

Prior airway exposure to allergen increases virus-induced airway hyperresponsiveness

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Background: Respiratory syncytial virus (RSV) bronchiolitis in early life can lead to changes in airway function, but there are likely additional predisposing factors, such as prior allergen exposure, determining which children develop wheezing and asthma.

Objective: To define the effects of prior airway exposure to sensitizing allergen on the development of airway inflammation and hyperresponsiveness (AHR) to subsequent RSV infection.

Methods: BALB/c mice were exposed to ovalbumin or PBS exclusively through the airways and subsequently infected with RSV or sham-inoculated. AHR, lung inflammation, and the frequency of cytokine-producing T lymphocytes in the lung were determined.

Results: In PBS-exposed mice, RSV infection induced AHR and an increased proportion of T_H1-type (IFN- γ and IL-12) cytokine-producing cells in the lungs. However, in mice previously exposed to ovalbumin through the airways and subsequently infected with RSV, the degree of AHR was significantly increased and was associated with an increased proportion of T_H2 (IL-4, IL-5) cytokine-producing T lymphocytes. This response was also associated with an increased accumulation of eosinophils, neutrophils, and CD8⁺ T cells in the lungs.

Conclusions: These data suggest that prior airway exposure to allergen may predispose sensitized hosts to a greater degree of altered airway function upon subsequent respiratory viral infection. (*J Allergy Clin Immunol* 2003;112:861-9.)

Key words: Airway hyperresponsiveness, asthma, allergen, viral infection, T lymphocytes, cytokines

Abbreviations used

AHR: Airway hyperresponsiveness
ANOVA: Analysis of variance
BAL: Bronchoalveolar lavage
IL: Interleukin
MCh: Methacholine
OVA: Ovalbumin
Penh: Enhanced pause
RSV: Respiratory syncytial virus

Respiratory syncytial virus (RSV) bronchiolitis remains a complex disease with predictably difficult interpretations of possible consequences and immunopathology. Some clinical studies have found an association with subsequent wheezing but not atopic sensitization,¹ whereas others have found an increased risk for both asthma and atopy after the disease.² RSV is the most common cause of bronchiolitis. However, the exact immunologic and physiologic mechanisms leading to postbronchiolitis wheezing and whether they are similar to those described in allergic/atopic asthma is still largely unknown.

A murine model has been used as one approach to elucidate the immunologic events that characterize RSV bronchiolitis. Studies from this laboratory have shown that RSV infection enhances subsequent allergic sensitization.³ This enhancement appeared to involve, at least partially, CD8⁺ T lymphocytes, eosinophils, and the production of interleukin (IL)-5.⁴⁻⁶ Data from human studies also suggest that there is at least a subset of infants who have an eosinophilic response during RSV bronchiolitis.⁷ Those with the highest eosinophil counts, even in the peripheral blood, demonstrated a statistically significant relation with the presence of wheezing at 7 years of age.⁸

The fact that infants with an eosinophilic response are also most likely to develop asthma in later life suggests that certain conditions preceding RSV infection may be important in determining outcome. In the review of their data, Sigurs² identified a family history of atopy or asthma as a risk factor for the development of postbronchiolitis asthma. Allergic airway sensitization may therefore be of importance in the sequence of events leading from bronchiolitis to asthma. We therefore hypothesized that

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prior airway exposure to allergen would alter the response to subsequent viral infection, resulting in increased airway hyperresponsiveness. To study possible mechanisms involved in linking allergic hosts and RSV infection, we developed an approach in which mice previously exposed to sensitizing allergen exclusively through the airways were subsequently infected with RSV, and immune/inflammatory responses and airway function were monitored.

METHODS

Animals

Female BALB/c mice, 6 to 10 weeks of age, free of specific pathogens, obtained from Jackson Laboratories (Bar Harbor, Maine) were maintained on OVA-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Virus preparation and infection of mice

Human respiratory syncytial virus (long strain, type A) was obtained from American Type Culture Collection (ATCC; Manassas, Va) and was propagated in monolayers of HEp-2 cells (ATCC) grown in Eagle's minimum essential medium (Gibco) supplemented with 2% FBS. At maximum cytopathic effect, the cells were harvested and disrupted by sonication in the same culture medium. The suspension was clarified by centrifugation at 2000g for 15 minutes at 18°C, and the resulting supernatant was layered on top of a sucrose gradient (30% sucrose in 50 mmol/L Tris-buffered-normal saline solution containing 1 mmol/L ethylenediaminetetraacetate [EDTA], pH 7.5) and further centrifuged at 100,000g for 2 hours at 10°C. The pellet containing virus was resuspended in 10 mmol/L PBS containing 15% sucrose and stored in aliquots at -70°C. Viral titers were determined by standard plaque assay combined with immunostaining for RSV. Uninfected HEp-2 cells were similarly processed to obtain a sham preparation. Stocks of RSV and sham preparations were free of endotoxin contamination as determined by a *Limulus* Amoebocyte Lysate (LAL)-based Pyrogen Plus Gel Clot assay with a sensitivity of 0.06 endotoxin units/mL (No. N283-06; BioWhittaker, Walkersville, Md).

Mice were infected under light anesthesia (Avertin 2.5%, 0.015 mL/g body wt) by intranasal inoculation of RSV (10^6 PFU in 25 μ L PBS). Controls for RSV infection consisted of mice inoculated intranasally with either UV-irradiated RSV (at the same dose) or with a sham preparation.

Experimental design

Mice were exposed to OVA (1% in PBS, Sigma, St Louis, Mo), without the use of adjuvant, or PBS exclusively through the airways for 30 minutes on 10 consecutive days with the use of an AeroSonic Nebulizer 5000 D from DeVilbiss (Sommerset, Pa). Twenty-four hours after the last OVA exposure, mice were infected with RSV or sham-inoculated. Airway responsiveness to inhaled methacholine (MCh) was measured on day 6, followed by assessment of lung inflammation in the same animals on day 7 after infection, as we previously showed airway hyperresponsiveness (AHR) and lung inflammation to peak at these time points after infection.³ In separate experiments, lung inflammation was assessed during the peak of viral replication on day 4 after infection. There were 4 experimental groups, each group containing 6 mice per experiment: (1) PBS-exposed, sham-infected (PBS-sham); (2) PBS-exposed, RSV-infected (PBS-RSV); (3) OVA-exposed, sham-infected (OVA-sham); and (4) OVA-exposed, RSV-infected (OVA-RSV).

Assessment of airway responsiveness to MCh

Airway responsiveness was assessed by whole-body plethysmography, with the use of a Buxco system (Troy, NY), as described previously.⁹ Penh (enhanced pause) was used as the indicator of airway responsiveness in this study. In the plethysmograph, mice were exposed for 3 minutes to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (Sigma) in PBS, with the use of an AeroSonic 5000 D ultrasonic nebulizer (DeVilbiss). After each nebulization, recordings were taken for 3 minutes. The Penh values measured during each 3-minute sequence were averaged and were expressed for each dose of MCh as the percentage of baseline Penh values after PBS exposure (% baseline Penh). Baseline Penh values did not differ significantly between any of the 4 groups.

Histopathology and morphometric analysis of mucus production

Histopathologic examination of lung tissue was carried out on formalin-fixed, paraffin-embedded lung tissue sections. The sections were stained for mucus with periodic acid Schiff (PAS). Under the experimental conditions of this study, PAS-positive epithelial cells were detected only in central intrapulmonary airways in all groups of animals. The data were analyzed morphometrically by digital image analysis using NIH Scion Image software (version 1.62, developed at the US National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). For normalization, the data were divided by the perimeter of the epithelium basement membrane (BM) for the corresponding airway. The measured values were averaged for each animal, and the mean values were determined for each group.

Isolation of lung cells

Lung cells were isolated by collagenase digestion as previously described and pooled from 6 to 12 mice in each group.¹⁰ After collagenase digestion, >90% of the cells were viable, based on trypan blue dye exclusion. Cytospin slides were stained with Leukostat (Fisher Diagnostics, Pittsburgh, Pa), and differential cell counts were performed in a blinded fashion by counting 200 cells under light microscopy.

For cell surface marker and intracellular cytokine analysis, mononuclear cells were enriched by density gradient centrifugation (Organon Teknica, Durham, NC). Cells were washed 3 times with PBS, resuspended in 10% DMSO in FBS, and kept frozen until needed for analysis. This study involved flow cytometric analysis of specimens at the Center for Disease Control (CDC) (Atlanta, Ga) and physiologic and immunologic characterization of pulmonary leukocytes at National Jewish Medical and Research Center (Denver, Colo). The frozen cells were analyzed by flow cytometry in a blinded manner. Flow cytometric analysis of cryopreserved cells is commonly used to examine intracellular cytokine expression and showed results similar to freshly prepared cells.^{11,12} Recovery of cryopreserved cells yielded a viable cell population of >90%, based on trypan blue dye exclusion.

Flow cytometry

Lung cells were examined for phenotype and intracellular cytokine expression without stimulation *ex vivo* (as described¹³). Single-cell suspensions of leukocytes were first incubated with 10% normal mouse serum (Jackson Laboratories) in Dulbecco PBS (GIBCO) to prevent nonspecific binding of antibodies to Fc receptors. For phenotyping, the cells were stained with the appropriate combinations of FITC- or PE-labeled anti-CD3 (145-2C11), anti-CD4 (RM4-5), anti-CD8 α (53-6.7), or similarly labeled isotype-

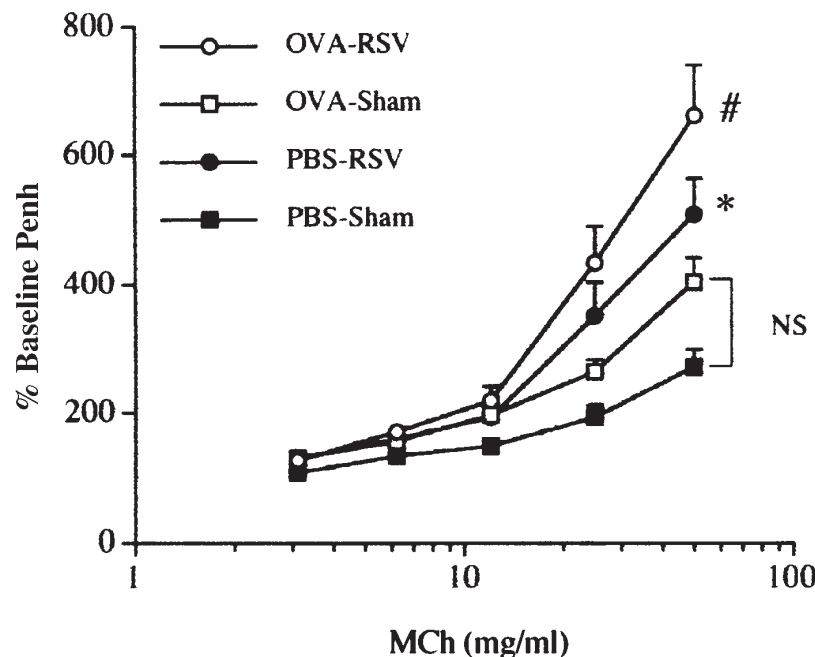


FIG 1. RSV-induced airway responsiveness to inhaled MCh is enhanced by prior allergen airway exposure. BALB/c mice were exposed to aerosolized OVA or PBS exclusively through the airways for 30 minutes daily on 10 consecutive days. On day 11, mice were either sham-inoculated or RSV-infected. Airway responsiveness to inhaled MCh was assessed on day 6 after RSV infection by barometric whole-body plethysmography. Data are expressed as percentage of baseline Penh values obtained after inhaled PBS challenge. *PBS-sham*, PBS-exposed; *sham-inoculated*, OVA-sham; *OVA-exposed*, sham-inoculated; *PBS-RSV*, PBS-exposed; *RSV-infected*, OVA-RSV; *OVA-exposed*, RSV-infected. There were no significant differences in baseline Penh values among groups. *Significantly different from PBS-sham; #significantly different from all other groups ($P < .05$). NS, Not significant.

matched control antibodies (all from PharMingen, San Diego, Calif) optimally diluted in PBS containing 1% bovine serum albumin (BSA, Sigma, St Louis, Mo). For intracellular cytokine staining, the cells were incubated in PBS containing GolgiStop (PharMingen) to block cytokine secretion and stained with FITC-conjugated anti-CD3 for 30 minutes on ice. The cells were washed once with Cytofix/Cytoperm fixation/permeabilization buffer (PharMingen) and resuspended in the same solution for a 30-minute permeabilization on ice. After washing with Perm/Wash buffer (PharMingen), the cells were incubated for intracellular cytokine staining with PE-conjugated anti-IL-4 (BVD4-1D11), anti-IL-5 (TRFK5), anti-IL-12 (C15.6), anti-IFN γ (XMG1.2) or PE-conjugated, isotype-matched rat control antibody (all from PharMingen) optimally diluted in Perm/Wash buffer. After 2 washes with Perm/Wash buffer, the cells were resuspended in PBS containing 1% BSA and analyzed by flow cytometry in 2-color mode on a FACScan with CellQUEST software (Becton-Dickinson, Mountain View, Calif). A lymphocyte gate was used to select 10,000 events for CD3⁺ lymphocytes.

Statistical analysis

Data are presented as mean $\pm$ SEM values obtained from 2 independent experiments, with $n = 6$ animals per group in each experiment. The data were analyzed by ANOVA with the Fisher PLSD test for multiple comparisons when the variance was equal for all groups. When the variance was not equal, statistical significance was determined by nonparametric analysis of 1-way ANOVA, with the use of Kruskal-Wallis and Wilcoxon tests.

RESULTS

Airway hyperresponsiveness

Repeated exposure to OVA exclusively through the airways for 10 consecutive days induces limited AHR to inhaled MCh and results in a low-level airway eosinophilia and inflammatory response.³ The possible changes superimposed by viral infection, therefore, are better detected in this system than in a more robust allergic model initiated by systemic sensitization to allergen together with adjuvant.⁹

As shown in Fig 1, animals exposed to OVA alone developed dose-dependent increases in airway responsiveness to inhaled MCh that were slightly higher but not statistically different from the increases seen in PBS-exposed animals. However, mice exposed to RSV alone had significant increases in airway responsiveness to MCh compared with sham-inoculated animals; inoculation with UV-irradiated virus was equivalent to sham inoculation (data not shown), as previously described.³ In PBS-exposed, RSV-infected mice, the degree of AHR was higher than the OVA-sham group ($P < .05$, ANOVA and Tukey-Kramer test) and corresponded to the peak response that was previously observed at day 6 after infection.³ Interestingly, the degree of AHR was further

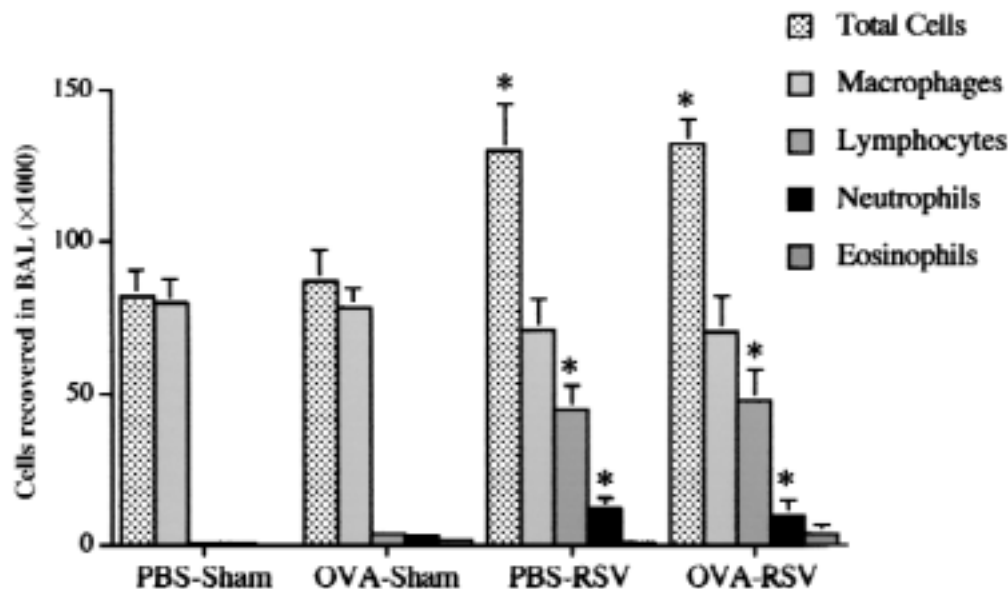


FIG 2. BAL cell composition. Same groups of mice as in Fig 1. *Significant differences compared with PBS-sham or OVA-sham ($P < .05$, ANOVA with Fisher PLSD tests for multiple comparisons).

TABLE I. Lung granulocytic cell inflammatory response

Cell type (%)	Days after infection	PBS-sham	OVA-sham	PBS-RSV	OVA-RSV
Neutrophil	4	6.7 ± 0.8	8.5 ± 1.1	10.4 ± 1.3	12.2 ± 1.3*
	7	3.1 ± 0.4	5.5 ± 1.3	8 ± 2.0	7.4 ± 1.2
Eosinophil	4	2.6 ± 0.6	4.7 ± 1.0	4.4 ± 0.5	6.2 ± 0.7*
	7	2.5 ± 0.5	5.5 ± 0.4*	5.2 ± 0.6*	8.8 ± 0.6†

*Significantly different from PBS group ($P < .05$, Kruskal-Wallis and Wilcoxon tests).

†Significantly different from all other groups ($P < .05$, Kruskal-Wallis and Wilcoxon tests).

increased in mice previously exposed to repeated OVA inhalations and subsequently infected with RSV. In these mice, the response to inhaled MCh was significantly increased as compared with the response seen in the RSV- or OVA-exposed mice ($P < .05$, ANOVA and Tukey-Kramer test).

Lung cellular inflammation

Fig 2 illustrates the changes in the cellular profile of the bronchoalveolar (BAL) fluids recovered from different groups of mice after either the 10-day OVA exposure, RSV infection, or both. With the exception of increased numbers of lymphocytes after RSV infection, the changes were only minor after repeated inhalational exposures to OVA. In addition, the cellular inflammatory response to RSV was not modified in the BAL by prior exposure to OVA.

To detect further effects on lung cellular inflammatory responses, cells were isolated from the lungs by collagenase digestion and examined. Table I shows that both neutrophils and eosinophils were significantly increased in the lungs of the OVA-RSV group, with neutrophils

peaking on day 4 and eosinophils on day 7 after infection ($P < .05$, Kruskal-Wallis and Wilcoxon tests). On day 7, RSV induced a mild (approximately twice the levels seen in control mice) but significant eosinophilia, which was similar to the OVA-sham group. The T-lymphocyte phenotype response was mostly characterized by significant increases in the proportion of CD8-positive T cells in the lungs of RSV-infected animals on day 7 after infection (Table II). However, this response was not significantly enhanced by prior exposure to OVA.

Lung histopathology

After 10-day OVA inhalation exposure, mice had a low-grade inflammatory response and low numbers of mucus-producing goblet cells (10.93 ± 1.81 vs 4.05 ± 1.16 PAS+/mm BM for PBS, $P = .009$) (Fig 3). On day 7 after RSV infection alone, mice had a characteristic mononuclear cell infiltration in the airways with sparse but significantly increased numbers of mucus-producing goblet cells in central airways (18.68 ± 1.83 PAS+/mm BM, $P = .004$ compared with OVA-exposed sham-inoculated mice). However, in mice previously exposed to

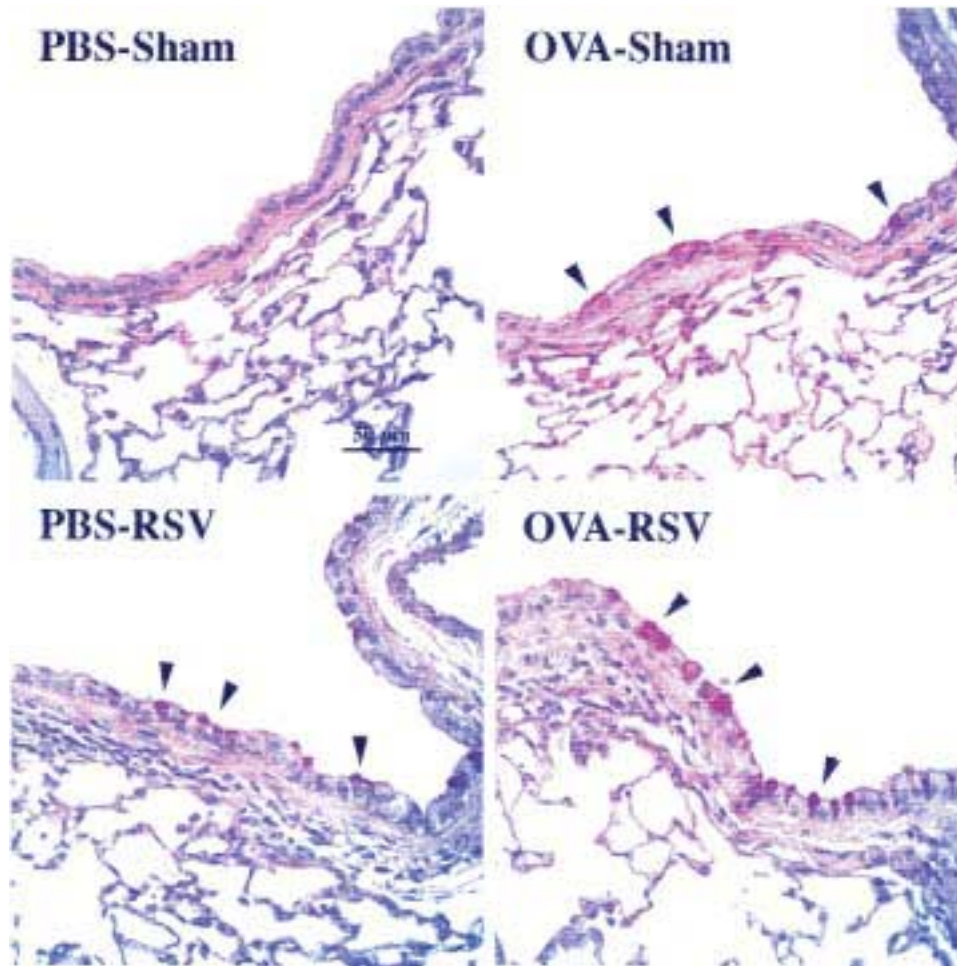


FIG 3. Lung histopathology. Formalin-fixed, paraffin-embedded lung tissue sections were stained with PAS reaction to detect mucus-producing goblet cells. Abbreviations as in Fig 1. Prior airway exposure to OVA increased RSV-induced airway tissue inflammation and markedly enhanced mucus production in central intrapulmonary airways (arrowheads).

TABLE II. Lung T-cell phenotype response

Cell surface marker	Days after infection	% Positive cells (mean $\pm$ SEM)			
		PBS-sham	OVA-sham	PBS-RSV	OVA-RSV
CD3	4	24.1 $\pm$ 2.7	28.3 $\pm$ 1.1	22.1 $\pm$ 1.3	27.2 $\pm$ 1.6
	7	24.4 $\pm$ 2.1	24.6 $\pm$ 1.4	25.2 $\pm$ 1.3	29.7 $\pm$ 1.1
CD4	4	19.5 $\pm$ 2.5	23.1 $\pm$ 1.5	17.3 $\pm$ 1.4	23.0 $\pm$ 1.3
	7	20.3 $\pm$ 1.6	21.2 $\pm$ 1.0	17.9 $\pm$ 1.7	21.3 $\pm$ 1.5
CD8	4	6.2 $\pm$ 1.0	7.8 $\pm$ 0.8	6.2 $\pm$ 0.8	7.8 $\pm$ 0.8
	7	6.8 $\pm$ 0.6	5.4 $\pm$ 0.5	9.7 $\pm$ 1.3*	10.4 $\pm$ 0.5*

*Significantly different from PBS group ($P < .05$, Kruskal-Wallis and Wilcoxon tests).

OVA, RSV infection resulted in further increases in airway mucus production (24.68 ± 1.36 PAS+/mm BM, $P = .019$ compared with PBS-exposed, RSV-infected mice), with increased airway tissue infiltration of inflammatory mononuclear cells and granulocytes.

Frequency of cytokine-producing lymphocytes

To investigate potential mechanisms underlying the changes in airway function induced by either the individ-

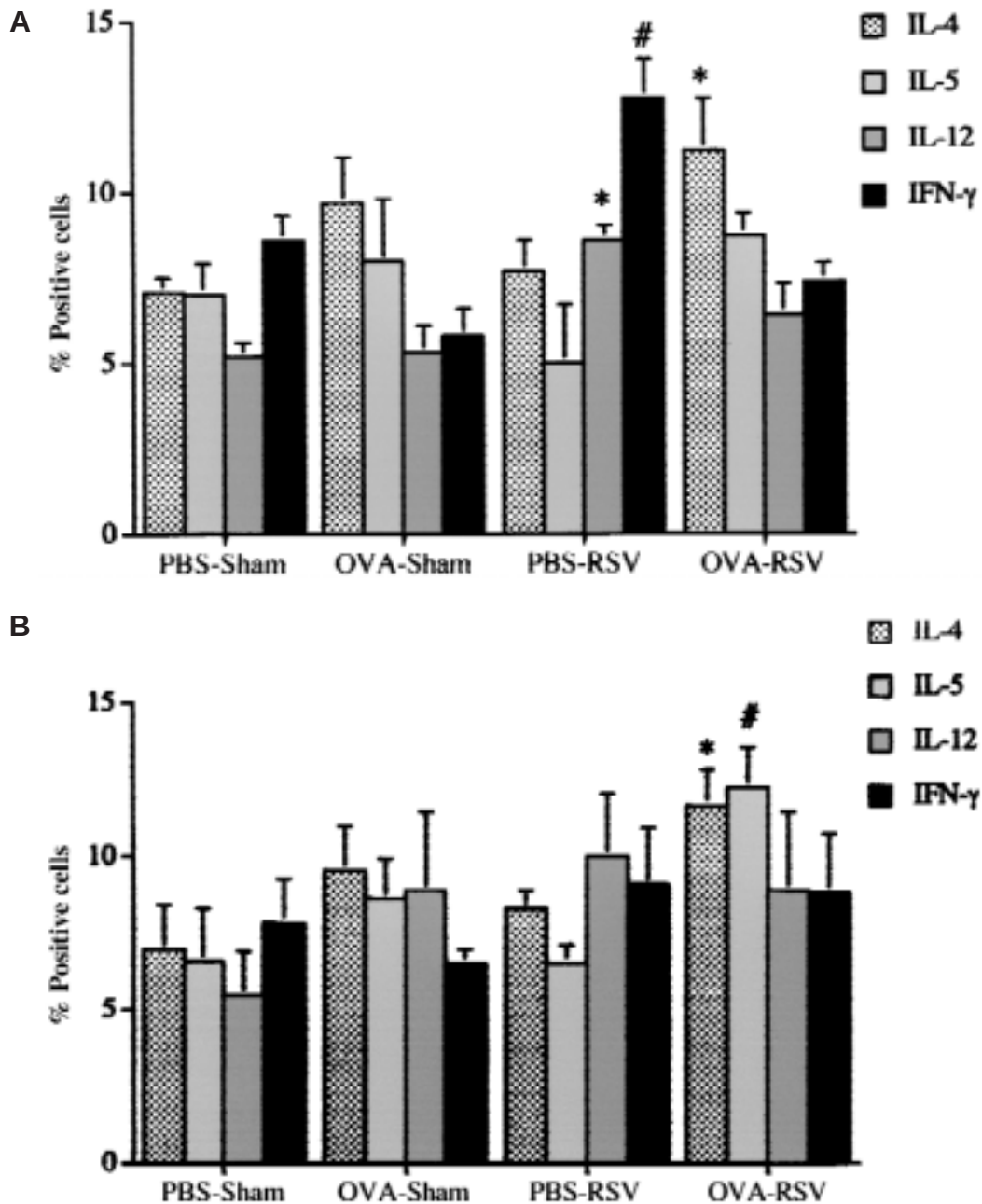


FIG 4. Frequency of T_H1 and T_H2 cytokine-producing T cells in the lung. Cytokine-producing T cells were detected in the lung digests obtained on day 4 (**A**) and day 7 (**B**) after infection by double immunostaining for surface expression of CD3 and intracellular expression of IL-4, IL-5, IL-12, or IFN- γ without restimulation of cells in vitro. *Significantly different from PBS group; #significantly different from all the other groups ($P < .05$, Kruskal-Wallis test).

ual or the combined effects of allergen exposure and RSV infection, the frequency of cytokine-producing lymphocytes was determined among mononuclear cells isolated from lung tissue digests. The cells were analyzed for specific intracellular cytokine staining without additional stimulation to more directly reflect their in vivo functional status. As shown in Fig 4, no significant changes in the frequency of cytokine-producing cells

were induced by 10 days of repeated allergen inhalation exposure. RSV infection alone resulted in significant increases in the proportion of predominantly T_H1 -type cytokine-producing cells (IFN- γ and IL-12). The frequency of these cells in the lungs was highest on day 4 after RSV infection (Fig 4, A). Such increases, however, were not seen in the lungs of RSV-infected mice that were previously exposed to allergen; here the frequency

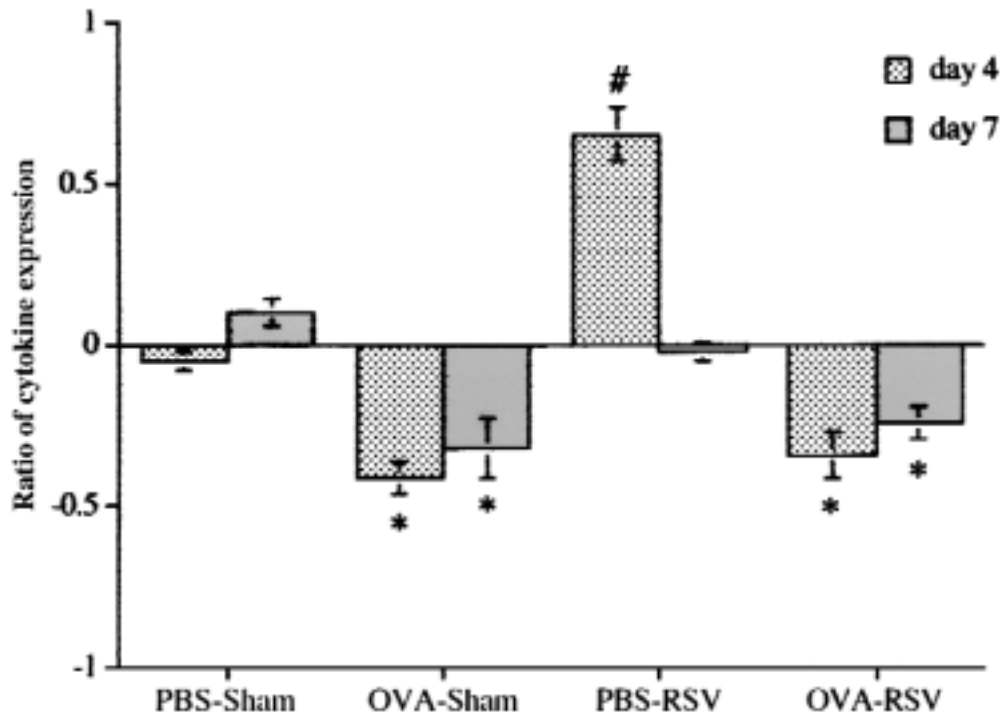


FIG 5. Ratio of T_H1 to T_H2 cytokine-expressing lung T cells. Data (mean ± SEM) reflect normalized ratio of IFN- γ to IL-4 cytokine-producing T-cell frequencies in the lung. Normalized ratio was obtained by using the formula [(frequency of IFN- γ + T cells/ frequency of IL-4+ T cells)-1], where 1 corresponds to equal frequencies for both cell populations. *Significantly different from PBS group; #significantly different from all the other groups ($P < .05$, ANOVA with Fisher PLSD tests for multiple comparisons).

of T_H2-type cytokine-producing cells (IL-4 and IL-5) predominated (Fig 4, A and B). These differences were best reflected in terms of the ratio of IFN- γ -producing to IL-4-producing T-cell proportions in the lung (Fig 5). The data revealed a significant shift in the balance in lung T-cell cytokine response in animals exposed to either OVA or RSV alone or to RSV after prior exposure to allergen through the airways.

DISCUSSION

It is now well established that mice exposed to OVA exclusively through the airways (in the absence of sensitization together with adjuvant) or to acute RSV infection can have alterations in lung function associated with lung inflammation and cytokine production, albeit to lower levels. The current study highlights the findings in allergen-exposed, RSV-infected mice, a combination that clearly enhances the changes in airway responsiveness elicited with either allergen exposure alone or RSV infection alone. Associated with the increased AHR was an increase in the frequency of T_H2-type (IL-4 and IL-5) cytokine-producing lymphocytes revealed in the lungs of OVA-exposed animals on subsequent RSV infection.

RSV infection alone did not significantly induce expression of T_H2 cytokines but was mainly associated with an increased frequency of T_H1-type (IFN- γ and IL-12) cytokine-producing lymphocytes in the lungs. It is noteworthy that in this study, allergen exposure occurred exclusively through inhalation in the absence of adjuvant or systemic sensitization. Direct airway exposure to repeated inhalations of sensitizing allergen has been shown to elicit a mild allergic response associated with some degree of altered airway function.³ The current data support the notion that prior airway exposure to inhaled allergen may predispose to the development of exaggerated airway responsiveness upon subsequent RSV infection. These results are in agreement with earlier animal studies in guinea pigs¹⁴ and in mice,¹⁵ suggesting that prior allergic sensitization can potentiate the physiologic changes induced by RSV infection.

In addition to altered airway function, we analyzed the inflammatory cellular responses and cytokine production that are compartmentalized to airway tissue and that may underlie the physiologic changes in airway function. The data demonstrate that RSV-induced changes in BAL cellularity were not markedly enhanced by prior allergen exposure. In the absence of adjuvant or systemic sensitization,

only minor changes were demonstrated in the profile of resident inflammatory cells recovered in the BAL fluid. In contrast, examination of lung tissue digests revealed significant changes in the numbers of infiltrating neutrophils and eosinophils after allergen exposure and RSV infection. The percentages of eosinophils and neutrophils in the collagenase-treated lung digests were comparable in mice exposed to OVA alone and mice infected with RSV only but were increased in OVA-exposed, RSV-infected mice. Our earlier studies suggested that eosinophils may have at least a partial role in the development of AHR after acute RSV infection.^{3,4} Whether neutrophils play a comparable role in this response is not known.

The cytokine data suggest that in addition to T_H1 responses, under certain conditions, such as in previously allergen-exposed mice, RSV infection can also induce T_H2 responses. A role for the T_H2 -type cytokines IL-4 and IL-5 has been demonstrated in the immunologic cascade, resulting in allergen-driven AHR.^{16,17} Other studies have shown that both T_H1 and T_H2 responses can play significant roles in the development of allergen-induced altered airway function.¹⁸ Our previous studies have indicated a critical role for IL-5 and less so for IL-4 in the development of RSV-induced AHR.¹⁹ We have recently shown that RSV-induced AHR, unlike allergen-induced AHR, is independent of IL-13.^{20,21} Thus, the increases in AHR after allergen exposure and RSV infection appear to be through independent mechanisms, contributing to their additive effects.

There is some controversy about the profile of cytokine responses that develop in human infants during acute RSV bronchiolitis and its specificity to the disease. In one study, RSV infection and associated bronchiolitis was associated with a T_H2 -type response,²² whereas in other studies, a T_H1 -type cytokine response was associated with RSV, and bronchiolitis and IFN- γ was shown to be elevated in children with virus-induced wheezing.^{23,24} Data from the current study in mice suggest that the T_H1 -type and T_H2 -type cytokine responses are not mutually exclusive and that there is an overall enhancement of proinflammatory cytokine production, results that are similar to findings described in some human studies.^{25,26} In a recent study by Matsuse et al,²⁷ examination of RSV infection in mice revealed that primary RSV infection elicited the greatest levels of IL-4, IL-5 and IFN- γ production, whereas in allergen-sensitized mice, RSV infection resulted in reduced production of IL-4 and IL-5. However, repeated RSV infection resulted in increased levels of IL-4 and IL-5. Our study revealed somewhat different results in cytokine expression. However, these differences may be explained by differences in the sensitization protocol used. In the study by Matsuse et al,²⁷ systemic allergen sensitization was used, whereas in our study, allergen exposure occurred exclusively through the airways in the absence of systemic sensitization and without the use of any adjuvant. The immune response after airway exposure to allergen and RSV infection probably reflects a more localized response than that after systemic allergen sensitization (together with adjuvant) and subsequent infection.

In summary, the current study illustrates and confirms the importance of allergen inhalation exposure as a possible risk factor for the development of increased airway dysfunction upon subsequent RSV infection.²⁸ The data highlight that RSV infection in OVA-exposed mice triggers a broad immune response. This is compartmentalized to airway tissue and is reflected by an increase in the frequency of T_H2 cytokine-producing cells, associated with increases in lung tissue eosinophils and neutrophils. Together, they may contribute to the alterations in airway function.

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