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Transfer of the Enhancing Effect of Respiratory Syncytial Virus Infection on Subsequent Allergic Airway Sensitization by T Lymphocytes

Jürgen Schwarze, Mika J. Mäkelä, Grzegorz Cieslewicz, Azzeddine Dakhama, Michael Lahn, Toshihide Ikemura, Anthony Joetham, and Erwin W. Gelfand

In mice, respiratory syncytial virus (RSV) infection enhances allergic airway sensitization, resulting in lung eosinophilia and in airway hyperresponsiveness (AHR). The mechanisms by which RSV contributes to development of asthma and its effects on allergic airway sensitization in mice are not known. We tested whether these consequences of RSV infection can be adoptively transferred by T cells and whether depletion of T cell subsets prevents the effects of RSV infection on subsequent airway sensitization. Mononuclear cells, T lymphocytes, or CD4 or CD8 T cells from peribronchial lymph nodes (PBLN) of RSV-infected mice were transferred into naive BALB/c mice which were then exposed to OVA via the airways. Additionally, RSV-infected mice were depleted of CD4 or CD8 T cells following acute RSV infection but prior to airway sensitization. Following sensitization, airway responsiveness to inhaled methacholine, numbers of lung eosinophils, and levels of IFN-γ, IL-4, and IL-5 in PBLN cell cultures were monitored. Transfer of T cells from RSV-infected mice resulted in increased eosinophil influx into the lungs, increased IL-5 production, and development of AHR following airway sensitization to allergen. Transfer of CD8 but not CD4 T cells from the PBLN of RSV-infected mice also resulted in AHR following 10 days of OVA exposure. Further, depletion of CD8 T cells prevented these consequences of RSV infection while CD4 T cell depletion reduced them. We conclude that T cells, in particular CD8 T cells, are critical in mediating RSV-induced development of lung eosinophilia and AHR following allergic airway sensitization. The Journal of Immunology, 1999, 163: 5729–5734.

R espiratory syncytial virus (RSV), the most common respiratory virus in children under 2 years (1), has been implicated in the development of childhood asthma (2, 3) and allergic sensitization (4). We have recently reported that RSV infection can enhance the consequences of subsequent airway sensitization to allergen in a murine model resulting in airway hyperresponsiveness (AHR) and influx of eosinophils and neutrophils into the lungs (5). RSV infection exerted its effect on airway sensitization after the resolution of the acute RSV-induced disease. The mechanisms by which RSV infection influences the development of airway inflammation and AHR following airway sensitization have not been determined. In this paper, we use this model to delineate the role of T cells in mediating RSV-induced effects on airway sensitization. In asthmatics, T cells of both CD4 and CD8 subsets are activated and their numbers are increased in the airways compared to nonasthmatic subjects (6–8). Further, increased production of Th1 and Th2-like cytokines has been observed in asthmatics (9–11). In murine models, both CD4 (12) and CD8 T cells (13) have been demonstrated to play an essential role in the development of allergen-induced AHR and airway inflammation, and we have recently shown that CD8 T cells are also critical for virus-induced AHR (14). Considering the role of T cells in the development of airway inflammation and AHR and taking into account that T cells are strongly activated during RSV infection (15), it seems likely that they may be involved in mediating the effects of RSV infection on subsequent airway sensitization to allergen.

To delineate the role of T cells in this model we used two approaches. In one approach, we adoptively transferred T cells from the peribronchial lymph nodes (PBLN), the draining lymph nodes of the lung, from RSV-infected mice into naive animals which were then sensitized to OVA via the airways. In a second approach, we depleted mice of CD4 or CD8 T cells following the acute phase of the RSV infection but prior to airway sensitization. Following airway sensitization to allergen in either experimental approach, airway responsiveness to inhaled methacholine (MCh) was assessed using barometric whole body plethysmography and influx of inflammatory cells into the lungs and cytokine production in PBLN cell cultures was monitored.

Material and Methods

Animals

Female BALB/c mice, 8–12 wk of age, free of specific pathogens, and obtained from The Jackson Laboratory (Bar Harbor, ME), 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

Human respiratory syncytial virus 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 the American Type Culture
Collection (ATCC; Manassas, VA) in medium containing FCS from Life Technologies (Grand Island, NY). The virus was purified as described (16). Briefly, cells and supernatant were harvested, the cells were disrupted by ultrasonic manipulation, and the suspension was clarified by centrifugation (8000 × g for 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 (St. Louis, MO)) and centrifuged 100,000 × g for 1 h at 10°C. The pellet was resuspended in PBS, aliquoted, and frozen at −70°C. The suspension was adjusted to contain 4 × 10^6 PFU of RSV/ml as assessed by quantitative plaque-forming assay.

**Infection of mice**

Mice were infected under light anesthesia (2.5% Avertin (Aldrich, Milwaukee, WI), 0.015 ml/g body weight) by intranasal inoculation of RSV (10^6 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 (17): Briefly, on day 4 and day 8 postinfection, mice were sacrificed; the lungs were removed, homogenized, centrifuged; and the supernatant was added to Hep 2 cell cultures. Infection could be demonstrated by cellular pathogenic effects in all infected animals tested on day 4 but not in mice sham-infected with PBS. On day 8 live virus could not be isolated anymore from the lungs of RSV-infected mice. No difference was detected between RSV-infected mice treated with anti-CD4, anti-CD8 or control Ab.

**Experimental protocols**

Mice were infected with RSV or sham-infected on day 0. Seven or 14 days postinfection, mice were sacrificed. PBLN was harvested, mononuclear cells were (MNC) isolated, and T cells were purified. MNC (10^7/mouse) or T cells (5 × 10^7/mouse) were adoptively transferred into naive mice by i.v. injection. Control T cells were purified (>90% CD3+ T cells) from spleens of naive mice, because only small numbers of T cells, insufficient for transfer, could be isolated from the PBLN of noninfected mice. In additional experiments, CD4+ and CD8+ T cells were isolated from the PBLN of infected mice using magnetic beads 14 days after RSV exposure. These cells were >96% CD4 or CD8, respectively, with no cross contamination. In these experiments, 1.5 × 10^7 cells were transferred. Twenty-four hours after cell transfer, mice were exposed to OVA (Sigma) via the airways for 10 consecutive days using an Aerologic Nebulizer 5000 D from DeVilbiss (Somerset, PA), as described (1%, in 7 ml PBS, 20 min/day) (18). A control group of mice was not exposed to allergen following adoptive cell transfer.

In a separate set of experiments, mice were infected with RSV and depleted of CD4 or CD8 T cells by i.p. injection of anti-CD4 from clone GK 1.5 (200 μg/dose) obtained from ATCC, or from ascites containing anti-CD8 from clone YTS 169 (200 μl/dose), kindly provided by Dr. Terry Potter (National Jewish Medical and Research Center, Denver, CO). Rat IgG was used as a control. The Abs were injected on days 15, 17, and 19 postinfection. From day 21 to day 30 postinfection, mice were exposed to OVA via inhalation as described above. In an attempt to sustain depletion during sensitization, mice were further treated with the respective Abs on days 23, 26, and 29 postinfection using the same doses as prior to sensitization. The extent of CD4 or CD8 T cell depletion was determined in PBLN cells harvested on day 21 postinfection before sensitization and on day 32 following sensitization. MNC were incubated in staining buffer (PBS, 2% FCS, and 0.2% sodium azide) with anti-CD4 (FITC-RM4-4, PharMingen, San Diego, CA) or anti-CD8 (FITC-53-6.7, PharMingen) at 4°C. Stained cells were analyzed using an Epics cytofluorograph (Coulter Electronics, Hialeah, FL). In both experimental approaches, airway responsiveness to inhaled MCh was assessed 48 h after completion of airway sensitization and the following day animals were sacrificed to harvest lungs and PBLN.

**Cell Preparation**

PBLN or spleens were harvested and MNC were purified by passing the tissue through a stainless steel mesh, followed by density gradient centrifugation (Organon Teknika, Durham, NC). Cells were washed three times with PBS and resuspended in RPMI 1640 medium (Life Technologies). For T cell purification, spleens and PBLN were carefully teased apart, RBC were lysed with Grey’s solution, and lysis-resistant cells were passed through nylon wool columns (19). The cell suspension from spleens contained >90%, and from PBLN >95%. CD3+ T cells as determined by FACS analysis following staining with anti-CD3 (FITC-145-2C11, PharMingen).

**In vitro cytokine production**

MNC were cultured for 48 h in 96-well round-bottom plates at a concentration of 400,000 cells/well in the presence or absence of 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 these culture supernatants were assessed by ELISA as described (22). Briefly, Immulon-2 plates from Dynatech (Chantilly, VA) were coated with anti-IFN-γ (R4-6A2, PharMingen), anti-IL-4 (11B11, PharMingen), or anti-IL-5 Abs (TRFK-5, Dr. R. Coffman, Palo Alto, CA) and blocked with PBS and 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-HRP (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.

**Statistical analysis**

Pairs of groups were compared by Student’s t test, comparison of more than two groups was performed by Tukey-Kramer HSD test. The p values for significance were set at 0.05. Values for all measurements are expressed as the mean ± SD except for values of airway responsiveness (Penh) and of cytokine concentrations, which are expressed as the mean ± SEM.

**Results**

**Transfer of MNC or T lymphocytes from PBLN of RSV-infected mice into naive mice prior to airway sensitization results in AHR**

Mice were sensitized to OVA via the airways following RSV infection, following sham-infection, or following adoptive transfer of MNC or T lymphocytes from the PBLN of RSV-infected mice or from noninfected controls. Airway responsiveness to MCh was assessed by barometric whole body plethysmography following airway sensitization, 11 days after cell transfer. As demonstrated previously, airway sensitization following RSV infection resulted in increased airway responsiveness, whereas airway sensitization in the absence of prior RSV infection did not result in an increased response to MCh. Penh in response to 50 mg/ml MCh increased 12.8 ± 2.3-fold in RSV-infected and sensitized mice compared to a 4.8 ± 0.5-fold increase in mice sensitized without prior infection. Adoptive transfer of 10^7 MNC from RSV-infected mice, 14 days postinfection, into naive mice prior to airway sensitization resulted in a 9.2 ± 0.9-fold increase in Penh in response to 50 mg/ml MCh (Fig. 1A). Virtually identical results were obtained following transfer of 5 × 10^6 T cells. In contrast, transfer of MNC or T cells from noninfected mice did not result in increased airway responsiveness to MCh, nor did adoptive transfer of MNC from RSV-infected mice without subsequent allergen exposure (Fig. 1A). Surprisingly, transfer of MNC or T cells from RSV-infected mice obtained only

**Determination of airway responsiveness**

Airway responsiveness was assessed using a single chamber whole body plethysmograph obtained from Buxco (Troy, NY) as described (20). Enhanced pause (Penh) was used as the 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 AeroSonic 5000 D ultrasonic nebulizer (DeVilbiss). 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 (PenhPBS). The PenhPBS values varied very little between groups (0.78 ± 0.22).

**Lung cell isolation**

Lung cells were isolated by collagenase digestion as previously described (21) and counted with a hemocytometer. Cytospin slides were stained with Leukostat from Fisher Diagnostics (Pittsburgh, PA), and differential cell counts were performed in a blinded fashion by counting at least 300 cells under light microscopy.

**In vitro cytokine production**

MNC were depleted of CD4 or CD8 T cells by i.p. injection of anti-CD4 from clone GK 1.5 (200 μg/dose) obtained from ATCC, or from ascites containing anti-CD8 from clone YTS 169 (200 μl/dose), kindly provided by Dr. Terry Potter (National Medical and Research Center, Denver, CO). Rat IgG was used as a control. The Abs were injected on days 15, 17, and 19 postinfection. From day 21 to day 30 postinfection, mice were exposed to OVA by inhalation as described above. In an attempt to sustain depletion during sensitization, mice were further treated with the respective Abs on days 23, 26, and 29 postinfection using the same doses as prior to sensitization. The extent of CD4 or CD8 T cell depletion was determined in PBLN cells harvested on day 21 postinfection before sensitization and on day 32 following sensitization. MNC were incubated in staining buffer (PBS, 2% FCS, and 0.2% sodium azide) with anti-CD4 (FITC-RM4-4, PharMingen, San Diego, CA) or anti-CD8 (FITC-53-6.7, PharMingen) at 4°C. Stained cells were analyzed using an Epics cytofluorograph (Coulter Electronics, Hialeah, FL). In both experimental approaches, airway responsiveness to inhaled MCh was assessed 48 h after completion of airway sensitization and the following day animals were sacrificed to harvest lungs and PBLN.
7 days after infection failed to significantly increase responsiveness to MCh (Fig. 1B), in contrast to the cells obtained 14 days after infection (Fig. 1A).

In additional experiments, 14 days after RSV infection, PBLN were harvested and CD4 and CD8 T cells isolated and transferred into naive recipients subsequently exposed to OVA or PBS. As shown in Fig. 2, only the transfer of CD8 T cells from the infected mice resulted in the development of AHR following exposure to OVA. Transfer of CD4 T cells was not effective.

Transfer of MNC or T cells from PBLN of RSV-infected mice into naive mice prior to airway sensitization results in the influx of eosinophils into the lung

To assess whether changes in airway responsiveness were associated with changes in pulmonary inflammatory cells following RSV infection or adoptive transfer, lung cells were isolated from these animals and differential cell counts were performed. RSV infection prior to airway sensitization resulted in an influx of eosinophils into the lung (from $0.66 \pm 0.28 \times 10^6$ eosinophils/lung following sensitization alone to $1.57 \pm 0.33 \times 10^6$ eosinophils/lung after RSV infection and sensitization). Adoptive transfer of MNC or T cells derived from the PBLN of RSV-infected mice 14 days postinfection into naive mice prior to airway sensitization resulted in significant increases in numbers of lung eosinophils as well (Fig. 3). Transfer of either MNC or T cells from noninfected mice or from RSV-infected mice harvested 7 days postinfection did not result in lung eosinophilia following sensitization (day 7 T cell transfer; $0.67 \pm 0.28 \times 10^6$ eosinophils/lung; day 7 MNC transfer, $0.55 \pm 0.1 \times 10^6$ eosinophils/lung). Adoptive transfer of MNC from RSV-infected mice without subsequent allergen exposure also did not result in increased lung eosinophils ($0.59 \pm 0.34 \times 10^6$ eosinophils/lung). RSV infection prior to airway sensitization also resulted in the influx of neutrophils into the lungs from $1.64 \pm 0.56$ to $3.22 \pm 0.68 \times 10^6$ neutrophils/lung. Transfer of MNC or T cells from RSV-infected mice failed to significantly alter lung neutrophil numbers (Fig. 3).

Transfer of mononuclear PBLN cells from RSV-infected mice into naive mice prior to airway sensitization results in increased production of IL-5

To monitor local cytokine production, MNC isolated from PBLN following adoptive transfer and airway sensitization were cultured and stimulated with phorbol dibutyrate/ionomycin. Concentrations of IFN-γ, IL-4, and IL-5 in culture supernates were measured by...
ELISA. Transfer of MNC from RSV-infected mice 14 days postinfection and subsequent airway sensitization resulted in increased production of IL-5 in culture (Fig. 4). In contrast, no such effect was observed following transfer of control cells from noninfected animals or from RSV-infected mice 7 days postinfection. Differences in IL-4 and IFN-γ production did not reach statistical significance between groups, but interestingly there was also a noticeable increase in IFN-γ levels following MNC transfer 14 days after RSV infection and subsequent airway sensitization (from 1035.2 ± 826.2 pg/ml to 3429.3 ± 2844.9 pg/ml).

Depletion of CD8 T cells following RSV-infection but prior to airway sensitization prevents, whereas depletion of CD4 T cells reduces, both AHR and lung eosinophilia

To investigate the involvement of individual T cell subsets in mediating the effects of RSV infection on subsequent allergic sensitization via the airways, mice were depleted of CD4 or CD8 T cells beginning day 15 postinfection, i.e., following the acute phase of RSV-induced disease. From day 21 postinfection, mice were sensitized to OVA via the airways over 10 days. Anti-CD4 treatment resulted in a 98% depletion of CD4 T cells in the PBNL assessed prior to OVA exposure and in a 72% depletion when assessed following the completion of airway sensitization, without affecting numbers of CD8 T cells. Following anti-CD8 treatment, CD8 T cells were depleted by 96% throughout the experimental protocol and numbers of CD4 T cells were not altered. Airway responsiveness to MCh and numbers of lung inflammatory cells were monitored following completion of airway sensitization. RSV infection prior to airway sensitization resulted in increases in numbers of lung eosinophils and neutrophils and in AHR as reported above. Depletion of CD8 T cells prevented the development of AHR (Fig. 5A) and lung eosinophilia and neutrophilia (Fig. 5B). Depletion of CD4 T cells, on the other hand, reduced the increases in airway responsiveness by 51.2 ± 16.9% (Fig. 5A). Numbers of lung eosinophils were reduced to a lesser extent than following CD8 T cell depletion and increases in neutrophil numbers were also prevented (Fig. 5B).

**Discussion**

In the present study, we monitored airway responsiveness, pulmonary inflammation, and local cytokine production in a murine model of RSV infection and subsequent sensitization to allergen via the airways. We recently reported that RSV infection preceding allergic airway sensitization enhances airway responsiveness to MCh and inflammatory cell influx into the lung; replicating RSV was required for eliciting these effects (5). In this paper, we utilized this model to define the role of MNC and ultimately T lymphocytes in mediating the effects of RSV infection on airway sensitization. To address this issue, we used two approaches: 1) adoptive transfer of MNC, T cells or CD4 and CD8 T cells from the PBNL of RSV-infected mice followed by airway sensitization, and 2) depletion of CD4 or CD8 T cells following RSV infection but prior to airway sensitization. Following the completion of airway sensitization in either approach, airway responsiveness to aerosolized MCh was assessed using barometric whole body plethysmography, and pulmonary inflammation was monitored.

As previously reported, RSV infection prior to airway sensitization resulted in significant increases in airway responsiveness to MCh, and this was associated with the infiltration of both eosinophils and neutrophils in the lung while allergic sensitization via the airways induced little of these responses (5). Following OVA exposure alone, OVA-specific IgE and IgG1 Abs were detectable in the serum at similar levels in both groups, indicating successful sensitization (data not shown). Transfer of 10⁷ MNC into naive mice, isolated from PBNL and harvested from mice 14 days after RSV infection, enhanced the effects of subsequent airway sensitization resulting in AHR and lung eosinophilia. No such effect was observed if MNC from noninfected animals were transferred as controls or if the adoptive transfer from RSV-infected mice was not followed by allergen exposure via the airways. Further, transfer of 5 × 10⁷ T cells (half the number of MNC) resulted in virtually identical findings. Because T cells, which account for ~60% of the cells obtained from PBNL, can transfer these effects,
the differences seen in day 7 and day 14 transfer results. At the present time, we cannot exclude the possibility that a cell type other than CD8 T cells was primed by RSV infection to induce AHR and lung eosinophilia following sensitization mediated by CD8 T cells.

Transfer of CD4 T cells (PBLN) from RSV-infected mice failed to result in AHR on subsequent exposure to OVA. On the other hand, depletion of CD4 T cells in RSV-infected mice reduced AHR to a limited extent. These data indicate that although CD4 T cells may be involved in the development of AHR and lung eosinophilia following the combination of RSV infection and airway sensitization, the transfer of RSV-induced T cell effects appears to be mediated primarily by CD8 T cells. That CD4 T cell depletion only had a partial effect on OVA responsiveness in our study may very well be due to the incomplete depletion during airway sensitization. The balance between IFN-γ-producing and IL-5-producing CD8 T cells may account for the differences seen in day 7 and day 14 transfer results. At the present time, we cannot exclude the possibility that a cell type other than CD8 T cells was primed by RSV infection to induce AHR and lung eosinophilia following sensitization mediated by CD8 T cells.

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**FIGURE 5.** A, Depletion of CD8 T cells following RSV infection but prior to airway sensitization prevents development of AHR: depletion of CD4 T cells reduces AHR. BALB/c mice were sham-infected (O, n = 12) or infected with RSV and treated with anti-CD4 (R/aCD4-O, n = 12), anti-CD8 (R/aCD8-O, n = 12), or rat IgG (R/O, n = 12) as a control (starting 15 days postinfection) before and during airway sensitization to OVA via the airways. Following completion of airway exposure to OVA, airway responsiveness to increasing concentrations of inhaled MCh (3–50 mg/ml) was assessed and Penh values were calculated. Means ± SEM of Penh values from three independent experiments are expressed as the percentage of baseline Penh values observed after PBS exposure. Significant differences: *, p < 0.05, R/O vs O and R/aCD8-O; †, R/aCD4-O vs O and R/O. B, Depletion of CD8 T cells following RSV infection but prior to airway sensitization prevents eosinophil influx into the lung. Lung cells were isolated from the same mice (n = 12) described for A. Numbers of eosinophils and neutrophils per lung were determined. Illustrated are means ± SD of the numbers of these cells from three independent experiments. Significant differences: *, p < 0.05, R/O vs O; †, R/aCD8-O and R/aCD4-O vs R/O, p < 0.1; R/aCD4-O vs R/O.

Interestingly, transfer of MNC or T cells obtained only 7 days postinfection, that is during the height of RSV-induced disease, did not result in AHR, lung eosinophilia, or increased cytokine production following airway sensitization. Mononuclear PBLN cells harvested at this time point produce very high levels of IFN-γ but little IL-5 and IL-4 upon stimulation, as we reported previously (5). Such IFN-γ-producing (Th1) cells when adoptively transferred may fail to enhance or may inhibit Th2 cytokine responses induced by airway sensitization, rendering transfer of cells 7 days postinfection ineffective in inducing AHR.

Having demonstrated that T cells and T cells alone can play a critical role in mediating RSV-induced AHR following airway sensitization, we attempted to delineate the involvement of CD4 and CD8 T cells in this process. We first isolated CD4 and CD8 T cells from the PBLN of RSV-infected mice and transferred them into naive recipients prior to OVA inhalation. Only the CD8 but not CD4 T cells conferred the ability to develop AHR. We also approached this question by depleting mice of CD4 or CD8 T cells by Ab treatment following the acute phase of RSV infection but prior to airway sensitization. CD8 T cells were almost totally depleted throughout the experiment, whereas depletion of CD4 T cells was almost complete just prior to allergen exposure but could not be sustained during the sensitization phase. Following CD8 T cell depletion, no increase in airway responsiveness or eosinophil and neutrophil recruitment to the lungs was observed in RSV-infected and allergen-sensitized animals, confirming that the presence of CD8 T cells was important for the enhancing effects of RSV infection on subsequent airway sensitization. CD8 T cells have been demonstrated to be capable of IL-5 production (27) and to be essential for the development of allergen-induced AHR (13). Further, in a different viral infection model, CD8 T cells were driven to IL-5 production resulting in eosinophil recruitment (28). We have recently demonstrated that CD8 T cells are critical for the development of RSV-induced AHR during acute infection (14). IL-5-producing CD8 T cells have been shown to persist for longer periods of time in vivo and to produce IL-5 on restimulation (29).

One explanation of our results is that such IL-5-producing CD8 T cells persist following the acute infection, whereas cytotoxic IFN-γ-producing CD8 T cells, which may predominate during the acute phase of RSV infection (30–32), actually decrease in numbers over time. Persistence of IL-5-producing CD8 T cells may be critical in inducing eosinophilia and AHR in response to an inflammatory stimulus such as airway sensitization. The balance between IFN-γ-producing and IL-5-producing CD8 T cells may account for the differences seen in day 7 and day 14 transfer results. At the present time, we cannot exclude the possibility that a cell type other than CD8 T cells was primed by RSV infection to induce AHR and lung eosinophilia following sensitization mediated by CD8 T cells.

These data demonstrated that the effect of RSV infection on subsequent airway sensitization can be transferred to noninfected animals by T cells alone, emphasizing that T cells are the critical cell type in mediating RSV-induced effects on allergic airway sensitization. Transfer of cells 14 days postinfection was also associated with increased levels of IL-5 production when PBLN of recipient animals by T cells alone, emphasizing that T cells are the critical cell type in mediating RSV-induced effects on allergic airway sensitization. Transfer of cells 14 days postinfection was also associated with increased levels of IL-5 production when PBLN of recipient mice were cultured. IL-5 has been shown to be essential for eosinophil recruitment and development of AHR induced by allergen (23–25) and following RSV infection (26). Our cytokine data suggest that T cells capable of producing IL-5 or capable of inducing other cells to do so evolve following RSV infection and were transferred in these experiments. The concomitant increase in IFN-γ production following transfer of cells 14 days postinfection may indicate that different T cell subsets, able to induce the production of a variety of cytokines, were adoptively transferred. Interestingly, transfer of MNC or T cells obtained only 7 days postinfection, that is during the height of RSV-induced disease, did not result in AHR, lung eosinophilia, or increased cytokine production following airway sensitization. Mononuclear PBLN cells harvested at this time point produce very high levels of IFN-γ but little IL-5 and IL-4 upon stimulation, as we reported previously (5). Such IFN-γ-producing (Th1) cells when adoptively transferred may fail to enhance or may inhibit Th2 cytokine responses induced by airway sensitization, rendering transfer of cells 7 days postinfection ineffective in inducing AHR.

Having demonstrated that T cells and T cells alone can play a critical role in mediating RSV-induced AHR following airway sensitization, we attempted to delineate the involvement of CD4 and CD8 T cells in this process. We first isolated CD4 and CD8 T cells from the PBLN of RSV-infected mice and transferred them into naive recipients prior to OVA inhalation. Only the CD8 but not CD4 T cells conferred the ability to develop AHR. We also approached this question by depleting mice of CD4 or CD8 T cells by Ab treatment following the acute phase of RSV infection but prior to airway sensitization. CD8 T cells were almost totally depleted throughout the experiment, whereas depletion of CD4 T cells was almost complete just prior to allergen exposure but could not be sustained during the sensitization phase. Following CD8 T cell depletion, no increase in airway responsiveness or eosinophil and neutrophil recruitment to the lungs was observed in RSV-infected and allergen-sensitized animals, confirming that the presence of CD8 T cells was important for the enhancing effects of RSV infection on subsequent airway sensitization. CD8 T cells have been demonstrated to be capable of IL-5 production (27) and to be essential for the development of allergen-induced AHR (13). Further, in a different viral infection model, CD8 T cells were driven to IL-5 production resulting in eosinophil recruitment (28). We have recently demonstrated that CD8 T cells are critical for the development of RSV-induced AHR during acute infection (14). IL-5-producing CD8 T cells have been shown to persist for longer periods of time in vivo and to produce IL-5 on restimulation (29).

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In summary, we present a murine model of RSV-induced enhancement of inflammatory cell recruitment to the lungs and of increased airway responsiveness following allergic airway sensitization. Utilizing adoptive transfer and T cell subset depletion in this model, we demonstrate that the effects of RSV infection on subsequent airway sensitization can be transferred by CD8 T cells from RSV-infected mice or that depletion of CD8 T cells in RSV-infected mice prevents these effects of RSV infection; CD4 T cell transfer was ineffective and CD4 T cell depletion only partially reduced AHR. We conclude that T cells, and in particular the combination of CD8 and CD4 T cells, are essential in mediating the effects of RSV infection on subsequent airway (allergic) sensitization.

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