN- γ (156.3), TNF- α (44.6); WT-Ag: IL-13 (1.1), IL-5 (64.2), IL-2 (3.66), IL-4 (51.74), IL-10 (377.5), IL-12 (20.3), IFN- γ (337.5), TNF- α (33.5); AKO-Ag: IL-13 (30), IL-5 (525), IL-2 (62), IL-4 (145.3), IL-10 (1012), IL-12 (39.5), IFN- γ (360), TNF- α (132); DKO-Ag: IL-13 (8.5), IL-5 (430), IL-2 (16.33), IL-4 (18.4), IL-10 (383), IL-12 (15.7), IFN- γ (42.3), TNF- α (26.6).

Table IV. Comparison of levels of specific IgE^a

	Ratio of the Values on Fourth Day to Zero Day of Administration				IFN- γ /IL-4	Ratio of the Values on 10th Day to Zero Day of Administration				IFN- γ /IL-4
	Anti BSA-IgE	Anti BSA-IgG	PEC	EPO		Anti BSA-IgE	Anti BSA-IgG	PEC	EPO	
WT-BSA	0.88	0.65	1.62	1.05	3.0	0.87	0.83	1.5	0.91	2.6
AKO-BSA	1.45	12.2	1.41	1.04	2.41	1.63	1.44	0.83	1.25	1.51
DKO-BSA	1.12	1.30	0.71	0.94	1.5	1.31	12.8	0.92	1.04	1.66
WT-SP-A			1.16	1.18	13.7			1.00	1.79	3.12
AKO-SP-A			0.38	1.02	2.9			0.14	0.63	2.72
WT-SP-D			1.0	1.93	3.81			2.5	0.92	7.0
DKO-SP-D			0.41	0.84	1.4			1.25	0.59	1.5
WT-rhSP-D			1.28	0.71	3.9			2.0	0.70	4.58
DKO-rhSP-D			0.85	1.52	2.8			0.57	0.80	3.06

^a Comparison of levels of specific IgE, specific IgG Abs, peripheral eosinophilic count (PEC), eosinophilic peroxidase activity, and IFN- γ /IL-4 ratio of control WT and KO mice on treatment with BSA, SP-A, SP-D, and rhSP-D on days 4 and 10. Each value represents a mean of nine readings (triplicate values from three animals of each group). The deviations were calculated for each mean value and were within $\pm 5\%$. The values for WT mice are pooled from WT (AKO type) and WT (DKO type).

Administration of SP-A to AKO mice leads to reduced eosinophilia and IL-5 levels but did not lower levels of IL-13

Administration of SP-A to AKO-C mice led to a decrease in peripheral eosinophilia (7.14-fold) and EPO activity (1.58-fold) with respect to WT-C mice (Table IV). Administration of SP-A to AKO-C mice led to a decrease in IL-5 (2.01-fold), IL-2 (1.96-fold), IL-10 (1.85-fold) on day 10 (Fig. 1). The ratio of IFN- γ to IL-4 did not change significantly. Lung histopathology of SP-A-treated AKO mice showed reduced infiltrations of eosinophils on both days 4 and 10 and were comparable to WT-C mice (Fig. 4).

The immune responses to Afu sensitization in AKO and DKO mice are distinct

Following Afu sensitization, WT mice showed a significant increase in Afu IgG Abs (8.28-fold), peripheral eosinophil count (2.78-fold), and a decrease in EPO activity (1.26-fold) (Table II). However, Afu IgE Abs did not show a significant increase. Histopathological examinations of lung sections of WT-Ag mice showed severe eosinophilia (Fig. 1). WT-Ag mice showed a decrease in all the cytokine levels in lung suspension following challenge with Afu allergens (TNF- α : 1.63-fold, IFN- γ : 1.25-fold, IL-12: 1.75-fold, IL-13: 2.73-fold, IL-4: 1.67-fold, IL-10: 1.96-fold, IL-5: 2.13-fold, and IL-2: 4.1-fold) (Fig. 5). However, the Th1

type of cytokines showed less decrease than Th2 type and the ratio of IFN- γ to IL-4 increased on allergen challenge (from 4.957 to 6.522, 1.32-fold increase) (Table II).

On repeated Ag sensitization, DKO mice showed a decrease in Afu-IgE (1.44-fold) and EPO activity (1.54-fold), but an increase in Afu-IgG Ab (10.11-fold) and peripheral eosinophil count (1.81-fold) (Table II). Lung sections of DKO-Ag mice showed significantly dense infiltrations of eosinophils than DKO-C mice, AKO-Ag, and WT-Ag (Fig. 1). DKO showed a decrease in all the cytokines and behaved in a more pronounced but similar manner to WT mice following allergen challenge (IFN- γ : 3.45-fold, IL-13: 4.11-fold, IL-4: 8.45-fold, and IL-2: 2.61-fold) (Fig. 5; Table III). However, the Th1 cytokines showed less decrease than Th2 type, and an increase in IFN- γ to IL-4 ratio (1.29-fold) (Table II).

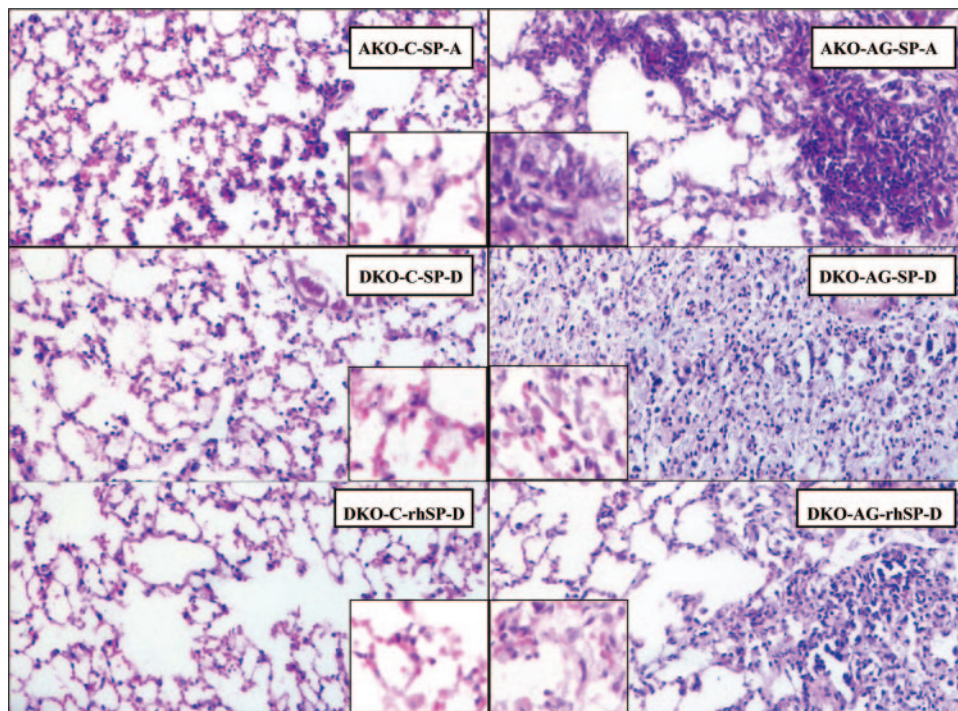
Sensitized AKO (AKO-Ag) mice showed an increase in Afu-IgE (1.31-fold) and Afu-IgG Ab (11.94-fold) and peripheral eosinophil count (2-fold) (Table II). Lung sections of AKO-Ag mice showed increased eosinophil infiltrations in comparison to AKO-C mice but were less than WT-Ag mice (Fig. 1). AKO showed an increase in TNF- α (1.71-fold), IFN- γ (1.63-fold), IL-12 (1.38-fold), IL-4 (1.49-fold), IL-10 (1.34-fold), and IL-2 (1.2-fold), while a decrease in IL-13 (1.32) and no change in IL-5 levels (Fig. 5; Table III). However, ratio of IFN- γ to IL-4 did not change significantly

Table V. Ratio of cytokine levels of lung suspensions of Afu-sensitized and control mice groups on fourth day to their levels on zero day^a

Group	IL-13	IL-5	IL-4	IL-2	IL-10	IL-12	IFN- γ	TNF- α
WT-Ag BSA	5.27	2.18	2.06	10	2.57	1.7	-1.11	1.75
WT-C BSA	2.28	1.17	-1.35	-2	-1.02	-1.19	-2.5	-1.25
WT-Ag SP-A	17	3.41	2.23	12	1.54	-1.33	1.3	1.75
WT-C SP-A	-4.66	1.28	-3.5	-1.1	-1.19	-1.19	-1.34	1.30
WT-Ag SP-D	1.25	3.66	-1.5	6.75	1.47	1.22	1.07	1.5
WT-C SP-D	2.9	3.86	-1.11	3	1.34	-1.03	1.05	1.54
WT-Ag rSP-D	1.6	1.11	-1.71	4.5	1.57	-1.88	-2.15	1.43
WT-C rSP-D	-2.6	-1.41	-2.12	1.7	-1.2	-3.6	-2.27	1.01
AKO-Ag BSA	-8.2	-3.46	-2.15	-2.3	-2.63	-3.1	-1.24	-1.85
AKO-C BSA	-2.46	1.17	-1.07	-1.4	1.08	-1.07	1.06	-1.15
AKO-Ag SP-A	2.9	1.15	-1.18	1.29	-1.1	1.08	-1.18	-1.81
AKO-C SP-A	-1.13	1.23	1.2	-1.06	-1.11	1.16	1.31	-1.26
DKO-Ag BSA	1.22	-1.5	6.75	1	1.94	1.28	3	1.78
DKO-C BSA	-2.25	-1.46	1.30	-1.21	1.15	1.14	1.43	1.33
DKO-Ag SP-D	-3.4	-3.37	8	2.44	1.23	1.59	1	1.9
DKO-C-SP-D	-16.36	-2.29	-1.75	-1.55	-1.47	-1.07	-1.52	1.96
DKO-Ag-rSP-D	2.09	1.94	10.8	2.4	2.13	2.58	20.5	2.53
DKO-C-rSP-D	1.71	1.17	1.5	1.78	1.64	1.232	3.4	5.0

^a Each value represents a mean of nine readings (triplicate values from three animals of each group). The deviations were calculated for each mean value and were within $\pm 5\%$. The negative sign indicates a decrease in the level of cytokine in the mice group on fourth day with respect to the level in the respective mice group on zero day. The values for WT mice are pooled from WT (AKO type) and WT (DKO type).

FIGURE 4. Histopathological examination of the lung sections (H&E stain) observed at $\times 40$ magnification, from the SP-A-treated control SP-A gene-deficient mice (AKO-C-SP-A), SP-A-treated *Afu*-sensitized SP-A gene-deficient mice (AKO-Ag-SP-A), SP-D-treated control SP-D gene-deficient mice (DKO-C-SP-D), SP-D-treated *Afu*-sensitized SP-D gene-deficient mice (DKO-Ag-SP-D), rhSP-D-treated control SP-D gene-deficient mice (DKO-C-rhSP-D), rhSP-D-treated *Afu*-sensitized SP-D gene-deficient mice (DKO-Ag-rhSP-D), on day 10 of the treatment study. The *insets* are at $\times 400$ magnification to show the presence of eosinophils in the infiltrated cells. Each picture is a representative of six sections (three each from two animals of each group).



(from 2.428 to 2.476, 1.01-fold increase), suggesting that AKO are differentially responsive to allergen challenge than both DKO and WT mice (Table II).

Treatment of Afu-sensitized WT, AKO, and DKO mice with BSA, SP-A, SP-D, and rhSP-D

Administration of BSA to WT-Ag mice led to an increase in peripheral eosinophil count (1.37-fold) and EPO activity (2.79-fold) (Table VI), increased lung infiltrations of eosinophils, and an increase in IL-13 (5.27-fold), IL-2 (10-fold), IL-10 (2.57) on day 4. IL-13 (14.5-fold), IL-5 (4.36), IL-2 (8-fold), and IL-10 (2.22-fold) showed increases on day 10, suggesting that BSA treatment served as a short-term Ag challenge for the *Afu*-sensitized mice. The levels of anti-BSA IgG and IgE Abs in these mice were not significantly elevated.

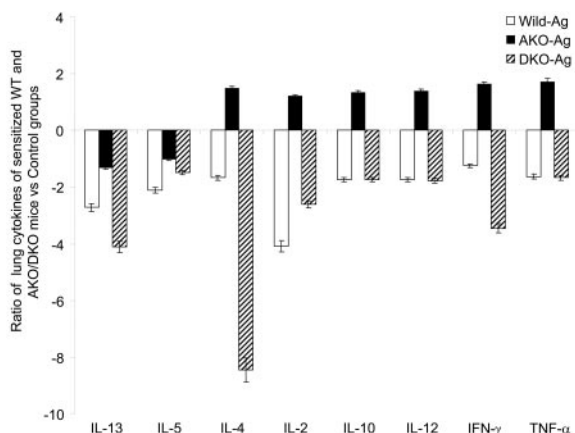


FIGURE 5. Ratio of lung cytokine levels of WT, AKO, and DKO mice sensitized with 3wcf of *A. fumigatus* (Ag) to their respective control groups on day 0 of treatment study. Groups include sensitized $\square$ WT (WT-Ag); $\blacksquare$ AKO (AKO-Ag); and $\square$ DKO (DKO-Ag) mice. Each value represents a mean of nine readings (triplicate values from three animals of each group).

Administration of SP-A, SP-D, and rhSP-D to WT-Ag mice led to decrease in *Afu*-IgE (0.78-, 0.87-, 0.7-fold) and peripheral eosinophilia (2.56-, 2.12-, and 4.16-fold, respectively), yet an increase in EPO activity (1.435-, 1.32-, 2.32-fold) (Table VI). Lung histopathology showed decreased eosinophil infiltrations following treatment (Fig. 4). In general, all groups of treated mice showed an increase in levels of IL-2 and a decrease in ratio of IFN- γ to IL-4. SP-A treatment resulted in increase in all the cytokines, with a significant increase in IL-13 (17-fold), IL-5 (3.41-fold), IL-4 (2.23-fold), IL-2 (12-fold), and TNF- α (1.75-fold) except IL-12 on day 4, followed by an increase in all the cytokines, IL-13 (5.8-fold), IL-5 (2.71-fold), IL-4 (2.05-fold), IL-2 (7-fold) on day 10 (Fig. 6). The ratio of IFN- γ to IL-4 decreased significantly from 6.522 on day 0 to 2.92 (2.23-fold) (Table VI).

SP-D treatment to WT-Ag mice led to increase in all the cytokines, with significant increase in IL-5 (3.66-fold), IL-2 (6.75-fold) except IL-4 (1.5-fold decrease) on day 4, followed by a decrease in IFN- γ (3.29-fold) and a further increase in IL-5 (6-fold) (Fig. 7). The IFN- γ to IL-4 ratio increased on day 4 and decreased on day 10 (from 6.522 on day 0 to 13.33, i.e., 2.04-fold increase and 1.63 i.e., 4-fold decrease) on days 4 and 10, respectively (Table VI). Administration of rhSP-D to WT-Ag mice led to an increase in IL-2 (4.5-fold), and a decrease in IL-4 (1.71-fold decrease), IL-12 (1.88-fold) and IFN- γ (2.15-fold) on day 4 followed by a decrease in IL-13 (1.87-fold), IL-2 (2.0-fold), IL-4 (8.4-fold), and IFN- γ (9.33-fold) (Fig. 8). The IFN- γ to IL-4 ratio decreased from 6.522 on day 0 to 3.9 (1.67-fold) and 4.5 (1.45-fold) on days 4 and 10, respectively (Table VI).

Administration of BSA to sensitized AKO-Ag and DKO-Ag mice led to decrease in peripheral eosinophilic count (2.08- and 3.12-fold, respectively) and EPO activity (1.69- and 1.14-fold, respectively) (Table VI). Lung histopathology showed decreased eosinophilic infiltrations in BSA-treated AKO-Ag and DKO-Ag mice. No significant increase in levels of anti-BSA IgG or IgE Ab was observed in these mice. BSA treatment to AKO-Ag mice showed a decrease in all the cytokines on day 4: IL-13 (8.28-fold),

Table VI. Comparison of levels of specific IgE^a

	Ratio of the Values on Fourth Day to Zero Day of Administration					Ratio of the Values on 10th Day to Zero Day of Administration				
	Afu IgE (α -BSA IgE)	Afu IgG (α -BSA IgG)	PEC	EPO	IFN- γ /IL-4	Afu IgE (α -BSA IgE)	Afu IgG (α -BSA IgG)	PEC	EPO	IFN- γ /IL-4
WT-BSA	1.06 (1.0)	1.26 (1.1)	1.37	2.78	3.1	0.94 (1.0)	1.12 (1.2)	1.37	2.51	3.27
AKO-BSA	0.99 (0.8)	1.04 (0.9)	0.88	0.74	2.04	1.40 (1.1)	1.03 (1.0)	0.48	0.58	2.5
DKO-BSA	0.99 (0.9)	1.10 (1.0)	0.88	1.24	1.4	1.07 (1.0)	1.08 (1.1)	0.32	0.87	1.85
WT-SP-A	0.89	0.94	1.22	2.03	3.8	0.78	0.90	0.38	1.43	2.92
AKO-SP-A	1.16	0.95	0.44	0.94	3.1	0.94	0.97	0.24	0.98	2.77
WT-SP-D	0.87	0.99	0.47	1.32	13.3	0.91	0.98	0.73	0.60	1.63
DKO-SP-D	1.53	1.48	0.41	1.22	0.62	0.94	0.98	0.62	0.61	3.75
WT-rhSP-D	0.70	1.02	0.22	0.68	3.9	0.80	1.11	0.45	0.24	4.5
DKOrhSP-D	0.94	0.89	0.24	2.32	1.8	0.70	0.82	0.4	0.49	3.0

^a Comparison of levels of specific IgE, specific IgG Abs, peripheral eosinophil count, EPO activity, and IFN- γ /IL-4 ratio of Afu-sensitized WT and KO mice on treatment with BSA, SP-A, SP-D, and rhSP-D on fourth day and 10th day. Each value represents a mean of nine readings (triplicate values from three animals of each group). The deviations were calculated for each mean value and were within $\pm 5\%$. "α" refers to Abs. The values for WT mice are pooled from WT (AKO type) and WT (DKO type).

IL-5 (3.46-fold), IL-4 (2.15-fold), IL-2 (2.38-fold) IL-10 (2.63-fold) and IL-12 (3.1-fold). The levels of IL-13 (11.6-fold), IL-5 (3.46-fold), IL-4 (4.75-fold), IL-2 (31-fold), IL-10 (2.16-fold), IL-12 (2.04-fold), IFN- γ (2.4-fold) and TNF- α (5.47-fold) further decreased on day 10. IFN- γ to IL-4 ratio did not change significantly (Table VI). BSA treatment to DKO-Ag mice showed a significant increase in IL-4 (6.75-fold) and IFN- γ (3-fold) on day 4 and IL-13 (2.64-fold), IL-4 (6.75-fold), and IFN- γ (3.75-fold) on day 10. IFN- γ to IL-4 ratio decreased from 2.298 on day 0 to 1.4 (1.64-fold) and 1.85 (1.24-fold) on day 4 and 10, respectively (Table VI).

Administration of SP-D or rhSP-D has therapeutic effects on Afu-sensitized DKO mice

Administration of SP-D or rhSP-D to DKO-Ag mice led to decrease in peripheral eosinophilic count (1.61- and 2.5-fold, respectively), EPO activity (1.6- and 2.04-fold, respectively), while a decrease in Afu IgE (0.94-, 0.7-fold) (Table VI). Lung sections of SP-D or rhSP-D-treated DKO-Ag mice showed reduced eosinophilic infiltrations on day 10 in comparison to untreated DKO-Ag mice on day 0 and rhSP-D administration was more effective in reducing eosinophilia than SP-D (Fig. 4).

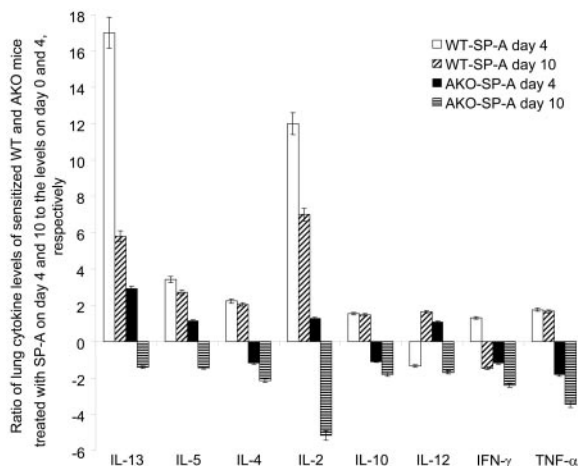


FIGURE 6. Ratio of lung cytokine levels of sensitized WT and AKO mice treated with SP-A on days 4 and 10 to the levels on days 0 and 4, respectively. □, WT mice treated with SP-A on day 4; ▨, WT mice treated with SP-A on day 10; ■, AKO mice treated with SP-A on day 4; and ▩, WT mice treated with SP-A on day 10. Each value represents a mean of nine readings (triplicate values from three animals of each group).

DKO-Ag-SP-D mice showed a decrease in IL-13 (3.4-fold) and IL-5 (3.37-fold) while an increase in IL-4 (8-fold), and IL-2 (2.44-fold) (Fig. 7). The IFN- γ to IL-4 ratio decreased on day 4 and increased on day 10 (from 2.298 on day 0 to 0.625 (3.67-fold) and 3.75 (2.23-fold) on days 4 and 10, respectively (Table VI). rhSP-D administration to DKO-Ag led to an increase in all the cytokines, with significant increases in IL-4 (10.8-fold), IL-10 (2.13-fold), IL-2 (2.4-fold), TNF- α (2.53-fold), IL-12 (2.58-fold), and IFN- γ (20.5-fold) on day 4. On day 10, however, cytokine levels decreased: IL-13 (2.7-fold), IL-5 (2.31-fold), IL-10 (2.17-fold), and IL-2 (2.22-fold) except IFN- γ , which increased by 9-fold (Fig. 8). The IFN- γ to IL-4 ratio decreased followed by an increase from 2.298 on day 0 to 1.8 (1.27-fold) and 3.0 (2.23-fold) on days 4 and 10, respectively (Table VI).

Administration of SP-A to AKO-Ag mice led to decrease in peripheral eosinophilic count (2.27-fold) on day 4 followed by further decrease on day 10 (4.16-fold) (Table VI). SP-A treatment led to an increase in levels of IL-13 (2.9-fold) on day 4 and a decrease in levels of IL-4 (2.14-fold), IL-2 (5.16-fold), IFN- γ (2.4-fold), and TNF- α (3.45-fold) on day 10 (Fig. 6). The IFN- γ to IL-4 ratio did not change significantly (Table VI). Lung sections showed significantly increased eosinophilic infiltrations on days 4 and 10 in comparison to AKO-Ag mice on day 0 and showed

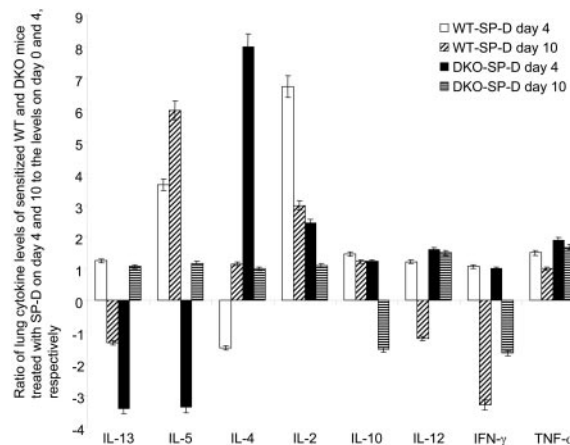


FIGURE 7. Ratio of lung cytokine levels of sensitized WT and DKO mice treated with SP-D on days 4 and 10 to the levels on days 0 and 4, respectively. □, WT mice treated with SP-D on day 4; ▨, WT mice treated with SP-D on day 10; ■, DKO mice treated with SP-D on day 4; and ▩, DKO mice treated with SP-D on day 10. Each value represents a mean of nine readings (triplicate values from three animals of each group).

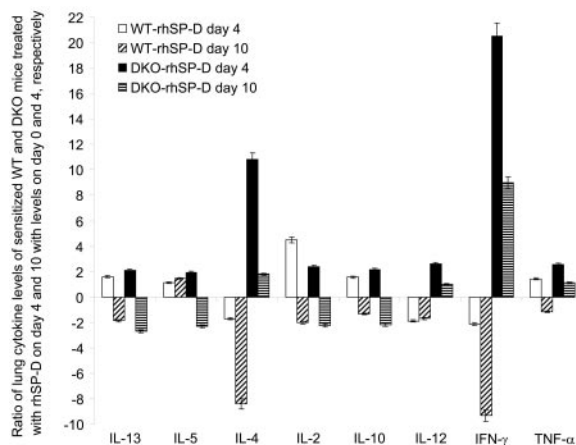


FIGURE 8. Ratio of lung cytokine levels of sensitized WT and DKO mice treated with rhSP-D on days 4 and 10 to the levels on days 0 and 4, respectively. □, WT mice treated with rhSP-D on day 4; ▨, WT mice treated with rhSP-D on day 10; ■, DKO mice treated with rhSP-D on day 4; ▩, DKO mice treated with rhSP-D on day 10. Each value represents a mean of nine readings (triplicate values from three animals of each group).

collapse of the alveolar structure (Fig. 4). It is important to note here that although the peripheral eosinophil count decreased with SP-A administration to *Afu*-sensitized AKO mice, the pulmonary eosinophilia worsened.

Discussion

In view of the important roles of SP-A and SP-D in pulmonary immune response, we had earlier examined the effect of SP-A, SP-D, and rhSP-D in a murine model of *Afu*-induced pulmonary hypersensitivity (8). *Afu* is the fungus most commonly implicated in causing both IgE-mediated and non-IgE-mediated hypersensitivity in humans leading to development of ABPA, which is characterized by activated Th2 cells and asthma. Intranasal administration of SP-A, SP-D, or rhSP-D (three doses on consecutive days) significantly lowered eosinophilia and specific Ab levels in ABPA mice (8). Lung sections of the ABPA mice showed extensive infiltration of lymphocytes and eosinophils, which were considerably reduced following treatment (8). The levels of IL-2, IL-4, and IL-5 were decreased, while that of IFN- γ was raised in supernatants of the cultured spleen cells, indicating a marked Th2→Th1 shift (8). This study highlighted a central role for SP-A and SP-D in regulation of pulmonary hypersensitivity (8). As a logical next step, we wished to examine the nature of immune response in AKO and DKO mice when challenged with *Afu* allergens to validate whether deficiency of these proteins made mice more susceptible to pulmonary hypersensitivity.

AKO and DKO show intrinsic hypereosinophilia

Both AKO and DKO mice showed elevated peripheral and pulmonary eosinophilia and a significant increase in EPO activity in comparison to the WT mice. A significant monocytic infiltration has been reported in the peribronchiolar and perivascular regions of the lungs in DKO mice (24). In addition, an increased accumulation of alveolar macrophages and lymphocytes was observed in DKO mice (32, 36). Because treatment with SP-A, SP-D or rhSP-D has been shown to lower IL-5, peripheral and pulmonary eosinophilia in the *Afu*-sensitized WT BALB/c mice (8), an alteration in the peripheral and pulmonary eosinophil counts in the AKO and DKO mice, was not surprising. A significantly raised level of IL-5 and IL-13 in both AKO and DKO mice may be one

of the mechanisms causing hypereosinophilia (37, 38). SP-A can inhibit IL-8 expression and production from eosinophils, thus probably preventing the autocrine cycle for recruitment of human eosinophils by inhibiting IL-8, a chemotactic cytokine (39). Eosinophils are the important effector cells for the pathogenesis of allergic inflammation via the secretion of highly cytotoxic granular proteins and Th2 type of cytokines. Blood and tissue eosinophilia is a common manifestation of late-phase allergic inflammation causing tissue damage. Hypereosinophilia exhibited by both AKO and DKO mice suggests that SP-A and SP-D have a role in regulating the eosinophil infiltration and modulation in the lung in response to environmental stimuli.

Genetic deficiency of SP-A or SP-D shifts the cytokine profile of C57BL/6 mice toward Th2 type

The cytokine profile of both AKO and DKO mice suggested a Th2 bias (elevated IL-13 and IL-5 levels) and down-regulation of Th1 cytokine, IFN- γ (more pronounced in DKO than AKO mice). IL-13 and IL-5 have important roles in allergen induced asthma and airway hyperresponsiveness (AHR). Overexpression of IL-13 in mice leads to 70-fold increase in SP-D, 3-fold increase in SP-A, and 6-fold increase in the phospholipid pool (38). Remarkably similar to DKO mice, IL-13 overexpressing mice have characteristic foamy macrophages, type II cell hypertrophy, fibrosis, massive inflammation involving eosinophilia, protease-dependent acquired emphysema, and AHR (38). IL-13, produced in the airway by a variety of cells (T cells, eosinophils, and mast cells), mediates mucus production and AHR through its combined actions on epithelial cells and smooth muscle cells independently of IL-5 and eotaxin (40–41). IL-13 also directly promotes eosinophil survival, activation, and recruitment (42–44). Alveolar macrophages of DKO mice show increased expression of reactive oxygen species (ROS), hydrogen peroxide, MMP-9, MMP-12, and NF- κ B (45). Because IL-13 has been reported to inhibit the production of proinflammatory mediators by monocytes and macrophages, including ROS, through a mechanism that probably involves NF- κ B, it appears that the increased levels of IL-13 are produced in DKO mice to regulate their increased oxidative state (46–50). However, IL-13 and SP-D have also been described as potent stimulators of MMP in the lung (37, 51). It is likely that certain physiological effects and hypereosinophilia observed in AKO and DKO mice arise due to overexpression of IL-13, although AKO mice do not show abnormalities, such as foamy macrophages, type II cell hypertrophy, and fibrosis similar to DKO mice. However, sequential targeting of both SP-A and SP-D genes (double knockout) show exaggerated alveolar proteinosis and emphysema compared with DKO mice, suggesting that SP-A deficiency may contribute to physiological abnormalities in the lungs (52).

Transgenic mice overexpressing IL-5 also exhibit intrinsic AHR (even in the absence of any antigenic stimuli) and increased numbers of eosinophils and lymphocytes in the lung tissue (53). The observation that AKO and DKO mice have elevated IL-5 levels, which is lowered by therapeutic delivery of SP-A or SP-D/rhSP-D, appears to suggest that SP-A and SP-D inhibit allergen mediated eosinophilia in the lungs through down-regulation of IL-5. It is worth noting that mice genetically deficient in GM-CSF also show pulmonary alveolar proteinosis associated with a marked increase in phospholipid pool similar to DKO mice. GM-CSF-deficient mice showed 50-fold increase in SP-D, while only a 3-fold increase in SP-A, similar to IL-13 overexpressing mice. It has been proposed that GM-CSF mediates some of the physiological changes seen in DKO mice as ablation of GM-CSF in DKO mice leads to alleviation of macrophage proliferation and type II cell hypertrophy (54). It is also possible that the actions of GM-CSF

and SP-D leading to similar pathophysiological changes are distinct (55). It is to be noted that *IL-5*, *IL-13*, and *GM-CSF* genes are situated on the same chromosomal location (5q31). Furthermore, GM-CSF, along with IL-5, is known to regulate IL-13 secretion from human eosinophils (56).

Distinct immune response to BSA by WT, AKO, and DKO

Intranasal administration of BSA on days 1–3 was included in the study as a control protein similar to our earlier studies. However, C57BL/6 mice responded to the BSA administered as a short-term allergen challenge with a characteristic Th2 response with increased peripheral eosinophil count and pulmonary eosinophilia, which has also been reported earlier (57). WT mice, however, showed no significant increase in anti-BSA IgG or IgE Abs. Interestingly, BSA-specific IgG and IgE Abs were observed in both BSA-treated AKO and DKO mice with AKO mice also showing a significant down-regulation of IFN- γ to IL-4 ratio (shift to Th2 response) with an increase in peripheral eosinophil count and pulmonary eosinophilia while DKO mice showed only an increase in pulmonary eosinophilia. These observations suggest that both AKO and DKO mice show a different pulmonary immune response to short-term sensitization than the WT mice and both SP-A and SP-D have important roles in regulation of humoral immune response to short-term allergen sensitization in the lung.

Afu sensitization provokes distinct immunological responses in AKO and DKO mice

Following *Afu* sensitization, C57BL/6 mice, which were used as a control for AKO and DKO mice (both in C57BL/6 background) showed no change in *Afu*-IgE, and an increase in *Afu*-IgG, peripheral and pulmonary eosinophilia. Allergen challenge led to a decrease in IFN- γ and IL-4 in lung suspensions (hence an increase in IFN- γ to IL-4 ratio). IL-2, IL-5, and IL-13 levels decreased significantly in the lung and spleen suspensions, suggesting the Th1 predominance in the mouse strain. This is consistent with the observation that response to allergenic challenge varies in different strains of mice and C57BL/6 mice show a predominantly Th1 response to a high dose of allergen sensitization (57).

Both AKO and DKO mice showed comparable increase in *Afu*-IgG and peripheral eosinophil count, and DKO mice showed more severe pulmonary eosinophilia than AKO mice, following *Afu* sensitization. AKO mice showed a corresponding increase in *Afu*-IgE level as well. The EPO activity was down in DKO mice, while in AKO mice, it remained unchanged. AKO mice showed an increase in all the cytokines in lung suspension (<2-fold) except IL-13 and IL-5. Increased levels of Th2 cytokines in *Afu*-sensitized AKO mice than WT and DKO mice suggests that the phenotype of these mice is more complex than previously reported. However, AKO-Ag mice consistently showed Th1 predominance in BAL as well as in lung and spleen suspensions. DKO mice showed a decrease in all cytokine levels in lung suspension, similar to WT mice, but in a more pronounced manner. Both lung and spleen suspensions of DKO-Ag mice showed a Th1 response; however, BAL had an increase in IL-13, IL-4, and IL-2 levels, similar to WT-Ag mice. A recent study showed similar results wherein, following OVA sensitization and challenge in vivo, SP-D^{-/-} mice expressed higher BAL eosinophils, IL-10 and IL-13 concentrations and lower IFN- γ expression at early time points compared with WT mice (58). It is evident that AKO mice are almost nonresponsive to the *Afu* sensitization, while DKO mice show a pronounced response. *Afu* sensitization led to a similar increase in the ratio of IFN- γ and IL-4 in both WT and DKO mice (no significant increase in AKO mice). Significant down-regulation of *Afu*-IgE Ab was specific to DKO mice. AKO mice, in contrast, showed a significant increase in

Afu-IgE Ab. This differential responsiveness to *Afu* sensitization in AKO and DKO mice may be accounted for by a 50% decrease in SP-A levels in DKO mice and a 7-fold increase in SP-D levels in AKO mice (59). It appears that both SP-A and SP-D contribute to the homeostasis to the allergenic challenge in an interdependent manner and absence of any one of them disturbs this balance.

Administration of SP-A, SP-D, or rhSP-D to the Afu-sensitized WT and KO mice can partially rescue them

As previously reported (8), *Afu*-IgE, and *Afu*-IgG, peripheral and pulmonary eosinophilia in WT-Ag were down-regulated by SP-A, SP-D, or rhSP-D treatment (the increased IFN- γ to IL-4 ratio was also reversed). SP-D was able to restore IL-5 and IL-2 levels increased by allergen challenge. *Afu* sensitization led to a decrease in IL-13, while an increase in IL-2, IL-4, IL-10, IL-12, IFN- γ , and TNF- α in AKO mice. SP-A treatment containing the peripheral eosinophil count, however, showed increased pulmonary eosinophilia with extensive tissue damage, possibly caused by increased levels of IL-13 and IL-5 in AKO mice. SP-A was also not able to bring down increased *Afu*-IgG and *Afu*-IgE levels, suggesting that SP-A treatment (3 μ g per mice for 3 consecutive days) is not leading to complete alleviation of the *Afu*-induced changes in AKO mice.

SP-D treatment to DKO-Ag mice restored IL-2, IL-4, IL-12, and TNF- α levels. IL-13 and IL-5 levels showed a further decrease with treatment, the levels being significantly lower than the DKO-C mice. IL-13, IL-5, and IL-2 levels in rhSP-D-treated DKO-Ag mice were comparable to WT-C mice, while IL-10 and IL-12 went down significantly compared with all other groups. Thus, SP-D and rhSP-D were more effective than SP-A in rescuing the respective gene-deficient mice from the effects of *Afu* sensitization. Previously, coadministration of SP-D has been shown to normalize viral clearance and cytokine response in DKO mice challenged with influenza A virus (IAV) (60). Expression of SP-D/conglutinin chimeric protein in epithelial cells of DKO mice substantially corrected the increased lung phospholipids and increased the clearance of IAV but could not ameliorate the ongoing lung inflammation, enhanced metalloproteinase expression, and alveolar destruction (28).

It appears likely that SP-A and SP-D influence the lung immunity by directly or indirectly modulating the nuclear factors. DKO mice have been shown to have elevated levels of transcripts for NF- κ B and AP-1 (45). NFAT1 (NF regulating expression of many genes encoding immunoregulatory cytokines)-deficient mice also show increased levels of IL-4, IL-5, and IL-13 as well as enhanced eosinophilia, similar to AKO and DKO mice. It is possible that NFAT1 is involved in the downstream signaling of SP-A and/or SP-D (61). In conclusion, the present study reports that both SP-A and SP-D have important roles in the regulation of cytokine milieu and eosinophilia in the lungs, and their absence leading to inherent hypersensitivity in mice highlights their essential role in host defense against allergic airway challenges. Thus, both these versatile macromolecules enable the lung to achieve homeostasis probably through distinct mechanisms. It is important to note here that both AKO and DKO mice are different and the anatomical and functional abnormalities reported only in DKO mice, and not in AKO mice, may be underlying issues for their behavior and susceptibility to *Afu* sensitization.

Acknowledgments

The SP-A and SP-D gene knockout mice were kindly provided by Dr. S. Hawgood (Cardiovascular Research Institute and Department of Pediatrics, University of California San Francisco, CA). We are grateful to Dr. Howard Clark for his technical help with breeding knockout mice.

Disclosures

The authors have no financial conflict of interest.

References

- Holmskov, U., S. Thiel, and J. C. Jensenius. 2003. Collectins and ficolins: humoral lectins of the innate immune defense. *Annu. Rev. Immunol.* 21: 547–578.
- Kishore, U., T. Madan, P. U. Sarma, M. Singh, B. C. Urban, and K. B. M. Reid. 2002. Protective roles of pulmonary surfactant proteins, SP-A and SP-D, against lung allergy and infection caused by *Aspergillus fumigatus*. *Immunobiology*. 205: 610–618.
- Whitsett, J. A. 2002. Intrinsic and innate defenses in the lung: intersection of pathways regulating lung morphogenesis, host defense, and repair. *J. Clin. Invest.* 109: 565–569.
- Kishore, U., and K. B. M. Reid. 2001. Structures and functions of mammalian collectins. In *Mammalian Carbohydrate Recognition Proteins*, in the series *Results and Problems in Cell Differentiation*. Vol. 33, P. Crocker, ed. Springer-Verlag, Berlin, p. 225–248.
- Wright, J. R. 1997. Immunomodulatory functions of surfactant. *Physiol. Rev.* 7: 931–962.
- Crouch, E. C. 2000. Surfactant protein D and pulmonary host defense. *Respir. Res.* 1: 93–108.
- Gardai, S. J., Y. Q. Xiao, M. Dickinson, J. A. Nick, D. R. Voelker, K. E. Greene, and P. M. Henson. 2003. By binding SIRPα or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 115: 13–23.
- Madan, T., U. Kishore, M. Singh, P. Strong, H. Clark, E. M. Hussain, K. B. M. Reid, and P. U. Sarma. 2001. Surfactant protein A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. *J. Clin. Invest.* 107: 467–475.
- Griese, M. 1999. Pulmonary surfactant in health and human lung diseases: state of the art. *Eur. Respir. J.* 13: 1455–1461.
- Wright, J. R., P. Borron, K. G. Brinker, and R. J. Folz. 2001. Surfactant Protein A: regulation of innate and adaptive immune responses in lung inflammation. *Am. J. Respir. Cell Mol. Biol.* 24: 513–517.
- Mason, R. J., K. Greene, and D. R. Voelker. 1998. Surfactant protein A and surfactant protein D in health and disease. *Am. J. Physiol.* 275: L1–L13.
- Cheng, G., T. Ueda, T. Numao, Y. Kuroki, H. Nakajima, Y. Fukushima, S. Motojima, and T. Fukuda. 2000. Increased levels of surfactant protein A and D in broncho-alveolar lavage fluids in patients with bronchial asthma. *Eur. Respir. J.* 16: 831–835.
- Tanaka, H., H. Sugawara, T. Saikai, K. Tsunematsu, H. Takahashi, and S. Abe. 2000. Mushroom worker's lung caused by spores of *Hypsizygus marmoreus* (Bunashimeji): elevated serum surfactant protein D levels. *Chest* 118: 1506–1509.
- Hickling, T. P., R. B. Sim, and R. Malhotra. 1998. Human lung surfactant protein A exists in several different oligomeric states: oligomer size distribution varies between patient groups. *Mol. Med.* 4: 266–275.
- Malhotra, R., J. Haurum, S. Thiel, J. C. Jensenius, and R. B. Sim. 1993. Pollen grains bind to lung alveolar type II cells (A459) via lung surfactant protein A (SP-A). *Biosci. Rep.* 13: 79–90.
- Wang, J. Y., U. Kishore, B. L. Lim, P. Strong, and K. B. M. Reid. 1996. Interaction of human lung surfactant proteins A and D with mite allergens. *Clin. Exp. Immunol.* 106: 367–373.
- Madan, T., U. Kishore, A. Shah, P. Eggleton, P. Strong, J. Y. Wang, S. S. Agrawal, P. U. Sarma, and K. B. M. Reid. 1997. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. *Clin. Exp. Immunol.* 110: 241–249.
- Wang, J. Y., C. C. Shieh, P. F. You, H. Y. Lei, and K. B. M. Reid. 1998. Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release in children with asthma. *Am. J. Respir. Crit. Care Med.* 158: 510–518.
- Borron, P. J., E. C. Crouch, J. F. Lewis, J. R. Wright, F. Possmayer, and L. J. Fraher. 1998. Recombinant rat surfactant-associated protein D inhibits human T lymphocyte proliferation and IL-2 production. *J. Immunol.* 161: 4599–4603.
- Singh, M., T. Madan, P. Waters, S. K. Parida, P. U. Sarma, and U. Kishore. 2003. Protective effects of a recombinant fragment of human surfactant protein D in a murine model of pulmonary hypersensitivity induced by dust mite allergens. *Immunol. Lett.* 86: 299–307.
- Takeda, K., N. Miyahara, Y. H. Rha, C. Taube, E. S. Yang, A. Joetham, T. Kodama, A. M. Balhorn, A. Dakhama, C. Duez, A. J. Evans, D. R. Voelker, and E. W. Gelfand. 2003. Surfactant protein D regulates airway function and allergic inflammation through modulation of macrophage function. *Am. J. Respir. Crit. Care Med.* 168: 783–789.
- Korfhagen, T. R., A. M. LeVine, and J. A. Whitsett. 1998. Surfactant protein A (SP-A) gene targeted mice. *Biochim. Biophys. Acta* 1408: 296–302.
- Harrod, K. S., A. D. Mounday, B. R. Stripp, and J. A. Whitsett. 1998. Clara cell secretory protein decreases lung inflammation after acute virus infection. *Am. J. Physiol.* 275: L924–L930.
- Wert, S. E., M. Yoshida, A. M. LeVine, M. Ikegami, T. Jones, G. F. Ross, J. H. Fisher, T. R. Korfhagen, and J. A. Whitsett. 2000. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proc. Natl. Acad. Sci. USA* 97: 5972–5977.
- Ikegami, M., B. M. Elhalwagi, N. Palaniyar, K. Dienger, T. R. Korfhagen, J. A. Whitsett, and F. X. McCormack. 2001. The collagen-like region of surfactant protein A (SP-A) is required for correction of surfactant structural and functional defects in the SP-A null mouse. *J. Biol. Chem.* 276: 38542–38548.
- Borron, P. J., C. McIntosh, T. R. Korfhagen, J. A. Whitsett, J. Taylor, and J. R. Wright. 2000. Surfactant-associated protein A inhibits LPS-induced cytokine and nitric oxide production in vivo. *Am. J. Physiol.* 278: L840–L847.
- LeVine, A. M., K. E. Kurak, J. R. Wright, W. T. Watford, M. D. Bruno, G. F. Ross, J. A. Whitsett, and T. R. Korfhagen. 1999. Surfactant protein A binds group B *Streptococcus* enhancing phagocytosis and clearance from lungs of surfactant protein A-deficient mice. *Am. J. Respir. Cell Mol. Biol.* 20: 279–286.
- Zhang, L., K. L. Hartshorn, E. C. Crouch, M. Ikegami, and J. A. Whitsett. 2002. Complementation of pulmonary abnormalities in SP-D ($^{-/-}$) mice with an SP-D/conglutinin fusion protein. *J. Biol. Chem.* 277: 22453–22459.
- Fisher, J. H., V. Sheftelyevich, Y. S. Ho, S. Fligiel, F. X. McCormack, T. R. Korfhagen, J. A. Whitsett, and M. Ikegami. 2000. Pulmonary-specific expression of SP-D corrects pulmonary lipid accumulation in SP-D gene-targeted mice. *Am. J. Physiol.* 278: L365–L373.
- Li, G., J. Siddiqui, M. Hendry, J. Akiyama, J. Edmondson, C. Brown, L. Allen, S. Levitt, F. Poulain, and S. Hawgood. 2002. Surfactant protein A-deficient mice display an exaggerated early inflammatory response to a β -resistant strain of influenza A virus. *Am. J. Respir. Cell Mol. Biol.* 26: 277–282.
- Jain, D., C. Dodia, S. R. Bates, S. Hawgood, F. R. Poulain, and A. B. Fisher. 2003. SP-A is necessary for increased clearance of alveolar DPPC with hyper-ventilation or secretagogues. *Am. J. Physiol.* 284: L759–L765.
- Botas, C., F. Poulain, J. Akiyama, C. Brown, L. Allen, J. George, J. Clements, E. Carlson, A. M. Gillespie, C. Epstein, and S. Hagwood. 1998. Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. *Proc. Natl. Acad. Sci. USA* 95: 11869–11874.
- Strong, P., U. Kishore, C. Morgan, A. Lopez Bernal, M. Singh, and K. B. M. Reid. 1998. A novel method of purifying lung surfactant proteins A and D from the lung lavage of alveolar proteinosis patients and from pooled amniotic fluid. *J. Immunol. Methods* 220: 139–149.
- Kishore, U., J. Y. Wang, H. J. Hoppe, and K. B. M. Reid. 1996. The α -helical neck region of human lung surfactant protein D is essential for the binding of the carbohydrate recognition domains to lipopolysaccharides and phospholipids. *Biochem. J.* 318: 505–511.
- Shrive, A. K., H. A. Tharia, P. Strong, U. Kishore, I. Burns, P. J. Rizkallah, K. B. M. Reid, and T. J. Greenhough. 2003. High-resolution structural insights into ligand binding and immune cell recognition by human lung surfactant protein D. *J. Mol. Biol.* 331: 509–523.
- Fisher, J. H., J. Larson, C. Cool, and S. W. Dow. 2002. Lymphocyte activation in the lungs of SP-D null mice. *Am. J. Respir. Cell Mol. Biol.* 27: 24–33.
- Zheng, T., Z. Zhu, Z. Wang, R. J. Homer, B. Ma, R. J. Riese Jr., H. A. Chapman Jr., S. D. Shapiro, and J. A. Elias. 2000. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J. Clin. Invest.* 106: 1081–1093.
- Homer, R. J., T. Zheng, G. Chupp, S. He, Z. Zhu, Q. Chen, B. Ma, R. D. Hite, L. I. Gobran, S. A. Rooney, and J. A. Elias. 2002. Pulmonary type II cell hypertrophy and pulmonary lipoproteinosis are features of chronic IL-13 exposure. *Am. J. Physiol.* 283: L52–L59.
- Cheng, G., T. Ueda, H. Nakajima, A. Nakajima, S. Kinjo, S. Motojima, and T. Fukuda. 1998. Suppressive effects of SP-A on ionomycin-induced IL-8 production and release by eosinophils. *Int. Arch. Allergy Immunol.* 117(Suppl. 1): 59–62.
- Yang, M., S. P. Hogan, P. J. Henry, K. I. Mattheai, A. N. McKenzie, I. G. Young, M. E. Rothenberg, and P. S. Foster. 2001. Interleukin-13 mediates airways hyperactivity through the IL-4 receptor- α chain and STAT-6 independently of IL-5 and eotaxin. *Am. J. Respir. Cell Mol. Biol.* 25: 522–530.
- Wills-Karp, M., and M. Chiaramonte. 2003. Interleukin-13 in asthma. *Curr. Opin. Pulm. Med.* 9: 21–27.
- Horie, S., Y. Okubo, M. Hossain, E. Sato, H. Nomura, S. Koyama, J. Suzuki, M. Isobe, and M. Sekiguchi. 1997. Interleukin-13 but not interleukin-4 prolongs eosinophil survival and induces eosinophil chemotaxis. *Intern Med.* 36: 179–185.
- Luttmann, W., B. Knoechel, M. Foerster, H. Matthys, J. C. Virchow Jr., and C. Kroegel. 1996. Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability. *J. Immunol.* 157: 1678–1683.
- Pope, S. M., E. B. Brandt, A. Mishra, S. P. Hogan, N. Zimmermann, K. I. Mattheai, P. S. Foster, and M. E. Rothenberg. 2001. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *J. Allergy Clin. Immunol.* 108: 594–601.
- Yoshida, M., T. R. Korfhagen, and J. A. Whitsett. 2001. Surfactant protein D regulates NF- κ B and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. *J. Immunol.* 166: 7514–7519.
- de Vries, J. E. 1998. The role of IL-13 and its receptor in allergy and inflammatory responses. *J. Allergy Clin. Immunol.* 102: 165–169.
- Endo, T., F. Ogushi, and S. Sone. 1996. LPS-dependent cyclooxygenase-2 induction in human monocytes is down-regulated by IL-13, but not by IFN- γ . *J. Immunol.* 156: 2240–2246.
- Doherty, T. M., R. Kastelein, S. Menon, S. Andrade, and R. L. Coffman. 1993. Modulation of murine macrophage function by IL-13. *J. Immunol.* 151: 7151–7160.
- Sozzani, S., W. Luini, A. Borsatti, N. Polentarutti, D. Zhou, L. Piemonti, G. D'Amico, C. A. Power, T. N. Wells, M. Gobbi, P. Allavena, and A. Mantovani. 1997. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J. Immunol.* 159: 1993–2000.

50. Lentsch, A. B., T. P. Shanley, V. Sarma, and P. A. Ward. 1997. In vivo suppression of NF- κ B and preservation of I κ B α by interleukin-10 and interleukin-13. *J. Clin. Invest.* 100: 2443–2448.
51. Trask, B. C., M. J. Malone, E. H. Lum, H. G. Welgus, E. C. Crouch, and S. D. Shapiro. 2001. Induction of macrophage matrix metalloproteinase biosynthesis by surfactant protein D. *J. Biol. Chem.* 276: 37846–37852.
52. Hawgood, S., M. Ochs, A. Jung, J. Akiyama, L. Allen, C. Brown, J. Edmondson, S. Levitt, E. Carlson, A. M. Gillespie, A. Villar, C. J. Epstein, and F. R. Poulain. 2002. Sequential targeted deficiency of SP-A and SP-D leads to progressive alveolar lipoproteinosis and emphysema. *Am. J. Physiol.* 283: L1002–L1010.
53. Lee, J. J., M. P. McGarry, S. C. Farmer, K. L. Denzler, K. A. Larson, P. E. Carrigan, I. E. Brenneise, M. A. Horton, A. Haczku, E. W. Gelfand, G. D. Leikauf, and N. A. Lee. 1997. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J. Exp. Med.* 185: 2143–2156.
54. Hawgood, S., J. Akiyama, C. Brown, L. Allen, G. Li, and F. R. Poulain. 2001. GM-CSF mediates alveolar macrophage proliferation and type II cell hypertrophy in SP-D gene-targeted mice. *Am. J. Physiol.* 280: L1148–L1156.
55. Ikegami, M., W. M. Hull, M. Yoshida, S. E. Wert, and J. A. Whitsett. 2001. SP-D and GM-CSF regulate surfactant homeostasis via distinct mechanisms. *Am. J. Physiol.* 281: L697–L703.
56. Schmid-Grendelmeier, P., F. Altnauer, B. Fischer, C. Bizer, A. Straumann, G. Menz, K. Blaser, B. Wuthrich, and H. U. Simon. 2002. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J. Immunol.* 169: 1021–1027.
57. Morokata, T., J. Ishikawa, and T. Yamada. 2000. Antigen dose defines T helper 1 and T helper 2 responses in the lungs of C57BL/6 and BALB/c mice independently of splenic responses. *Immunol. Lett.* 72: 119–126.
58. Schaub, B., R. M. Westlake, H. He, R. Arestides, K. J. Haley, M. Campo, G. Velasco, A. Bellou, S. Hawgood, F. R. Poulain, D. L. Perkins, and P. W. Finn. 2004. Surfactant protein D deficiency influences allergic immune responses. *Clin. Exp. Allergy.* 34: 1819–1826.
59. LeVine, A. M., K. Hartshorn, J. Elliott, J. A. Whitsett, and T. R. Korfhagen. 2002. Absence of SP-A modulates innate and adaptive defense responses to pulmonary influenza infection. *Am. J. Physiol.* 282: L563–L572.
60. LeVine, A. M., J. A. Whitsett, K. L. Hartshorn, E. C. Crouch, and T. R. Korfhagen. 2001. Surfactant protein D enhances clearance of influenza A virus from the lung in vivo. *J. Immunol.* 167: 5868–5873.
61. Viola, J. P., A. Kiani, P. T. Bozza, and A. Rao. 1998. Regulation of allergic inflammation and eosinophil recruitment in mice lacking the transcription factor NFAT1: role of interleukin-4 (IL-4) and IL-5. *Blood* 91: 2223–2230.