IL-5 and Eosinophils Are Essential for the Development of Airway Hyperresponsiveness Following Acute Respiratory Syncytial Virus Infection

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IL-5 and Eosinophils Are Essential for the Development of Airway Hyperresponsiveness Following Acute Respiratory Syncytial Virus Infection1

Jürgen Schwarze,* Grzegorz Cieslewicz,* Eckard Hamelmann,* Anthony Joetham,* Leonard D. Shultz,† Marinus C. Lamers,‡ and Erwin W. Gelfand2*

Viral respiratory infections can cause bronchial hyperresponsiveness and exacerbate asthma. In mice, respiratory syncytial virus (RSV) infection, which induces an immune response dominated by IFN-γ, results in airway hyperresponsiveness (AHR) and eosinophil influx into the airways, both of which are prevented by pretreatment with anti-IL-5 Ab. To delineate the role of IL-5, IL-4, and IFN-γ in the development of RSV-induced AHR and lung eosinophilia, we tested the ability of mice deficient in each of these cytokines to develop these symptoms of RSV infection. Mice deficient in either IL-5, IL-4, or IFN-γ were administered infectious RSV intranasally, and 6 days later, airway responsiveness to inhaled methacholine was assessed by barometric body plethysmography, and numbers of lung eosinophils and production of IFN-γ, IL-4, and IL-5 by mononuclear cells from peribronchial lymph nodes were monitored. RSV infection resulted in airway eosinophilia and AHR in both IL-4- and IFN-γ-deficient mice, but not in IL-5-deficient mice. Reconstitution of IL-5-deficient mice with IL-5 restored these responses and enhanced the responses in IL-4-deficient mice. Anti-VLA-4 (very late Ag-4) treatment prevented lung eosinophilia and AHR following RSV infection and IL-5 reconstitution. We conclude that in response to RSV, IL-5 is essential for the influx of eosinophils into the lung and that eosinophils in turn are critical for the development of AHR. IFN-γ and IL-4 are not essential for these responses to RSV infection. The Journal of Immunology, 1999, 162: 2997–3004.

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2Address correspondence and reprint requests to Dr. Erwin W. Gelfand, Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO 80206; †The Jackson Laboratory, Bar Harbor, ME 04609; and *Max-Planck-Institut für Immunbiologie, Freiburg, Germany.

Viral respiratory tract infections can increase bronchial reactivity in normal subjects and exacerbate preexisting asthma (1, 2). Respiratory viruses are among the important triggers for acute asthma symptoms (3). Respiratory syncytial virus (RSV),3 the most common respiratory virus in the age group of less than 2 yr, is among the respiratory viruses implicated in asthma exacerbation in children (4). The immunologic and inflammatory cell requirements for the development of bronchial hyperresponsiveness triggered by viral respiratory infections are not well defined. Acute RSV infection in humans has been associated with influx of neutrophils (5) and eosinophils (6, 7) to the respiratory tract. To investigate the cellular mechanisms linking viral respiratory infections to the development of airway hyperresponsiveness (AHR), we employed a murine model of acute RSV infection that allows the investigation of airway responsiveness, the assessment of pulmonary inflammation, and the study of local cytokine production in the draining lymph nodes of the airways (8). We reported recently in this model using BALB/c mice that acute RSV infection results in eosinophil and neutrophil influx into the lung and in AHR to inhaled methacholine (MCh). In this model, we showed that treatment with anti-IL-5 Ab prevented both the influx of eosinophils and the development of AHR. To further delineate the role of eosinophils and of the cytokines IL-5, IL-4, and IFN-γ in the development of virus-induced AHR, we assessed in the present study the ability of mice deficient in IL-5, IL-4, or IFN-γ to develop inflammatory airway responses and AHR to MCh following RSV infection. In addition, to define the role of airway eosinophils in the development of AHR, we utilized a mAb to the α4 integrin subunit of the very late Ag (VLA)-4. VLA-4 is expressed on eosinophils and other circulating leukocytes, except neutrophils, and binds to VCAM-1 on endothelial cells (9, 10). In vitro studies demonstrated that VLA-4 interaction with VCAM-1 results in adhesion to endothelial and transendothelial migration (9, 11). Anti-VLA-4 Ab has previously been shown to inhibit eosinophil recruitment to the lung in murine models of allergic airway inflammation (12, 13).

Materials and Methods

Animals

Female IL-5-sufficient or IL-5-deficient C57BL/6 mice from our colony (initially derived at Max-Planck-Institut for Immunology, Freiburg, Germany), IL-4-sufficient and IL-4-deficient, and IFN-γ-sufficient and IFN-γ-deficient BALB/c mice (The Jackson Laboratory, Bar Harbor, ME), all 8 to 16 wk of age and free of specific pathogens, were used in this study. All 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

Human RSV, group A (Long strain), free of chlamydia or mycoplasma contamination, was obtained from the Viral Diagnostics Laboratory, Health Sciences Center, University of Colorado (Denver, CO). The virus was cultured on HEP 2 cells from American Type Culture Collection

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1Division of Basic Sciences, Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO 80206; †The Jackson Laboratory, Bar Harbor, ME 04609; and *Max-Planck-Institut für Immunbiologie, Freiburg, Germany.

2Address correspondence and reprint requests to Dr. Erwin W. Gelfand, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206. E-mail address: gelfande@njc.org

3Abbreviations used in this paper: RSV, respiratory syncytial virus; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; MCh, methacholine; PBLN, peribronchial lymph nodes; PFU, plaque-forming unit; VLA, very late antigen.
(ATCC, Manassas, VA) in medium containing FCS from Life Technologies (Grand Island, NY). It was purified as described (14). Briefly, cells and supernatant were harvested, the cells were disrupted by ultrasonic manipulation, and the suspension was clarified by centrifugation (8000 × g, 20 min). The supernatant was layered over 30% sucrose in STEU buffer (0.1 M sodium chloride, 0.01 M Tris, 0.001 M EDTA, and 1 M urea, all obtained from Sigma). The suspension was centrifuged (100,000 × g, 1 h, 10°C). The pellet was resuspended in 1.2 ml PBS, aliquoted, and frozen at −70°C. The suspension was adjusted to contain 4 × 10^6 PFU of RVSC/ml, as assayed by quantitative plaque-forming assay.

**Infection of mice**

Mice were infected under light anesthesia (2.5% Avertin, 0.015 ml/g body weight) by intra-nasal inoculation of RVSC (10^5 PFU in 50 µl PBS). Controls were sham infected with PBS in the same way. Efficacy of this infection procedure was regularly tested by qualitative plaque-forming assays (15); briefly, on day 4 postinfection, mice were sacrificed; the lungs were removed, homogenized, and centrifuged; and the supernatant was added to Hep 2 cell cultures. Infection could be demonstrated by cell pathogenic effects in all infected animals tested, but not in mice sham infected with PBS.

**Experimental protocols**

Mice were infected on day 0. Airway responsiveness to MCh was assayed on day 6 postinfection and animals were sacrificed the following day for the removal of bronchial lymph nodes (PBLN) and lungs. In a separate set of experiments, IL-5-deficient and IL-4−/− mice were reconstituted with 40 ng of murine IL-5 (kindly provided by Dr. James Lee, Mayo Clinic, Rochester, MN) given i.v. on days 0, 2, and 4 after RVSC inoculation, or following sham infection. Additionally, on days 2 and 4 postinfection, some RVSC-infected and IL-5-reconstituted IL-5-deficient mice were treated i.v. with 100 µg of anti-IFN-γ (α chain) Ab (rat IgG2b) isolated from supernatants of PS/2 cells obtained from ATCC or with rat IgG2b as a control.

**Determination of airway responsiveness**

Airway responsiveness was assessed using a single chamber whole body plethysmograph obtained from Buxco (Troy, NY), as described (16). In this system, a spontaneously breathing mouse is placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber are recorded. The resulting box pressure signal is caused by volume and resultant pressure changes during the respiratory cycle of the animal. From these box pressure signals the phases of the respiratory cycle, tidal volumes, and enhanced pause (Penh) can be calculated. Penh is a dimensionless value that represents the function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated animals (16) and was used as a measure of airway responsiveness in this study. In the plethysmograph, mice were exposed for 3 min to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (Sigma) in PBS using an Aerasonic ultrasonic nebulizer (DeVilbiss, Somerset, PA). After each nebulization, recordings were taken for 3 min. The Penh values measured during each 3-min sequence were averaged and are expressed for each MCh concentration as the percentage of baseline Penh values following PBS exposure.

**Lung and bone marrow cell isolation**

Lung cells were isolated by collagenase digestion, as previously described (17), and counted with a hemocytometer. Bone marrow from the right femur was collected by saline irrigation. Cells from either preparation were resuspended in RPMI 1640 medium (Life Technologies, Gaithersburg, MD).

**In vitro cytokine production**

Mononuclear cells were cultured for 48 h in 96-well round-bottom plates at a concentration of 400,000 cells/well in the absence or presence of UV-inactivated RSV (equivalent of 10^6 PFU/well) or the combination of ionomycin (0.5 µM) from Calbiochem (La Jolla, CA) and phorbol 12, 13-dibutyrate (10 ng/ml; Sigma). Supernatants were harvested and frozen at −20°C. The concentrations of IFN-γ, IL-4, and IL-5 in the supernatants were assessed by ELISA, as described (18). Briefly, Immulon-2 plates from Dynatech Laboratories (Chantilly, VA) were coated with anti-IFN-γ (RA-6A2; PharMingen, San Diego, CA), anti-IL-4 (11B11; PharMingen), or anti-IL-5 Abs (TRFK-5; Dr. R. Coffman) and blocked with PBS/10% FCS overnight. Samples were added; biotinylated anti-IFN-γ (XMG 1.2; PharMingen), anti-IL-4 (BVD6-24G2; PharMingen), or anti-IL-5 Abs (TRFK-4; PharMingen) were used as detecting Abs; and the reactions were amplified with avidin-horseradish-peroxidase (Sigma). Cytokine levels were calculated by comparison with known cytokine standards (PharMingen). The limit of detection in the assay was 4 pg/ml for each cytokine.

**Results**

**Acute RSV infection does not result in increased airway responsiveness to MCh in IL-5-deficient mice**

IL-5-deficient or IL-5-sufficient mice were infected by intranasal installation of RSV (10^5 PFU). The airway response to MCh in mice infected with RSV and in sham-infected controls was assessed by barometric whole body plethysmography on day 6 after RSV inoculation. The airways of IL-5-sufficient mice infected with RSV were significantly more reactive than the airways of sham-infected controls (Fig. 1A). Penh to 100 mg/ml MCh increased 4.2 ± 0.6-fold over PBS in infected mice compared with a 2.2 ± 0.2-fold increase in mice sham infected with PBS. In contrast, in IL-5-deficient mice, acute RSV infection did not result in increased airway responsiveness to MCh.

**Acute RSV infection does not result in influx of pulmonary eosinophils to the lung in IL-5-deficient mice**

To investigate changes in numbers of pulmonary inflammatory cells during acute RSV infection, lung cells were isolated and differential cell counts were performed. In IL-5-sufficient mice acutely infected with RSV, the numbers of eosinophils and neutrophils were 4.5- and 1.6-fold, respectively. In IL-5-deficient mice, RSV infection did not result in increased numbers of neutrophils in the lung.

**Acute RSV infection causes an increase in IFN-γ, but not in IL-5 production in IL-5-deficient mice**

We measured the production of IFN-γ, IL-4, and IL-5 in 48-h cultures of mononuclear cells obtained from PBLN, harvested on day 7 postinfection, and stimulated with UV-inactivated RSV or phorbol dibutyrate/ionomycin. In IL-5-sufficient mice, production of IFN-γ and IL-5 was significantly increased following RSV infection (Table I). In IL-5-deficient mice on the other hand, no IL-5 could be detected, but RSV infection still resulted in increased IFN-γ production. Indeed, in IL-5-deficient mice, IFN-γ levels were higher than in IL-5-sufficient mice. Only very low levels of IL-4 were detected in any of the groups.
Reconstitution of IL-5-deficient mice with IL-5 during RSV infection restores eosinophil influx and development of AHR

IL-5-deficient mice infected with RSV or sham infected were reconstituted with IL-5. In sham-infected mice, this treatment resulted in increases in numbers of eosinophils in the bone marrow (from 0.14 ± 0.10 to 0.45 ± 0.14 × 10⁶ eosinophils/femur, p < 0.05, both n = 6), but not in the lung, and airway responsiveness to MCh was not increased. In contrast, in RSV-infected IL-5-deficient mice, reconstitution with IL-5 not only resulted in bone marrow eosinophilia (from 0.16 ± 0.11 to 0.66 ± 0.16 × 10⁶ eosinophils/femur, p < 0.05, both n = 6), but also in eosinophil influx to the lung (Fig. 2b) and in the development of AHR to MCh (Fig. 2a). The Penh to 100 mg/ml MCh increased 5.8 ± 2.7-fold over PBS in this group of animals.

Anti-VLA-4 Ab treatment prevents eosinophil influx and development of AHR following reconstitution with IL-5

RSV-infected IL-5-deficient mice were reconstituted with IL-5 and at the same time treated with anti-VLA-4 Ab or rat IgG2b as a control. Treatment with anti-VLA-4, but not with rat IgG, prevented increases in numbers of lung eosinophils (Fig. 2b) and the development of AHR to MCh observed in RSV-infected IL-5-deficient mice following IL-5 reconstitution (Fig. 2c).

RSV infection in IFN-γ-deficient mice results in increased IL-5 production, eosinophil influx into the lung, and AHR to MCh

IFN-γ-deficient and IFN-γ-sufficient BALB/c mice were infected intranasally with RSV. On day 6 of the acute infection, airway responsiveness to MCh was assessed, and on day 7 lung inflammatory cells were enumerated. RSV infection in both IFN-γ-deficient and IFN-γ-sufficient mice resulted in AHR to MCh (Fig. 3a) and in increases in numbers of lung eosinophils and neutrophils (Fig. 3b). Compared with IFN-γ-sufficient mice, the dose-response curve to MCh was shifted to the left, and the influx of eosinophils into the lung was greater in IFN-γ-deficient mice following RSV infection. Furthermore, RSV infection resulted in increases in IL-5 and IL-4 production in PBLN cell cultures of IFN-γ-deficient mice (Table II). In contrast, in IFN-γ-sufficient BALB/c mice, decreases in IL-4 and IL-5 production were observed following RSV infection (IL-4, from 71 ± 24.7 to 10.8 ± 1.6 pg/ml; IL-5, from 1996.3 ± 536.7 to 686 ± 322.8 pg/ml; both p < 0.05, n = 12).

AHR to MCh and lung eosinophilia following RSV infection in IL-4-deficient mice are enhanced by IL-5 treatment

IL-4-deficient and IL-4-sufficient BALB/c mice were infected intranasally with RSV, and some IL-4-deficient mice were treated with IL-5 during the infection. Airway responsiveness to MCh was assessed on day 6 postinfection, and on day 7 lung inflammatory cells were counted. RSV infection resulted in AHR to MCh (Fig. 4a) and in increases in numbers of lung eosinophils and neutrophils (Fig. 4b) in both IL-4-deficient and IL-4-sufficient mice. In IL-4-deficient mice, the numbers of eosinophils and neutrophils...
FIGURE 2. A. Reconstitution with IL-5 in IL-5-deficient mice results in AHR following RSV infection (R). IL-5-deficient mice were infected with RSV or sham infected (C) with PBS and treated i.v. with IL-5 (IL-5+/C + IL-5, n = 8; IL-5−/− C + IL-5, n = 8) or with PBS as a control (IL-5−/− R, n = 8; IL-5−/− C, n = 8). On day 6 postinfection, airway responsiveness was assessed. Means ± SEM of Penh values from two independent experiments are illustrated. Significant differences, *p < 0.05, IL-5+/− R + IL-5 vs all other groups. B. Anti-VLA-4 treatment of IL-5-deficient mice reconstituted with IL-5 prevents RSV-induced lung eosinophilia. Lung cells were isolated from RSV-infected (IL-5−/− R, n = 8) or noninfected (IL-5+/− C, n = 8) IL-5-deficient mice following IL-5 reconstitution (IL-5−/− C + IL-5, IL-5−/− R + IL-5, both n = 8) and simultaneous treatment with anti-VLA-4 or rat IgG2b as a control (IL-5−/− R + IL-5 + anti-VLA-4, IL-5−/− R + IL-5 + rat IgG2b, both n = 8). Illustrated are means ± SD of lung eosinophil numbers from two independent experiments. Significant differences, p < 0.05; *IL-5−/− C + IL-5 vs IL-5−/− C, and IL-5−/− C + IL-5; †IL-5−/− R + IL-5 + anti-VLA-4 vs IL-5−/− R + IL-5 + rat IgG2b and IL-5−/− R + IL-5. C. Anti-VLA-4 treatment of IL-5-deficient mice reconstituted with IL-5 prevents development of RSV-induced AHR. IL-5-deficient RSV-infected mice reconstituted with IL-5 were treated with anti-VLA-4 (IL-5−/− R + IL-5 + anti-VLA-4, n = 8) or rat IgG2b as a control (IL-5−/− R + IL-5 + rat IgG2b, n = 8). On day 6 postinfection, airway responsiveness was assessed. Means ± SEM of Penh values from two independent experiments are illustrated. Significant differences, *p < 0.05; *IL-5−/− R + IL-5 + rat IgG2b vs IL-5−/− R + IL-5 + anti-VLA-4.

FIGURE 3. A. RSV infection (R) results in AHR in IFN-γ-deficient mice. IFN-γ-deficient or IFN-γ-sufficient mice were infected with RSV (IFN-γ−/− R, n = 8; IFN-γ+/− R, n = 8) or sham infected (C) with PBS (IFN-γ−/− C, n = 8; IFN-γ+/− C, n = 8). On day 6 postinfection, airway responsiveness to increasing concentrations of inhaled MCh (3–50 mg/ml) was assessed. Means ± SEM of Penh values from two independent experiments are illustrated. Significant differences, p < 0.05; *, IFN-γ−/− R vs IFN-γ+/− C; †, IFN-γ−/− R vs IFN-γ−/− C, IFN-γ−/− R vs IFN-γ+/− R. B. RSV infection results in eosinophil and neutrophil influx into the lung in IFN-γ-deficient mice. Lung cells were isolated from the same IFN-γ-deficient or IFN-γ-sufficient mice 7 days after RSV infection (IFN-γ−/− R, n = 8; IFN-γ−/− R, n = 8) or sham infection (IFN-γ−/− C, n = 8; IFN-γ+/− C, n = 8). Numbers of eosinophils and neutrophils per lung were determined. Illustrated are means ± SD of the numbers of these cells from two independent experiments. Significant differences, p < 0.05; †, IFN-γ−/− R vs IFN-γ+/− C; *, IFN-γ−/− R vs IFN-γ+/− C, IFN-γ−/− R vs IFN-γ+/− R.
augmented airway responsiveness to MCh and further increased the influx of eosinophils (but not neutrophils) into the lung in response to RSV (Fig. 4, A and B).

Discussion

In the present study, we monitored airway responsiveness, pulmonary inflammation, and local cytokine production in a murine model of RSV infection. We recently reported that RSV infection results in AHR and eosinophil influx into the lung in this model. In this study, we utilized this approach to address two specific issues: 1) whether development of virus-induced AHR is IL-5-, IL-4-, or IFN-γ-dependent, and 2) whether AHR is dependent on the influx of eosinophils to the airways under these conditions. To address these issues, we compared the responses to RSV infection in IL-5-, IL-4-, and IFN-γ-deficient mice. Airway responsiveness to aerosolized MCh was assessed using barometric whole body plethysmography in unrestrained animals, and pulmonary inflammation and cytokine production in the local draining lymph nodes of the lung, the PBLN, were monitored.

Acute RSV infection in IL-5-sufficient C57BL/6 mice resulted in significant increases in airway responsiveness to MCh, and this was associated with the infiltration of both eosinophils and neutrophils in the lung. These findings parallel those seen in BALB/c mice (8, 19) and in other models of respiratory tract viral infection (20). As has been reported previously (21) and in our studies of allergic sensitization of the airways, altered airway responsiveness to MCh develops to a lower degree and at higher concentrations of the bronchoconstrictor in C57BL/6 mice compared with BALB/c mice. This was also seen following RSV infection (Figs. 1, 3, and 4). Assessment of cytokine production by mononuclear cells from the PBLN during acute RSV infection in IL-5-sufficient C57BL/6 mice demonstrated that both IFN-γ and IL-5 production were increased. The increases in IFN-γ production during acute RSV infection are in keeping with observations made earlier that showed increased production of total IFN in tracheobronchial lavages from RSV-infected mice (22), and with our own observations in BALB/c mice that exhibited an increase in IFN-γ following RSV infection (8). In BALB/c mice, IL-5 production was decreased compared with noninfected controls, but RSV-infected BALB/c mice still produced substantial amounts of IL-5 (8). C57BL/6 mice are less prone to develop Th2 responses than are BALB/c mice (23, 24). This may explain the lower levels of IL-4 detected in noninfected and RSV-infected mice of this strain. In contrast to the sufficient mice, IL-5-deficient mice did not develop pulmonary eosinophilia or AHR following RSV infection. This was not due to a lack of infection since RSV infection in the lungs could be demonstrated by plaque-forming assay (data not shown) and resulted in an influx of neutrophils into the lung and increased production of IFN-γ in these animals. Indeed, the number of neutrophils and the levels of IFN-γ were higher in IL-5-deficient than in IL-5-sufficient mice. These observations extend our previous findings that anti-IL-5 Ab treatment reduces influx of eosinophils, but not of neutrophils to the lung, and prevents the development of AHR (8). These data suggest that the lack of IL-5 is the critical element

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</table>

a PBLN were harvested on day 7 postinfection from IL-4-deficient and IFN-γ-deficient mice and their cytokine-sufficient controls infected with RSV (IL-4+/+ R, IL-4−/− R, IFN-γ+/+ R, IFN-γ−/− R, IL-4+/+ C, IFN-γ+/+ C, IFN-γ−/− C, n = 8 for each group) or sham-infected with PBS (IL-4+/+ C, IL-4−/− C, IFN-γ+/+ C, IFN-γ−/− C, n = 8 for each group). Mononuclear cells were isolated and cultured in the absence or presence of UV-inactivated RSV or P/I for 48 h. Concentrations (pg/ml) of IFN-γ, IL-4, and IL-5 were determined in culture supernatants by ELISA. Illustrated are means ± SD of cytokine concentrations from two independent experiments. Significant differences, p < 0.05, IL-4+/+ R vs. IL-4−/− R or IL-4−/− R vs. IFN-γ−/− R vs IFN-γ+/+ R or IFN-γ−/− R vs. IFN-γ+/+ R, ± IFN-γ+/+ C, ± IFN-γ−/− C, ± IFN-γ+/+ R vs. IFN-γ−/− R.
that prevents eosinophilic airway inflammation and the development of AHR during RSV infection in these deficient mice. If so, then administration of IL-5 should render IL-5-deficient mice susceptible to the effects of RSV infection, and this was the case. Reconstitution of IL-5-deficient mice with IL-5 during RSV infection resulted in increases in numbers of eosinophils in the bone marrow, eosinophil influx into the lung, and the development of AHR. These consequences of IL-5 treatment were all dependent on RSV infection, except for the bone marrow eosinophilia that also occurred in sham-infected, IL-5-deficient mice reconstituted with IL-5. These data demonstrate that IL-5 is essential for the influx of eosinophils and for the development of AHR in response to RSV infection and, in turn, the absence of IL-5 appeared protective against the development of AHR. To delineate whether IL-5 itself is directly responsible for the development of increased airway responsiveness or whether the eosinophils that are recruited to the lung during RSV infection in the presence of IL-5 are themselves necessary for the development of AHR, we treated RSV-infected, IL-5-reconstituted, IL-5-deficient mice with anti-VLA-4 Ab. The adhesion molecule VLA-4, expressed on eosinophils and other circulating lymphocytes except neutrophils (9), is known to be important in the recruitment of mononuclear cells and eosinophils from the circulation to sites of inflammation (11–13). In the present study, anti-VLA-4 treatment prevented the influx of eosinophils into the lung and the development of AHR in infected and reconstituted IL-5-deficient mice. This indicates that eosinophil recruitment to the lung is essential for the development of RSV-induced AHR. One could argue that the inhibition of AHR by anti-VLA-4 treatment might also be due to impaired recruitment of lymphocytes that may secrete IL-5 or result in AHR by some other mechanism, e.g., IL-11 secretion (25). Given that anti-VLA-4 treatment was effective even following administration of IL-5, the lack of IL-5 secretion by lymphocytes cannot be the explanation for the inhibition of AHR. Furthermore, if non-IL-5- or noneosinophil-mediated mechanisms were primarily responsible for the development of RSV-induced AHR, one would have expected to observe AHR following RSV infection in IL-5-deficient mice that are otherwise immunologically competent.

Using genetically deficient BALB/c mice, we also tested whether RSV-induced AHR is dependent on the cytokines IFN-γ or IL-4. The Th1 cytokine IFN-γ is produced in large amounts during acute RSV infection in humans (26) and in mice (8, 21, 27). It may play a pivotal role in virus-induced inflammation (28). Increased amounts of IFN-γ have been observed in cells from bronchoalveolar lavage (BAL) of atopic asthmatics (29, 30). The role of IFN-γ in the development of allergen-induced AHR remains controversial. On one hand, administration of IFN-γ during airway sensitization has been shown to inhibit allergic airway inflammation and AHR (31, 32). On the other hand, in a study employing an Ab against IFN-γ, this cytokine seemed to play a critical role in the development of allergen-induced AHR (33). The role of IFN-γ in virus-induced AHR was investigated in the present study: RSV infection resulted in eosinophil and neutrophil influx into the lung and AHR in IFN-γ-deficient mice. Interestingly, IFN-γ-deficient mice developed AHR at lower MCh concentrations and recruited more eosinophils to the lung than IFN-γ-sufficient controls. Furthermore, IL-4 and IL-5 production was increased following RSV infection in IFN-γ-deficient mice, while others demonstrated a partial inhibition of eosinophil recruitment to the lung, but no inhibition of AHR or histologic damage of the airways in IL-4-deficient mice (41). IL-4 may be involved in the development of allergic asthma. Increased levels of IL-4 in BAL fluid (34) and increased expression of IL-4 mRNA in BAL cells (35) and bronchial biopsies (36) have been demonstrated in patients with atopic asthma. In murine models of allergic airway sensitization, IL-4 has been found to be crucial for the development of allergic airway inflammation (37, 38) and AHR (39, 40) in some studies, while others demonstrated a partial inhibition of eosinophil recruitment to the lung, but no inhibition of AHR or histologic damage of the airways in IL-4-deficient mice (41).
asthma via several mechanisms, some of which may be of importance in RSV-induced AHR as well. IL-4 is necessary for the production of allergen-specific IgE (42, 43), which in turn may be critical for the development of AHR by activation of mast cells (44) and eosinophils (45). RSV-specific IgE may play a role in the development of bronchiolitis and asthma following RSV infection in infants (46). Furthermore, IL-4 is known to be involved in up-regulation of VCAM-1 on vascular endothelial cells (9), facilitating eosinophil and lymphocyte extravasation; IL-4 is also involved in Th2 cell recruitment to sites of allergic inflammation (47). As we show in the present study, increased eosinophil recruitment is essential in RSV-induced AHR, as previously shown for allergen-induced AHR. RSV infection in IL-4-deficient mice resulted in recruitment of eosinophils and neutrophils to the lung and in development of AHR. Although numbers of lung eosinophils did not differ significantly between IL-4-sufficient and IL-4-deficient mice, increases in airway responsiveness were considerably smaller in IL-4-deficient mice. In addition, in IL-4-deficient animals, only very low levels of IL-5 were detected following RSV infection. Treatment of IL-4-deficient mice with IL-5 during infection resulted in a significant increase in airway responsiveness and in eosinophil influx when compared with untreated mice. These data suggest that although IL-4 may not be absolutely necessary for RSV-induced lung eosinophilia and AHR, it does contribute to the overall magnitude of AHR most likely through enhancing Th2 cell differentiation and enhanced IL-5 production.

In summary, we present a murine model of airway inflammation and AHR following acute RSV infection. Utilizing mice deficient in either IL-5, IL-4, or IFN-γ, we show that IL-5 is critical for the development of RSV-induced lung eosinophilia and AHR, and that prevention of eosinophil recruitment to the lungs by anti-IL-4A1 attenuates AHR. The presence of IL-4, while not essential, may enhance AHR in RSV infection by enhancing IL-5 production. In contrast, IFN-γ, the predominant cytokine in acute RSV infection, does not seem to be required for the development of AHR, and in RSV-infected IFN-γ-deficient mice the eosinophil inflammatory response and airway responsiveness to inhaled MCH are enhanced.

References


