

Respiratory syncytial virus–induced airway hyperresponsiveness is independent of IL-13 compared with that induced by allergen

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Background: IL-13 is a central mediator of allergen-induced airway hyperresponsiveness (AHR), but its role in respiratory syncytial virus (RSV)–induced AHR is not defined. The combination of allergen exposure and RSV infection is known to increase AHR and lung inflammation, but whether IL-13 regulates this increase is similarly not known.

Objective: Our objective was to determine the role of RSV infection and IL-13 on airway responsiveness and lung inflammation on sensitized and challenged mice.

Methods: Using a murine model of RSV infection and allergen exposure, we examined the role of IL-13 in the development of AHR and lung inflammation in IL-13 knockout mice, as well as using a potent IL-13 inhibitor (IL-13i). Mice were sensitized and challenged to allergen, and 6 days after the last challenge, they were infected with RSV. IL-13 was inhibited using an IL-13 receptor α_2 -human IgG fusion protein. AHR to inhaled methacholine was measured 6 days after infection, as was bronchoalveolar lavage fluid and lung inflammatory and cytokine responses.

Results: RSV-induced AHR was unaffected by the IL-13i, despite prevention of goblet cell hyperplasia. Similar results were seen in IL-13–deficient mice. In sensitized and challenged mice, RSV infection significantly increased AHR, and after IL-13i treatment, AHR was significantly reduced, but to the levels seen in RSV-infected mice alone.

Conclusions: These results indicate that despite some similarities, the mechanisms leading to AHR induced by RSV are different from those that follow allergen sensitization and challenge. Because IL-13 inhibition is effective in preventing the increases in AHR and mucus production in sensitized and challenged mice infected with RSV, IL-13i could play an important role in preventing the consequences of viral infection in patients with allergic asthma. (*J Allergy Clin Immunol* 2003;112:1078-87.)

Key words: Respiratory syncytial virus, airway hyperresponsiveness, IL-13

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and viral pneumonia in infants¹ and is suspected to play a role in the development of bronchial asthma.² In murine models, RSV infection can induce airway hyperresponsiveness (AHR) and airway inflammation, predominantly characterized by mononuclear cell infiltration.³⁻⁵ In RSV-infected mice, CD8 and T_H1 immune responses are more prominent than T_H2 immune responses; however, the latter might have some role in RSV-induced AHR.⁴⁻⁹ In allergen-induced AHR, T_H2 responses play a predominant role in airway inflammation and mucus production, which seems to be critically dependent on IL-13.¹⁰⁻¹⁴ In addition to T_H2 cells, IL-13 can be produced by mast cells¹⁵; basophils¹⁶; and CD8, T_H0, and T_H1 T cells at low levels.¹⁷⁻²⁰ As a result, it is possible that IL-13 might play a role in the development of AHR and lung disease in RSV-infected mice, but this is somewhat controversial.²⁰⁻²³

METHODS

Experimental protocols

Two protocols were used in this study. To determine the role of IL-13 in the development of AHR induced by RSV infection alone, WT and IL-13–deficient BALB/c mice were used. The mice were infected with RSV, and airway responsiveness to inhaled methacholine (MCh) was assessed on day 6 after intranasal inoculation of mice with RSV. Murine IL-13R α_2 -hIgG fusion protein (IL-13i), a potent inhibitor of IL-13 and allergen-induced AHR and inflammation,¹⁴ was prepared as previously described.²⁴ IL-13i was administered by intraperitoneal injection (300 μ g/mouse) 24 hours before infection and on days 1, 3, and 5 after inoculation. In the second protocol, the role of IL-13 in RSV infection in allergen-sensitized and challenged mice was evaluated. For this purpose, BALB/c mice were sensitized by intraperitoneal injection of 20 μ g of ovalbumin (OVA) in alum (Pierce, Rockford, Ill) on days 0 and 14, and the mice were challenged by inhalation of aerosolized OVA (1% in PBS) for 20 minutes by use of an ultrasonic nebulizer (Model NE-U07; Omron Co, Kyoto, Japan) on days 26, 27, and 28. IL-13i was administered just before each challenge. RSV was inoculated on day 30 followed by measurement of airway responsiveness to MCh on day 36. In this protocol, IL-13i was injected daily from days 26 to 29 and then every other day until the mice were killed.

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Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
MCh: Methacholine
OVA: Ovalbumin
PAS: Periodic acid-Schiff
RSV: Respiratory syncytial virus

Animals

Eight- to 12 week-old female BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, Me). Homozygous IL-13-deficient mice (BALB/c)²⁵ were kindly provided by Dr Dale T. Umetsu (Stanford University, Stanford, Calif).

Virus

Human RSV (long strain, type A) was purified in sucrose gradients as previously described.²⁶ Viral titer was determined by standard plaque assay combined with immunostaining for RSV. Mice were inoculated under light anesthesia (Avertin 2.5%, 0.015 mL/g body weight) by intranasal administration of RSV (10⁶ pfu) or sham inoculation using a virus-free preparation or UV-irradiated virus.³

Assessment of AHR

AHR, as a change in airway function after challenge with aerosolized MCh, was assessed using methods described by Takeda et al.²⁷ MCh aerosol was administered by nebulization in increasing concentrations. Maximum values of lung resistance or lowest levels of dynamic compliance were calculated and expressed.

BAL

Immediately after assessment of AHR, lungs were lavaged with the tracheal tube with 1 mL of Hanks' balanced salt solution (Gibco, Grand Island, NY). Total cell numbers were obtained (Coulter Counter, Coulter Co, Hialeah, Fla). Differential cell counts were performed by counting at least 200 cells on cytocentrifuged preparations (Model Cytospin 3; Shandon Ltd, Runcorn, Cheshire, UK) stained with Leukostat (Fisher Diagnostics, Fair Lawn, NJ).

Histologic and immunohistochemistry studies

After obtaining the BAL fluid, the lungs were fixed in formalin and processed into paraffin. Tissue sections were stained with hematoxylin and eosin for routine histologic studies and periodic acid-Schiff (PAS) for identification and quantification of mucus-containing goblet cells as described.¹⁴ Cells containing major basic protein in lung sections were identified by immunohistochemical staining as described with rabbit anti-mouse major basic protein (provided by Dr J. J. Lee, Mayo Clinic Scottsdale, Scottsdale, Ariz).²⁸ All sections were examined in a blinded fashion as to the treatments.

Cytokine measurements

Cytokine levels in BAL fluid and lung homogenates were determined by ELISA as described.¹⁴ Detection limits for the assays were as follows: IL-4 (7.8 pg/mL), IL-5 (15.6 pg/mL), IL-13 (1.5 pg/mL), IFN- γ (62.5 pg/mL), and IL-12 (62.5 pg/mL). For lung homogenation, lung tissue was frozen at -70°C immediately after the mice were killed. Lung tissue was mixed with a PBS-0.1% Triton-X100 solution containing proteinase inhibitors at a 1:2.5 ratio of weight per volume (PharMingen; BDPharMingen, San Diego, Calif). The specimens were homogenized and then centrifuged at

15,000 rpm for 15 minutes. The supernatants were frozen at -70°C until analysis.

Statistical analysis

Data are presented as mean $\pm$ SEM. For analysis of the role of IL-13i in RSV-infected BALB/c mice and allergen-sensitized and challenged mice after RSV infection, ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs were performed using the Tukey-Kramer honest significant difference test. For analysis of the effects of RSV infection between 2 groups, the Mann-Whitney test was used. Statistical significance levels were set at a *P* value of .05.

RESULTS

Kinetics of RSV-induced AHR and IL-13 production in BALB/c mice

IL-13 levels in BAL fluid of infected mice were just at or below the detection limit (1.5 pg/mL) in ELISA. When lung homogenates were examined, the levels of IL-13 peaked on day 6 after infection and rapidly decreased after that. Treatment of the mice with the IL-13i did not significantly alter IL-13 levels in lung homogenates as has been reported previously.²⁹⁻³¹ The results in Fig 1 demonstrate that RSV induced maximum AHR on day 6, confirming previous results,³ and this was concomitant with maximum levels of IL-13 in lung homogenates.

IL-13 is not required for the development of RSV-induced AHR and airway inflammation

As shown in Fig 2, treatment with IL-13i did not impair the ability of the mice to develop AHR after RSV infection. Increases in lung resistance (Fig 2, A) and decreases in dynamic compliance (Fig 2, B) were not significantly different than in infected mice receiving control IgG.

After RSV infection, an increase in BAL lymphocyte numbers was detected, as well as a small, but reproducible, increase in neutrophils (Fig 3). Treatment with IL-13i was without effect on any of these responses. Infection with RSV resulted in little or no change in BAL levels of IL-4, which were very low (Fig 4, A). After RSV infection, levels of IL-5 were decreased, and administration of IL-13i did not affect these levels (Fig 4, B). RSV infection did not significantly increase IL-13 levels in BAL fluid (Fig 4, C), and administration of IL-13i did not affect these levels; the levels were even slightly higher than in sham-infected mice. Levels of IL-12 were not different between sham-infected and RSV-infected mice with control antibody treatment. In contrast, treatment with IL-13i did result in a significant increase in the levels of IL-12 (Fig 4, D). RSV infection did lead to a significant increase in BAL levels of IFN- γ , and these levels were not further increased after IL-13i treatment (Fig 4, E).

RSV infection resulted in a significant perivascular and peribronchial inflammatory response, characterized by an increase in lymphocytes, eosinophils, and neutrophils. As previously reported,³⁻⁵ eosinophils were not detected in the BAL fluid. Immunohistochemical stain-

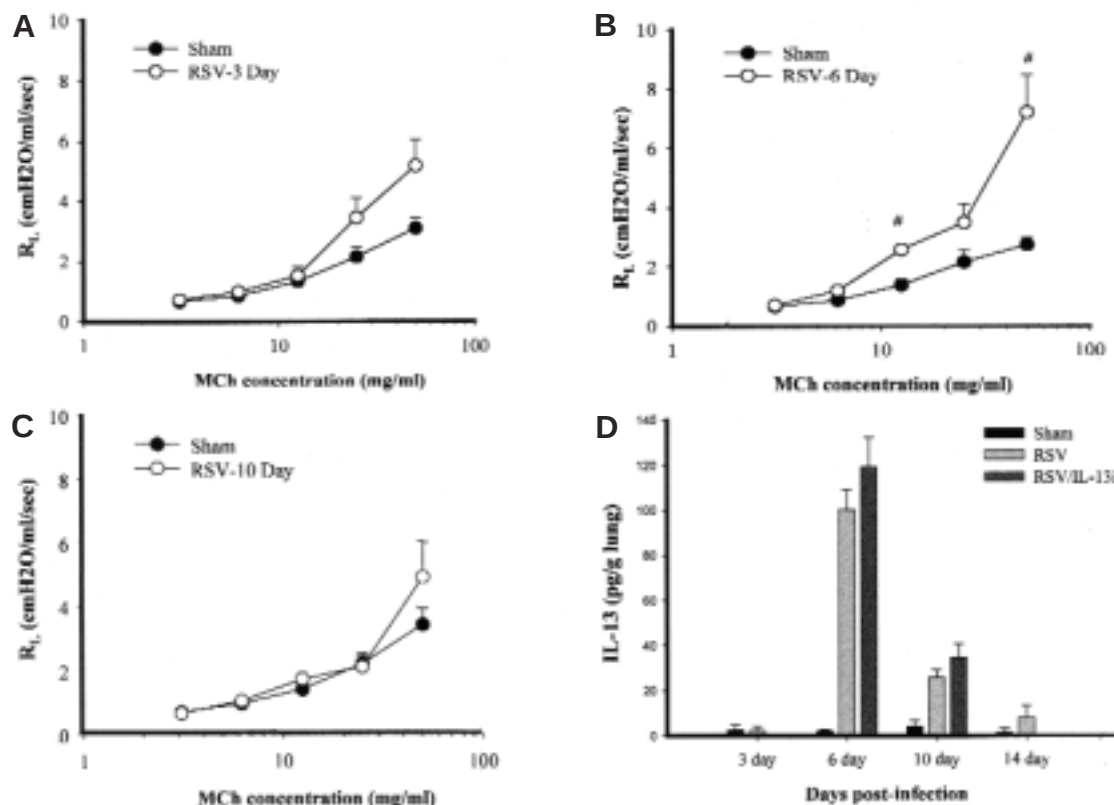


FIG 1. Kinetics of the development of airway hyperresponsiveness (AHR) (A-C) and IL-13 levels in lung homogenates (D) after respiratory syncytial virus infection. AHR is expressed as increases in airway resistance to inhaled methacholine on day 3 (A), day 6 (B), and day 10 (C). Each group consisted of 8 mice. # $P < .05$ compared with the sham-infected group.

ing of eosinophil major basic protein revealed small but significant numbers of eosinophils in peribronchial and perivascular airway tissue of RSV-infected mice (data not shown). The inflammatory response in the lung was not altered by treatment with IL-13i. To assess epithelial goblet cell hyperplasia, sections were stained with PAS (Fig 5, A-D), and the numbers of PAS-positive cells were quantified (Fig 5, E). RSV infection induced an increase in PAS-positive goblet cell numbers, and this increase was virtually eliminated in the IL-13i-treated group.

RSV-induced AHR and airway inflammation develop in IL-13-deficient mice

After RSV infection, IL-13-deficient mice developed significant increases in lung resistance (Fig 6, A), although the overall response to RSV or sham infection was lower than in WT mice (Fig 6, B). When compliance was measured, IL-13-deficient mice showed comparable decreases in dynamic compliance (Fig 6, C,D). The increase in numbers of BAL neutrophils was not affected by the absence of IL-13, and the lymphocyte count was even higher than in WT mice (Fig 7). IL-4 and IL-13 levels in BAL fluid were below the detection limits of ELISA in both the sham and RSV-infected IL-13-deficient mice. Levels of IL-5 were not different (sham: 29 ± 11 pg/mL vs infected: 27 ± 2 pg/mL). In the IL-13-defi-

cient mice, RSV induced a significant increase in IFN- γ compared with sham infection (588 ± 47 pg/mL vs 156 ± 48 pg/mL, $P < .01$), but IL-12 levels were not altered by RSV infection (63 ± 3 pg/mL vs 64 ± 23 pg/mL).

Histologic examination of the lung tissue in the deficient mice was similar to that seen in the IL-13i-treated WT animals. A perivascular and peribronchial inflammatory response was seen in both IL-13-deficient and IL-13-sufficient mice, but no PAS-positive cells were detected in the IL-13-deficient mice (Fig 8).

IL-13 is required for the increases in AHR after RSV infection of sensitized mice

Mice sensitized and challenged with OVA developed increases in AHR (OVA) that were almost completely inhibited after treatment with IL-13i (OVA/IL-13i) (Fig 9), as previously described.¹⁴ OVA-sensitized mice were infected with RSV 2 days after completion of the 3 days of OVA inhalation challenge (days 26-28), and airway responses to inhaled MCh were measured 6 days later (day 36). As shown previously,²⁶ OVA-sensitized and OVA-challenged mice infected with RSV (OVA/RSV/cIg) demonstrated a significant increase in lung resistance compared with nonsensitized but challenged RSV-infected mice (challenge/RSV) or sensitized/challenged but noninfected mice (OVA). In fact, the combi-

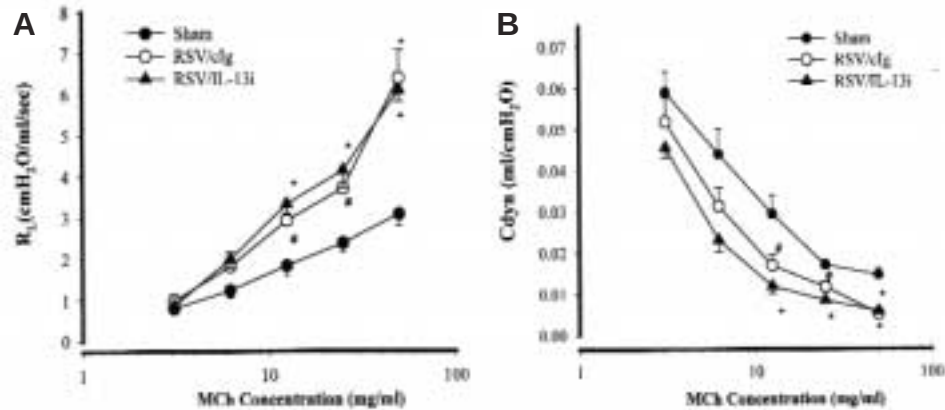


FIG 2. Airway hyperresponsiveness develops after respiratory syncytial virus infection and is not affected by administration of IL-13i. Results are expressed as changes in airway resistance (Fig 1, A) and dynamic compliance (Fig 1, B). Each group consisted of 8 mice. * $P < .01$ compared with the sham-infected group, # $P < .05$ compared with the sham-infected group.

nation of OVA sensitization and challenge followed by RSV infection resulted in the highest levels of AHR. Treatment of the mice with IL-13i (OVA/RSV/IL-13i) resulted in a decrease in AHR to the levels seen after RSV infection and challenge alone (Fig 9).

OVA sensitization and challenge resulted in a marked increase in eosinophils ($135 \pm 14/\text{mm}^3$), and this was inhibited by about 40% after treatment with IL-13i. RSV infection significantly decreased these numbers ($77 \pm 18/\text{mm}^3$), whereas IL-13i treatment further decreased the number of eosinophils in the BAL fluid ($26 \pm 5/\text{mm}^3$) (Table I). The numbers of lymphocytes were not further increased by RSV infection nor were they altered by IL-13i treatment. Levels of IL-4, IL-5, or IL-13 were not altered by RSV infection or IL-13i treatment (Table II). The levels of IL-4 and IL-5 were low in the sensitized and challenged mice, likely because they were measured beyond the peak response (day 36).³² IL-12 levels were significantly lower in OVA-sensitized/challenged mice than in the OVA-challenged only group and were increased by IL-13i treatment but were not significantly altered by IL-13i administration in the RSV-infected, OVA-sensitized and OVA-challenged mice. Infection with RSV did lead to an increase in IFN- γ levels, and the levels were unaffected by prior OVA sensitization and challenge or by IL-13i treatment.

DISCUSSION

Previous reports on the role of IL-13 in RSV-induced AHR have been somewhat contradictory.²⁰⁻²⁴ In this study, we demonstrated, using 2 independent approaches, that IL-13 is not essential to the development of AHR induced by RSV infection. We do not exclude a role for IL-13 in this RSV infection model, but it is not central to the development of AHR. In this regard, the results showed that IL-13 is indeed critical for triggering epithelial cell mucus production in RSV-infected mice. IL-13 was detected in lung homogenates of RSV-infected mice peaking at day 6 after infection but was not detected in BAL fluid. This contrasts with the higher levels of IL-13 in the BAL fluid

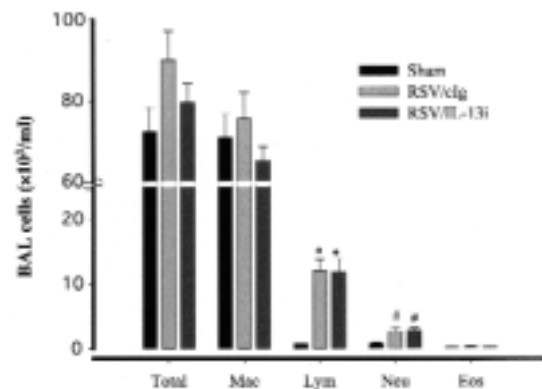


FIG 3. Bronchoalveolar lavage (BAL) fluid cell counts. The results are from the same mice shown in Fig 1. There were no differences in BAL fluid lymphocyte and neutrophil counts between the respiratory syncytial virus (RSV)/clg and the RSV/IL-13i group. * $P < .01$ compared with the sham-infected group, # $P < .05$ compared with the sham-infected group.

of OVA-sensitized and OVA-challenged mice.¹⁴ We speculate that the level of IL-13 production after RSV infection is sufficient to induce production of bronchial epithelial cell mucus production but is not essential to the development of AHR. The role of mucus production in the development of AHR is controversial. Mucus production has not been associated with development of AHR in allergen-induced models³³ or in a model in which cytomegalovirus infection is combined with allergen.³⁴ Walter et al³⁵ reported on the differences in genetic control of AHR and goblet cell hyperplasia in Sendai virus-infected mice. Furthermore, we have previously shown that mucus hyperproduction per se is not essential to development of AHR after allergen sensitization and challenge.³² These results are consistent with the results of Cohn et al,^{36,37} who showed that T_H2 cells and IL-13 are critical for epithelial cell mucus production and that IFN- γ might negatively regulate mucus production. However, they also showed that T_H1 cells can induce mucus production through the IL-13 pathway in the absence of IFN- γ and that the IL-13

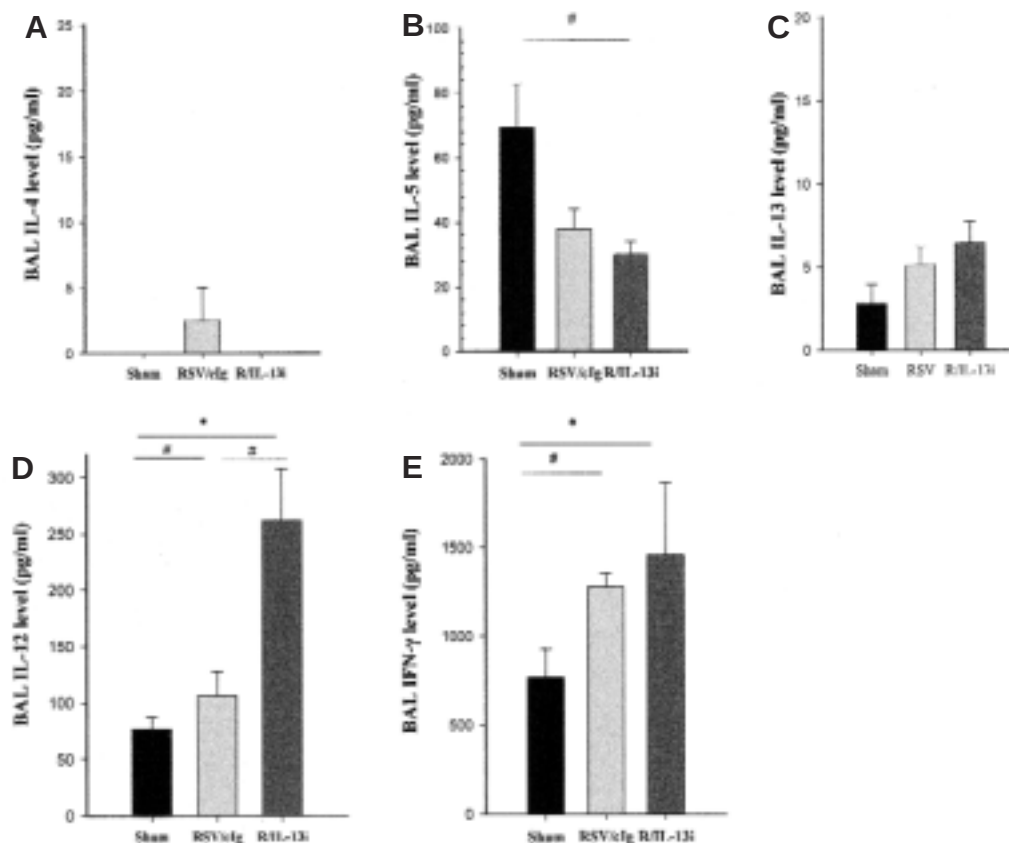


FIG 4. Levels of cytokines in the bronchoalveolar lavage fluid of respiratory syncytial virus-infected mice. Results are from the same mice shown in Fig 1. * $P < .01$, # $P < .05$.

TABLE I. Bronchoalveolar lavage fluid cell profiles of ovalbumin-sensitized/challenged mice after respiratory syncytial virus infection

BAL cells ($\times 10^3/\text{mL}$)	Challenge/sham	Challenge/RSV	OVA/cIg	OVA/IL-13i	OVA/RSV/cIg	OVA/RSV/IL-13i
Total cells	91.0 $\pm$ 4.5	121.8 $\pm$ 13.5	235.8 $\pm$ 18.5	153.0 $\pm$ 12.1	201.4 $\pm$ 17.2	151.3 $\pm$ 15.5*
Macrophages	77.5 $\pm$ 4.5	83.3 $\pm$ 9.0	40.1 $\pm$ 5.5	32.8 $\pm$ 11.4	47.4 $\pm$ 3.4	76.7 $\pm$ 13.4*
Lymphocytes	8.4 $\pm$ 2.8	33.3 $\pm$ 7.3	58.0 $\pm$ 5.9	30.6 $\pm$ 7.6	59.4 $\pm$ 6.3	46.3 $\pm$ 8.4
Eosinophils	0	0.3 $\pm$ 0.1	135.3 $\pm$ 13.7	85.0 $\pm$ 14.6*	77.0 $\pm$ 17.9*	26.4 $\pm$ 5.1†
Neutrophils	5.1 $\pm$ 2.5	8.8 $\pm$ 2.6	2.9 $\pm$ 0.8	4.6 $\pm$ 0.9	17.5 $\pm$ 3.7‡	2.7 $\pm$ 0.7

Mice are from the same groups described in Fig 9.

BAL, Bronchoalveolar lavage; OVA, ovalbumin; RSV, respiratory syncytial virus.

* $P < .05$ compared with OVA group.

† $P < .05$ compared with OVA/RSV/cIg and $P < .01$ compared with OVA.

‡ $P < .01$ compared with OVA and OVA/RSV/IL-13i groups.

TABLE II. Bronchoalveolar lavage fluid cytokine levels of respiratory syncytial virus-infected ovalbumin-sensitized/challenged mice

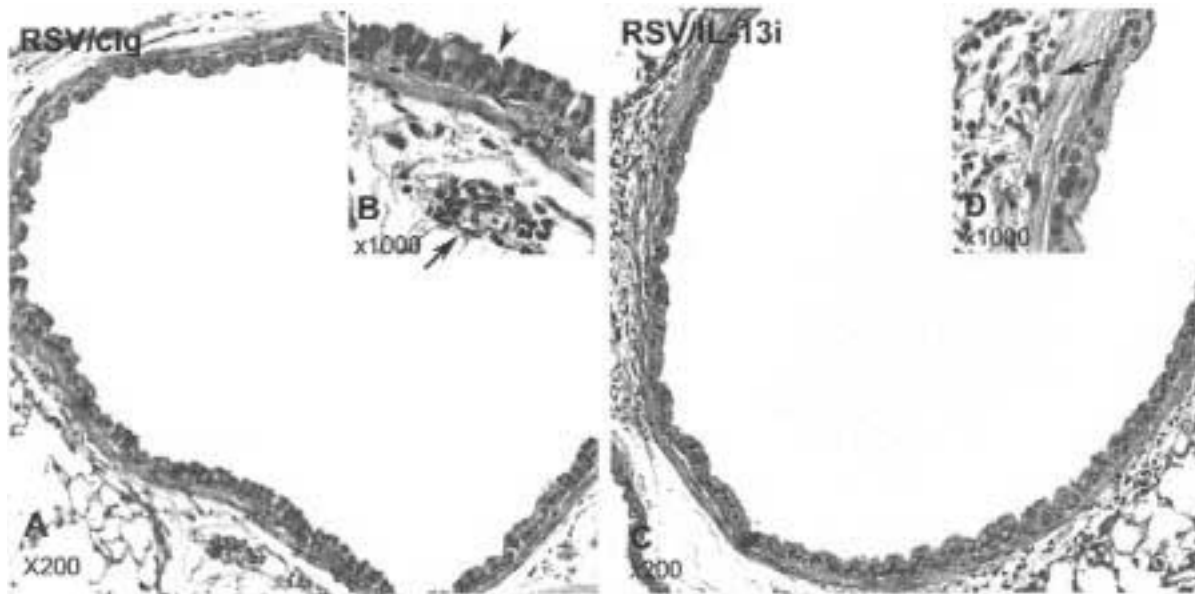
BAL cytokines (pg/mL)	Challenge/sham	Challenge/RSV	OVA/cIg	OVA/IL-13i	OVA/RSV/cIg	OVA/RSV/IL-13i
IL-4	2.1 $\pm$ 1.4	4.3 $\pm$ 1.7	1.1 $\pm$ 1.1	3.2 $\pm$ 2.1	0.9 $\pm$ 2.5	5.6 $\pm$ 2.2
IL-5	95.9 $\pm$ 8.1	127.3 $\pm$ 21.7	96.9 $\pm$ 8.0	83.7 $\pm$ 6.8	79.1 $\pm$ 5.4	89.6 $\pm$ 18.4
IL-13	0.6 $\pm$ 0.6	5.0 $\pm$ 2.0	58.5 $\pm$ 12.2	69 $\pm$ 16.1	51.2 $\pm$ 9.8	93.2 $\pm$ 21.4
IL-12	156.7 $\pm$ 55.0	85.3 $\pm$ 29	0*	67.8 $\pm$ 22.3†	17.5 $\pm$ 11.5*	63.1 $\pm$ 33.9
IFN-γ	440 $\pm$ 47	1959 $\pm$ 484†	132 $\pm$ 19	213 $\pm$ 27†	1054 $\pm$ 294†	1225 $\pm$ 250†

Mice are from the same groups described in Fig 9.

BAL, Bronchoalveolar lavage; OVA, ovalbumin; RSV, respiratory syncytial virus.

*Decreased compared with sham group.

†Increased compared with sham and OVA groups ($P < .05$).



Asthma, rhinitis, other
respiratory diseases

levels induced by T_H1 cells were about 100 times lower than those induced by T_H2 cells.¹⁹ It has generally been thought that IL-13 is primarily produced by T_H2 cells, but many cell types, including mast cells,¹⁵ macrophages,³⁵ CD8, and T_H1 cells,^{17,18} prominent cells in RSV pathology, can produce IL-13.

Although IL-13 can aggravate eosinophilic inflammation in allergen-induced models,¹⁰⁻¹⁴ it also might exhibit important anti-inflammatory properties. IL-13 can induce the expression of the IL-1R antagonist³⁸ and downregulate the production of proinflammatory cytokines and IL-12 in monocytes.^{39,40} IL-13 also has been reported to be an effective anti-inflammatory agent in experimental autoimmune encephalitis,^{41,42} LPS-induced lethal endotoxemia,⁴⁰ and collagen-induced arthritis models.⁴³ In this study, IL-12 levels were increased in IL-13i-treated mice, and BAL lymphomonocytosis induced by RSV infection was higher in IL-13-deficient mice, although there were no differences in BAL lymphomonocytosis between RSV alone and RSV/IL-13i-treated BALB/c mice. We showed that inhibition of IL-13 after RSV infection can prevent mucus production, but we cannot exclude the possibility of some enhancement of inflammation after IL-13i treatment.

Our results seem to contradict the reports of Tekkanat et al,^{20,21,44} which emphasized the role of IL-13 not only in epithelial cell mucus production but also in the development of AHR after RSV infection. They showed that inhibition of IL-13 could enhance IL-12 production and thus prevent the development of AHR in RSV-infected mice. Our study also showed that inhibition of IL-13 can enhance IL-12 secretion after RSV infection, but AHR was not attenuated. In OVA-sensitized and OVA-challenged mice that were administered IL-13i, a similar enhancement of IL-12 can be demonstrated.¹⁴ These results indicate the presence of an IL-13/IL-12 axis; however, on the basis of our findings, it did not seem that this pathway was critical for the regulation of AHR after RSV infection.

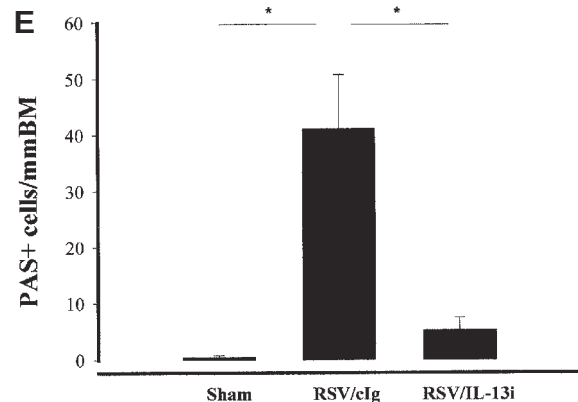


FIG 5. Lung histologic features in respiratory syncytial virus (RSV)-infected mice. Lung tissues were stained with periodic acid-Schiff (PAS). RSV causes peribronchovascular inflammation (long arrow) and epithelial mucus production (short arrow) (A and B). After IL-13i administration, bronchial epithelial cell mucus production was markedly decreased, but the peribronchovascular inflammation was not changed (C and D). E, Quantitative analysis of PAS-positive cells in the central airways. Data are from the same mice shown in Fig 1. Results are expressed as the number of PAS-positive cells per millimeter of epithelial basement membrane. * $P < .01$.

Several additional differences were found between our 2 studies. In their studies, the peaks of IL-13 and AHR were on day 10 to 12 after infection; however, in our system, these were on day 6 after infection, with a decrease by days 10 to 12. Furthermore, the concentrations of IL-13 in their study were about 30 to 40 times higher than we detected. They inoculated one-third the amount of RSV through a surgical tracheostomy compared with inoculation of 10^6 pfu RSV by the intranasal route. To inhibit IL-13, we injected soluble high-affinity decoy IL-13 receptor (IL-13R α 2)-human IgG fusion protein (IL-13i), which has been shown to target only IL-13.^{10,11,24} Tekkanat et al used a rabbit polyclonal anti-IL-13 serum.

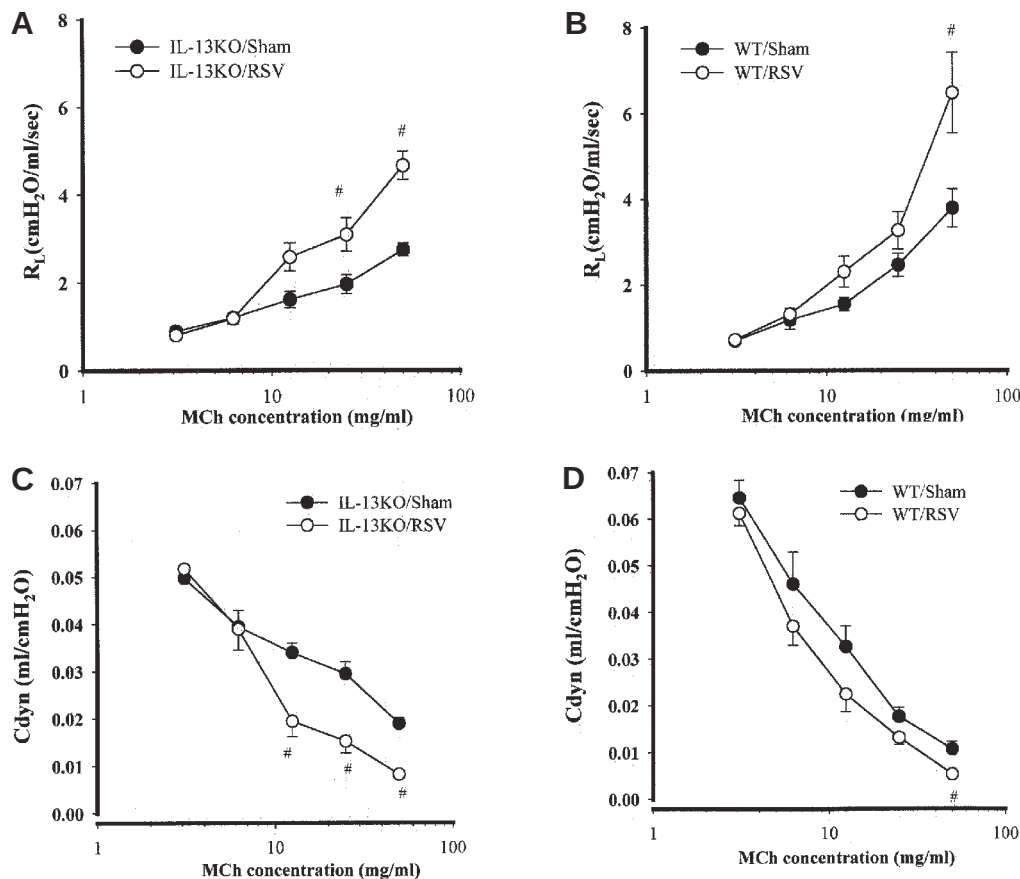


FIG 6. IL-13-deficient mice (A and C) and WT mice (B and D) develop airway hyperresponsiveness (AHR) after respiratory syncytial virus (RSV) infection. AHR in response to inhaled methacholine is expressed as changes in airway resistance (A and B) or dynamic compliance (C and D). Each group consisted of 8 mice. # $P < .05$ between sham-infected and RSV-infected mice.

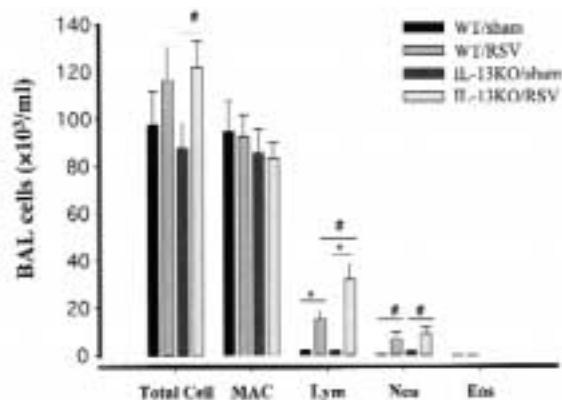


FIG 7. Bronchoalveolar lavage fluid lymphocytosis and neutrophilia after respiratory syncytial virus infection in IL-13 KO and WT mice. Data are from the same mice as used in Fig 6. * $P < .01$ compared with sham-infected mice, # $P < .05$ compared with sham-infected mice.

The potency of the IL-13i has been demonstrated in several studies of allergen-induced AHR,^{10,11,14} and in this study clearly abolished goblet cell/mucus changes induced by RSV. Finally, the results were confirmed in

IL-13-deficient mice, which showed no impairment of AHR development after RSV infection, in the absence of induction of goblet cell hyperplasia or mucus production.

As reported previously in different models,²⁹⁻³¹ administration of IL-13i did not significantly alter the levels of IL-13 in BAL fluid or lung homogenates, but the inhibition of IL-13 activity by the inhibitor was validated in the functional assays. Although the IL-13i was ineffective in preventing development of AHR after RSV infection, its efficacy was validated by preventing any increases in goblet cell numbers and by attenuating the increases in airway responsiveness after infection of allergen-sensitized and allergen-challenged mice. After infection of sensitized and challenged mice, RSV reduced the degree of lung eosinophilia after sensitization and challenge, a finding consistent with reports by others. Waris et al⁴⁵ reported that priming with RSV infection could reduce T_H2-like responses associated with formalin-inactivated RSV immunization. Because IFN- γ was increased in RSV-infected, OVA-sensitized, and OVA-challenged mice, this cytokine might decrease the allergen-induced eosinophilia.³⁷ Durbin et al⁴⁶ recently reported that in STAT1 KO mice, primary RSV

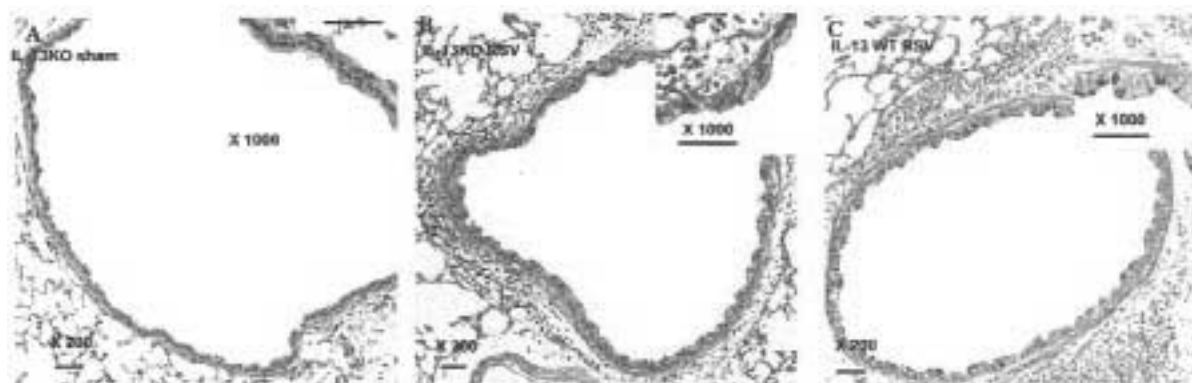


FIG 8. Lung histologic features of respiratory syncytial virus (RSV)-infected, IL-13-deficient mice. Lung tissues were stained with periodic acid-Schiff (PAS). **A**, Sham-infected IL-13-deficient mice. **B**, RSV causes peribronchial and perivascular inflammation but not epithelial mucus production in IL-13-deficient mice. **C**, Peribronchial and perivascular inflammation and epithelial mucus production in RSV-infected WT mice. Insets show PAS staining; original magnification, $\times 1000$.

infection can induce lung eosinophilia, and they suggested that IFN- α/β and IFN- γ might have a protective role in development of lung eosinophilia in this model. Cytomegalovirus infection before allergen challenge also attenuated the development of AHR and eosinophilia.³⁴ Whereas the role of IFN- γ in the regulation of T_H2 immune responses in allergen-triggered models has been well proved, its role in virus-induced AHR is more questionable.^{8,33,47} Of interest, although RSV infection decreased BAL eosinophilia, AHR was nonetheless enhanced by RSV infection in sensitized and challenged animals. These results are consistent with those of Peebles et al,²³ who reported that AHR could be aggravated if the mice were infected with RSV after OVA sensitization and challenge. However, if the mice were infected before OVA inhalation, RSV attenuated the development of AHR and allergic inflammation, a finding contradicted by our own studies.^{3,5} In this study we measured AHR on day 6 after infection, which was 8 days after the last of 3 OVA challenges. The peak level of AHR in OVA-sensitized and OVA-challenged mice is usually 24 to 48 hours after the challenge³² and that after RSV infection is on day 6 after infection. The pattern and extent of AHR might therefore be different, depending on the time points chosen for AHR measurements; we speculate that at this specific time point (8 days after challenge and 6 days after infection), the level of AHR detected best reflects mechanisms regulated by RSV infection.

Our results indicate that despite some overlap, the mechanisms leading to AHR induced by RSV seem to be different from those that follow allergen sensitization and challenge. Although T_H1 immune responses (IL-12, IFN- γ) induced by RSV infection are capable of attenuating allergic inflammation, RSV infection still contributes in an additive or synergistic manner to the development of AHR triggered by allergen sensitization and challenge. IL-13 inhibition might have little effect on the prevention of AHR after RSV infection alone; however, it is effective in preventing the production of mucus after RSV infection and can also prevent the increases in AHR in sensitized

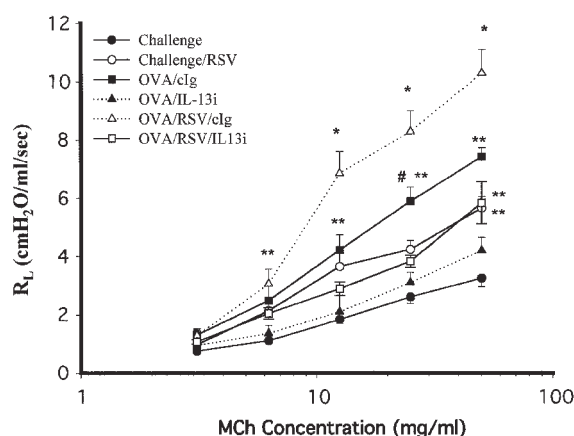


FIG 9. Airway hyperresponsiveness (AHR) was increased after respiratory syncytial virus (RSV) infection in ovalbumin (OVA)-sensitized and challenged mice. Mice were sensitized on days 0 and 14 and received aerosolized OVA challenges on days 26, 27, and 28 (OVA). A group of these mice received IL-13i treatment before the challenges (OVA/IL-13i). Some mice received OVA challenge alone (challenge). On day 30, 2 days after completion of the challenges, mice were inoculated with RSV (or sham-infected). AHR was monitored 6 days later (day 36). IL-13i or control Ig was injected daily on days 26 to 29 and every other day thereafter. Each group consisted of 8 mice. * $P < .01$ compared with all the other groups, # $P < .05$ compared with OVA/RSV/IL-13i, ** $P < .05$ compared with sham-infected mice.

and challenged mice infected with RSV. In this way, IL-13i could have an important role in preventing the consequences of viral infection in patients with allergic asthma.

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