

Respiratory Syncytial Virus Protects Against the Subsequent Development of Ovalbumin-Induced Allergic Responses by Inhibiting Th2-Type $\gamma\delta$ T Cells

Leiying Zhang,¹ Jing Liu,¹ Enhua Wang,² Bing Wang,³ Sheng Zeng,¹ Jianqi Wu,⁴ Yoshinobu Kimura,⁵ and Beixing Liu^{1,2*}

¹Department of Immunology, School of Basic Medical Science, China Medical University, Shenyang, China

²Institute of Pathology and Pathophysiology, School of Basic Medical Science, China Medical University, Shenyang, China

³Laboratory of Infectious Diseases, Center for Disease Control and Prevention, Shenyang, China

⁴Department of Clinical Medicine, Harbin Medical University, Harbin, China

⁵Department of Medical Technology, Gifu University of Medical Science, Gifu, Japan

Respiratory syncytial virus (RSV) infection has been hypothesized to be a risk factor for the development of allergy and asthma, but epidemiologic studies in humans still remain inconclusive. The association between RSV infection and allergic diseases may be dependent on an atopic background and previous history of RSV infection. It has been reported that RSV infection before sensitization to an allergen decreased the production of Th2-like cytokines in the lung and the levels of allergen-specific Th2-type antibodies in the serum. However, the underlying mechanisms are largely unknown. In the present study, the role of pulmonary $\gamma\delta$ T cells in RSV-affected, allergen-induced airway inflammation was investigated. BALB/c mice were sensitized to or challenged with ovalbumin (OVA) and infected with RSV either before or after the sensitization period. It became clear that sensitization and challenge of mice with OVA induced a large influx of $\gamma\delta$ T cells to the lungs. However, prior RSV infection inhibited the infiltration of $\gamma\delta$ T cells as well as activated $\gamma\delta$ T cells, characterized by expression of CD40L or CD69 molecular in the cell surface. Moreover, prior RSV infection elevated the type 1 cytokine gene expression but suppressed type 2 cytokine expression in the lung $\gamma\delta$ T cells. Adoptive transfer of $\gamma\delta$ T cells from OVA-sensitized and challenged mice increased airway inflammation, suggesting that $\gamma\delta$ T cells may play a proinflammatory role in allergic responses. These results described here support the idea of an unknown $\gamma\delta$ T cell-dependent mechanism in the regulation of RSV-affected, allergen-induced allergic airway responses. **J. Med. Virol. 85:149–156, 2013.** © 2012 Wiley Periodicals, Inc.

KEY WORDS: respiratory syncytial virus; ovalbumin allergic airway inflammation; pulmonary $\gamma\delta$ T cells

INTRODUCTION

Respiratory syncytial virus (RSV), a member of family Paramyxoviridae, genus *Pneumovirinae*, is the most common cause of respiratory tract infections in infants and young children [Gupta et al., 2011; González et al., 2012]. Because severe RSV infection induces asthma like symptoms, epidemiological studies have been carried out to investigate the effects of RSV infection in allergic respiratory diseases. Recent studies suggested that the timing between viral or bacterial infection and exposure to an allergen may be critical for the development of respiratory allergy in mice [Yamamoto et al., 2001; Chu et al., 2003; Barends et al., 2004]. It became clear that RSV infection before sensitization to an allergen decreased the production of Th2-like cytokines in the lung and the levels of allergen-specific Th2-type antibodies in the serum

Grant sponsor: Liaoning Provincial Educational Department for the Promotion of Science; Grant number: 2009A745.

Disclosure of conflicts of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

*Correspondence to: Prof. Beixing Liu, Department of Immunology, School of Basic Medical Science, China Medical University, No. 92, North Second Road, Heping District, Shenyang 110001, China. E-mail: bxliu@mail.cmu.edu.cn

Accepted 3 September 2012

DOI 10.1002/jmv.23435

Published online in Wiley Online Library (wileyonlinelibrary.com).

[Peebles et al., 2001; Hassantoufighi et al., 2007; Liu and Kimura, 2007]. However, the mechanisms that RSV infection protects against the subsequent development of allergen-induced allergic responses are still unknown.

It has been reported that when comparing asthmatic patients with control subjects, there was an increase in Th2 cytokines and a decrease in Th1 cytokines being produced by $\gamma\delta$ T cells after allergen challenge [Krug et al., 2001], leading investigators to hypothesize these cells may be playing a proinflammatory role in asthma. Studies in mouse models have also demonstrated a role for $\gamma\delta$ T cells in asthma. For instance, the presence of $\gamma\delta$ T cells results in enhanced allergic airway inflammation [Zuany-Amorim et al., 1998], in contrast, a deficiency in this T cell subset results in decreased eosinophils in the bronchoalveolar lavage (BAL) fluid and lung [Lahn et al., 1999], suggesting that $\gamma\delta$ T cells are contributing to allergic airway inflammation. However, Hamelmann et al. used a mouse model of airway inflammation to demonstrate that $\gamma\delta$ T cells affect airway hyperresponsiveness, but do not have a significant role in modulating airway inflammation [Hamelmann et al., 1996]. Therefore, it is important to gain more insight on the association between $\gamma\delta$ T cells and allergen-induced allergic responses, particularly RSV-influenced allergic airway inflammation.

In the present study, BALB/c mice were sensitized to or challenged with ovalbumin (OVA) and infected intranasally with live RSV either before or after the sensitization period. The effects of pulmonary $\gamma\delta$ T cells on RSV-affected, OVA-induced allergic airway inflammation were investigated.

MATERIALS AND METHODS

Animals

Female BALB/c mice, 6–10 weeks of age, free of specific pathogens, were purchased from Shanghai Laboratory Animal Center (Shanghai, China). Mice had fresh water and autoclaved food and were kept at 23°C under bio-clean conditions throughout all experiments. To avoid laboratory contamination, all virus- and allergen-inoculated mice were housed in negatively pressurized isolators equipped with a ventilation system through a high efficiency particulate air filter (AH model, Nihon-Ika, Osaka, Japan). This work was approved by the Institutional Animal Care and Use Committee of China Medical University, China.

Virus

Human RSV type A2 (RSV A2) was supplied kindly by Dr. B. Wang, Center for Disease Control and Prevention, Shenyang, China. The virus was propagated in monolayers of HEp2 cells (ATCC) grown in Eagle's minimum essential medium (Nissui

Pharmacia, Tokyo, Japan) supplemented with 2% heat-inactivated fetal calf serum (FCS, Greiner, Frickenhausen, Germany). At the time of maximum cytopathic effect, cells were harvested and disrupted by sonication in the same culture medium. The suspension was clarified by centrifugation at 2,000g for 20 min at 4°C and the resulting supernatant was layered on top of a sucrose gradient (30% sucrose in 50 mM Tris buffered-normal saline solution containing 1 mM ethylenediamine-tetraacetate [pH 7.5]) and centrifuged further at 100,000 g for 2 hr at 10°C. The pellet was resuspended in 10 mM phosphate-buffered saline (PBS) and stored in aliquots at –80°C. Viral titer was determined by standard plaque titration.

Experimental Design

Mice were sensitized by intraperitoneal injection of 20 μ g of OVA (grade V, Sigma Chemical Co., MO, USA) emulsified in 2.25 mg aluminum hydroxide in a total volume of 100 μ l on days 0 and 14, followed by airway challenge with nebulized OVA (1% in saline) for 20 min on days 28, 29, and 30, according to the schedule shown in Figure 1. One week before the first OVA sensitization or 1 week after the last OVA sensitization, mice, under light anesthesia (pentobarbital sodium, 0.025 mg/g body weight, intraperitoneal administration), were infected intranasally with RSV in 20 μ l of suspension containing 2×10^5 p.f.u. per mouse. Airway responsiveness and airway inflammation were assessed 2 days after the last nebulized OVA exposure. There were four experimental groups, each group containing five mice per experiment: (i) PBS-sensitized, PBS-challenged group (expressed as Mock); (ii) OVA-sensitized, OVA-challenged group (expressed as OVA); (iii) infection with RSV before sensitization to OVA (expressed as RSV + OVA); and (iv) infection with RSV after sensitization to OVA (expressed as OVA + RSV).

Determination of Airway Hyperresponsiveness

Airway responsiveness to aerosolized methacholine (Mch) in conscious, spontaneously breathing BALB/c mice was measured by barometric plethysmography (Buxco, Troy, NY, USA) as described previously [Hamelmann et al., 1997]. Pressure differences between the main chamber of the plethysmograph containing the animal and a reference chamber were measured (box pressure signal). Mice were challenged with aerosolized saline (for the baseline measurement) or Mch (3–50 mg/ml) for 2 min, and readings were taken and averaged for 3 min after each nebulization. Data are expressed as the fold increase above saline challenge baseline values using the dimensionless parameter enhanced pause (Penh). Baseline Penh values did not differ significantly between any of the four groups.

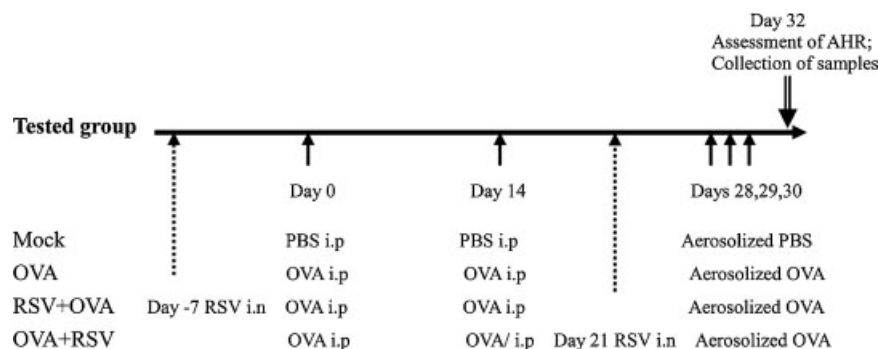


Fig. 1. Time line of experimental protocol. Mock group, mice were inoculated with PBS; OVA group, mice were sensitized by intraperitoneal injection (i.p.) of 20 μ g of OVA on days 0 and 14, followed by aerosolized airway challenge with nebulized 1% OVA on days 28, 29, and 30; RSV + OVA group, mice were infected intranasally (i.n.) with live RSV at an inoculum dose of 2×10^5 p.f.u. per mouse 1 week before OVA sensitization; OVA + RSV group, mice were infected intranasally with live RSV at an inoculum dose of 2×10^5 p.f.u. per mouse 1 week after the final OVA sensitization. Five mice were used in each group.

Histological Analysis of Airway Inflammation

BAL fluids were collected after lungs were instilled with 1 ml saline. Leukocytes were pelleted by centrifugation, cytospun on to glass slides and stained with hematoxylin and eosin (H&E). Two hundred cells were counted on each slide and the proportions of different leukocyte subtypes were calculated. Whole lungs were then dissected and fixed by inflation with formalin. The lungs were maintained in formalin for a further 24 hr before being processed into paraffin using standard histological techniques. Lung tissue sections (5 μ m thick) were stained with H&E for analysis of peribronchial cellular infiltration.

Identification of TCR $\gamma\delta$ T Cells in the Lung

Minced lung tissues were incubated at 37°C for 1 hr on a rocker with 200 mg/ml collagenase D and 40 mg/ml DNase I (Roche Molecular Biochemicals, Mannheim, Germany) in 10 ml of RPMI 1640 medium supplemented with 10% FCS, 2×10^{-5} M 2-mercaptoethanol and antibiotics. Single cell suspensions from the digested lung were collected through density-gradient centrifugation with lymphocyte separation solution (Antibody Institute, Gumma, Japan). The number of dispersed cells in each sample was calculated, and 0.5×10^6 cells were stained with 1 μ g PE-conjugated anti- $\gamma\delta$ TCR, FITC-conjugated anti-CD69, FITC-conjugated anti-CD40L and PECy5-conjugated anti-CD3 Abs (BD Biosciences Pharmingen, San Diego, CA, USA) for 20 min on ice. The percentages of TCR $\gamma\delta$ T cells, CD69⁺ $\gamma\delta$ T cells and CD40L⁺ $\gamma\delta$ T cells in the samples were then determined by flow cytometry.

Measurement of Cytokine Gene Expression in $\gamma\delta$ T Cells

Total RNA was extracted from pulmonary $\gamma\delta$ T cells with TRIzol reagent (Life Technologies, Carlsbad, CA,

USA) according to the manufacturer's instructions. The first-strand cDNA was synthesized from the RNA using SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, CA, USA). Quantitative real-time PCR was performed using SYBR Green Master Mix (Life Technologies, Carlsbad, CA, USA). Primer sets for individual genes are as follows: IL-4-F, ACAGGAGAAGGGACGCCAT and IL-4-R, GAAGCCCTACAGACGAGC TCA; IFN- γ -F, TCAAGTGGCATAGAT GTGGAAGAA and IFN- γ -R, TGGCTCTGCAGGATTT TCATG; IL-10-F, GGTTGCCAAGCCTTATCGGA and IL-10-R, ACCTGC TCCACTGCCTTGCT; and GAPDH-F, TTCACCACCATGGAGAAGGC and GAPDH-R, GGC-ATGGACTGTGGTCATGA. Real-time RT-PCR was run in a Corbett Rotor-Gene RG-3000 (Corbett Life Science, Sydney, NSW, Australia) under identical amplification conditions. Results are normalized to GAPDH expression and presented as fold increase in mRNA expression.

Adoptive Transfer of $\gamma\delta$ T Cells

Lymphocytes were prepared from the lungs of OVA-sensitized and challenged mice, and incubated with PE-conjugated anti-TCR $\gamma\delta$ mAb and FITC-conjugated anti-CD3 mAb (BD Biosciences Pharmingen, San Diego, CA, USA) for 20 min on ice. CD3⁺ $\gamma\delta$ ⁺ T cells were then sorted based on their expression of TCR $\gamma\delta$ and CD3 using a MoFlo cell sorter. Isolated CD3⁺ $\gamma\delta$ ⁺ T cells were washed in PBS and resuspended to 1×10^6 cells/ml PBS, and 2×10^4 cells/mouse in 200 μ l PBS were transferred intravenously into RSV-infected, OVA-exposed mice within 1 hr before the first airway challenge.

Statistical Significance

The two-tailed Mann-Whitney *U*-test and Student's *t*-test were used to determine whether there was a significant difference ($P < 0.05$) between the experimental and control group. Statistical analyses were

done by using StatView J4.11 software (SAS Institute, Cary, NC, USA).

RESULTS

Prior RSV Infection Causes Decreased Airway Hyperresponsiveness in OVA-Sensitized and Challenged Mice

To elucidate the effects of RSV infection on the development of OVA-induced airway hyperresponsiveness, live RSV was inoculated intranasally into BALB/c mice before or after sensitization to OVA, in accordance with the experimental protocol (Fig. 1). As shown in Figure 2, the mice exposed to OVA alone developed a dose dependent increase in airway responsiveness to inhaled methacholine. The value of enhanced pause (Penh) was diminished significantly by prior RSV infection. In contrast, RSV infection after sensitization to OVA showed no effect on OVA-induced airway hyperactivity, indicating that the timing between RSV infection and exposure to OVA may be critical for the development of respiratory allergy.

Prior RSV Infection Diminishes OVA-Induced Airway Inflammation

As shown in Figure 3, a large number of cells, mainly eosinophils and neutrophils, infiltrated the lungs of OVA-sensitized and challenged mice. Prior RSV infection diminished the inflammatory reactions. A marked decrease in the number of total cells, particularly eosinophils and neutrophils, was observed in the lungs of mice that inoculated with RSV before sensitization to OVA. No appreciable difference in the number of BAL cells could be found between OVA + RSV mice and control OVA mice.

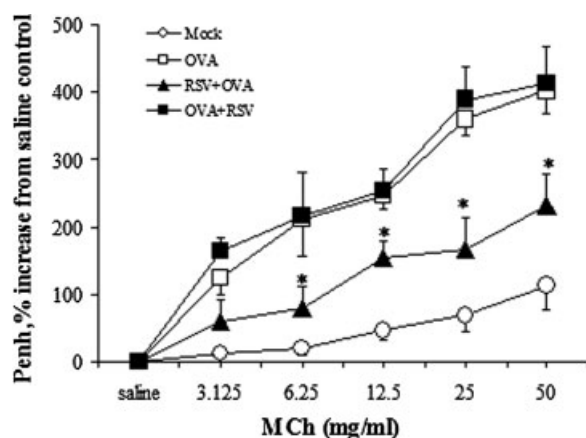


Fig. 2. OVA-induced airway responsiveness to inhaled Mch is diminished by prior RSV infection. Mice were infected with RSV before or after OVA sensitization. Airway responsiveness to inhaled Mch was assessed 2 days after the last nebulized OVA exposure. Data are expressed as percentage of baseline Penh values obtained after inhaled PBS challenge. There were no significant differences in baseline Penh values among groups. Representative data from three independent experiments containing five mice per group are shown. *Significant difference ($P < 0.05$, by Mann-Whitney U -test), compared with corresponding OVA group.

On the other hand, OVA mice and OVA + RSV mice exhibited a significant peribronchial inflammation with a large number of mononuclear cells in the lung tissue sections. However, RSV infection before sensitization to OVA diminished the inflammatory responses induced by subsequent inhaled allergen (Fig. 4).

Prior RSV Infection Inhibits the Influx of $\gamma\delta$ T Cells to the Lung

To determine whether $\gamma\delta$ T cells play a role in allergen-induced allergic responses, the percentage and number of $\gamma\delta$ T cells in the lung parenchyma of allergic mice were assessed by flow cytometer. As shown in Figure 5, sensitization and challenge of mice with OVA induced a massive influx of $\gamma\delta$ T cells to the lungs of mice. However, prior RSV infection inhibited the influx of $\gamma\delta$ T cells as well as activated $\gamma\delta$ T cells, characterized by expression of CD40L or CD69 molecular in the cell surface, to the lungs. In contrast, RSV infection after sensitization to OVA has a weak inhibitory effect on OVA-induced $\gamma\delta$ T cells infiltration. This result suggests that $\gamma\delta$ T cells may be involved in the development of RSV-affected, OVA-induced allergic airway inflammation.

Prior RSV Infection Influences Cytokine Gene Expression in $\gamma\delta$ T Cells

As shown in Figure 6, relative expression against GAPDH of IFN- γ mRNA in the lung $\gamma\delta$ T cells was enhanced by infection with RSV before sensitization to OVA. Moreover, prior RSV infection caused a decrease in the gene expression of Th2 cytokines (IL-4 and IL-10) in $\gamma\delta$ T cells. However, RSV infection after sensitization to OVA, did not alter Th1/Th2 cytokine production by $\gamma\delta$ T cells, suggesting that $\gamma\delta$ T cells may contribute to the development of RSV-affected, OVA-induced allergic responses.

Adoptive Transfer of $\gamma\delta$ T Cells Increases Airway Inflammation in BALB/c Mice

To confirm the role of $\gamma\delta$ T cells in the development of allergic airway inflammation, the lung $\gamma\delta$ T cells obtained from OVA-sensitized and challenged mice were transferred intravenously into the mice within 1 hr before the first airway challenge. As shown in Table I, adoptive transfer of the pulmonary $\gamma\delta$ T cells increased the number of inflammatory cells and the percentage of eosinophils, not only in the lungs of mock mice but also in the lungs of RSV + OVA mice ($P < 0.01$, compared with the corresponding non-transferred mice), indicating that $\gamma\delta$ T cells may play a proinflammatory role in the development of allergen-induced airway inflammation. However, adoptive transfer of the pulmonary $\gamma\delta$ T cells did not affect the cellular infiltration in the lungs of mice that inoculated with RSV after sensitization to OVA. No appreciable differences in the number and proportion of

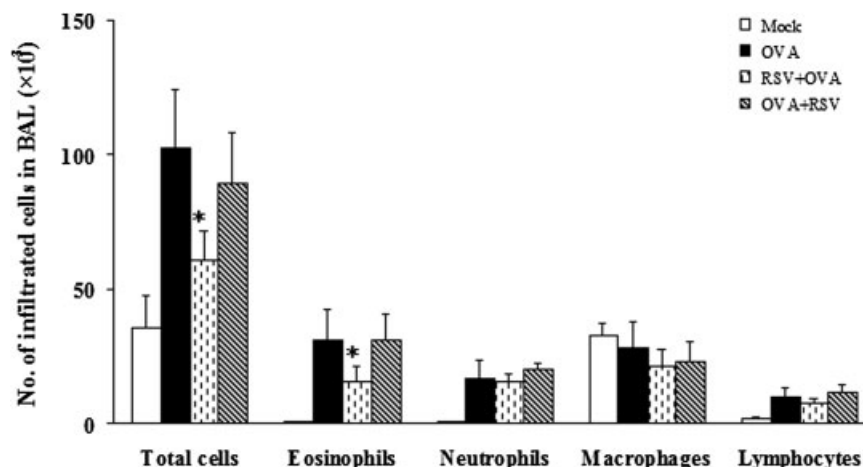


Fig. 3. Cellular infiltration in the lungs of RSV-infected, OVA-sensitized mice. BALB/c mice were infected intranasally with RSV before or after OVA sensitization. The bronchoalveolar lavage (BAL) fluids were collected 2 days after the last nebulized OVA exposure and analyzed for cellular infiltration. Data are means $\pm$ SD of results for each group of five mice teased. Representative data from three independent experiments are shown. *Significant difference ($P < 0.05$, by Mann-Whitney U -test), compared with corresponding OVA group.

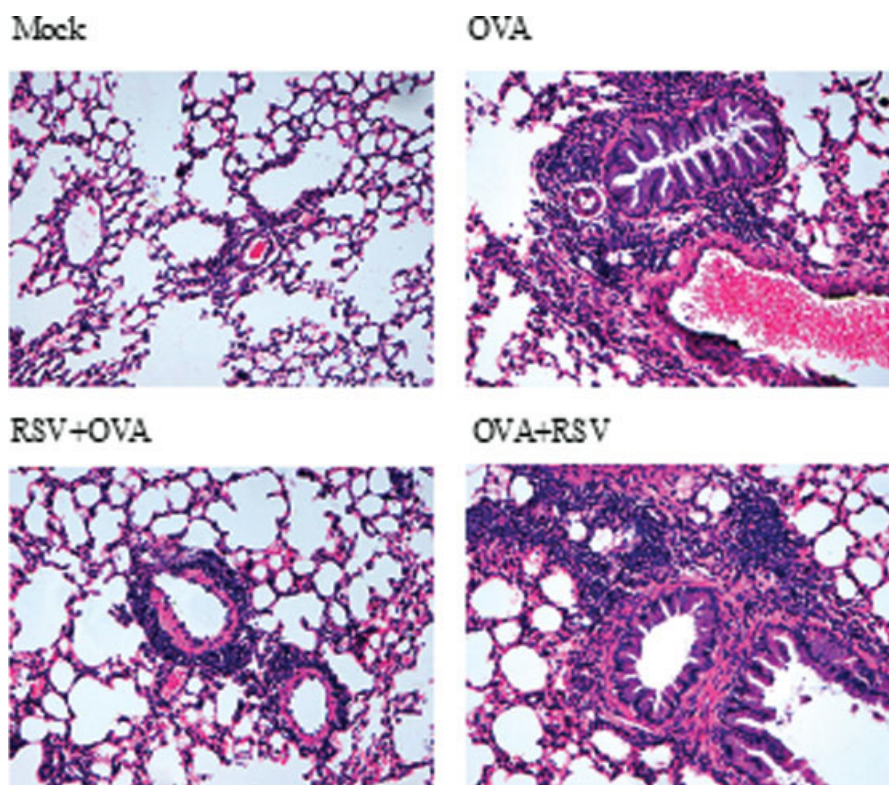


Fig. 4. Histological examination of lung tissues from BALB/c mice treated with OVA and RSV. Mice were infected intranasally with RSV before or after OVA sensitization. Lung pathologic lesions were analyzed 2 days after the last nebulized OVA exposure. The sections displayed are representative of those from three independent experiments containing five mice per group. Original magnification, $\times 20$. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/jmv>]

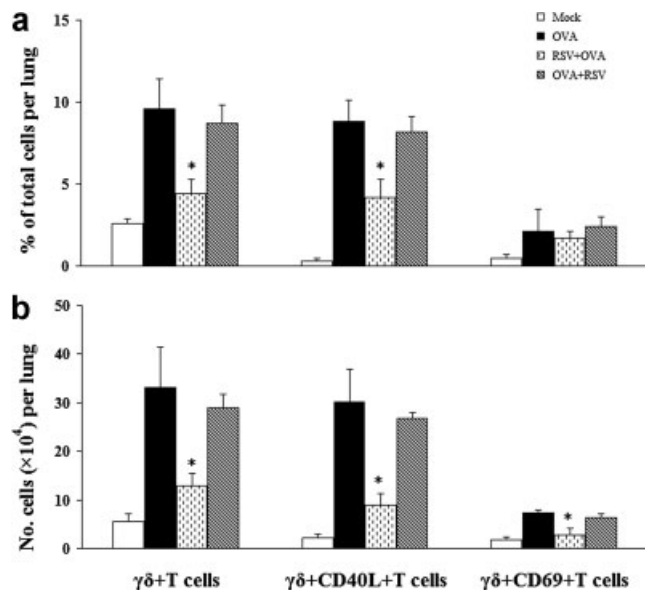


Fig. 5. Percentage (a) and absolute number (b) of $\gamma\delta$ T cells in the lungs of RSV-infected, OVA-sensitized mice. Data are means $\pm$ SD of results for each group of five mice teased. Representative data from three independent experiments are shown. *Significant difference ($P < 0.01$, by Mann-Whitney *U*-test), compared with corresponding OVA group.

leukocyte subtypes could be detected between the transferred mice and corresponding non-transferred mice.

DISCUSSION

It has been reported previously that the timing of RSV infection is critical for allergen-induced allergic responses. Infection with RSV before sensitization to an allergen, diminished the productions of Th2 cytokines in the lung and allergen-specific antibodies in the serum [Barends et al., 2004; Liu and Kimura, 2007], suggesting that under a certain condition, RSV infection may play a protective role in allergen-induced allergic reactions. However, the underlying mechanisms remain unknown. This article focuses on the role of pulmonary $\gamma\delta$ T cells in the development of RSV-affected, OVA-induced allergic airway inflammation.

Although the biological role and repertoire of $\gamma\delta$ T cells have not been defined fully, studies suggest that $\gamma\delta$ T cells are contributing to allergic airway inflammation. In murine models, $\gamma\delta$ T cells have been shown to be required for Th2-type eosinophilic inflammation and airway hyperresponsiveness to allergen challenge [Schramm et al., 2000]. An increase in $\gamma\delta$ T cells expressing Th2-type cytokines is reported in BAL fluids after allergen challenge of asthmatic patients [Pawankar et al., 1996; Spinozzi et al., 1996]. In this study, prior RSV infection inhibited the influx of $\gamma\delta$ T cells to the lungs of mice. The percentages and absolute numbers of total $\gamma\delta$ T cells as well as activated $\gamma\delta$ T cells were decreased significantly in the lungs of

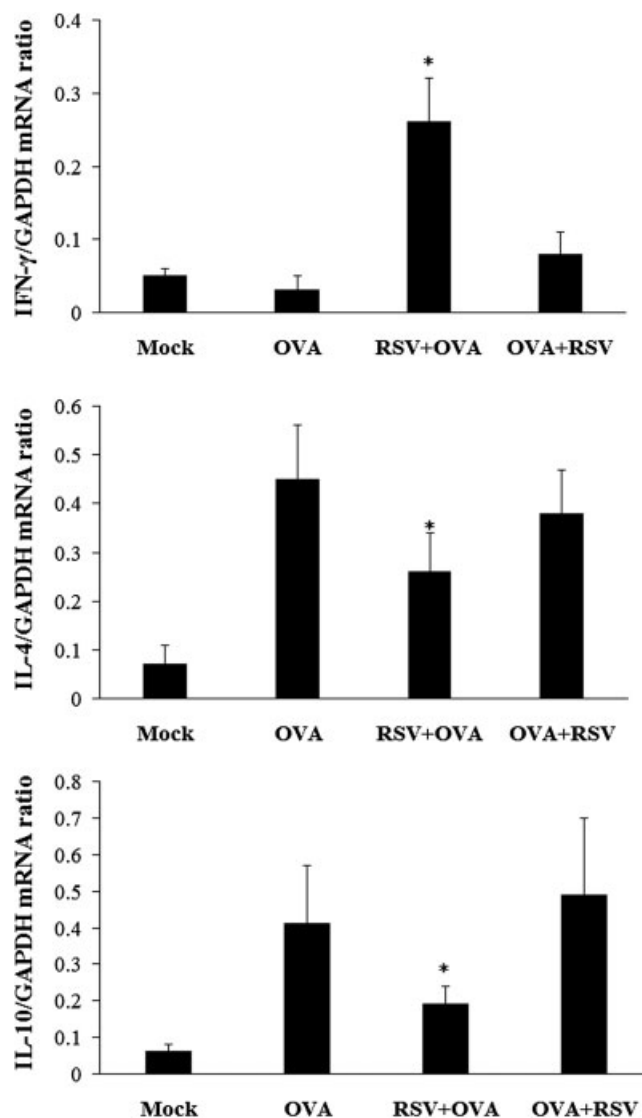


Fig. 6. Relative expressions against GAPDH of IFN- γ , IL-4 and IL-10 mRNA in $\gamma\delta$ T cells. $\gamma\delta$ T cells were isolated from the lungs of mice 2 days after the last nebulized OVA exposure and assayed for cytokine gene expression. Data are means $\pm$ SD of results for each group of five mice teased. Representative data from three independent experiments are shown. *Significant difference ($P < 0.01$, by Mann-Whitney *U*-test), compared with corresponding OVA group.

RSV + OVA mice (Fig. 5). Moreover, prior RSV infection caused a decrease in type 2 cytokine gene expression (IL-4 and IL-10 mRNA), but an increase in type 1 cytokine gene expression (IFN- γ mRNA) in the lung $\gamma\delta$ T cells (Fig. 6), suggesting that RSV infection protects against the subsequent development of OVA-induced allergic responses probably by diminishing the number and function of Th2-type $\gamma\delta$ T cells. It has been reported that RSV impaired T cell activation by preventing synapse assembly with dendritic cells [González et al., 2008; Munir et al., 2011]. However, in the present study, as an innate lymphocyte, $\gamma\delta$ T cell may be in response to RSV infection through distinct mechanisms, for instance, apoptosis or

TABLE I. Increased Cellular Infiltration in the Lungs of BALB/c Mice After Adoptive Transfer of $\gamma\delta$ T Cells

Tested group	Adoptive transfer of $\gamma\delta$ T cells	No. of total cells in BAL ($\times 10^3$)	% of cells		
			Eosinophils	Macrophages	Neutrophils
Mock	—	1.9 $\pm$ 0.6	0.4 $\pm$ 0.1	85.8 $\pm$ 0.2	0.2 $\pm$ 0.1
	+	3.3 $\pm$ 0.8*	5.5 $\pm$ 2.8*	76.7 $\pm$ 5.0	1.3 $\pm$ 1.1
OVA	—	34.2 $\pm$ 1.6	21.0 $\pm$ 5.6	59.0 $\pm$ 6.4	11.2 $\pm$ 2.6
	+	50.1 $\pm$ 18.3**	36.5 $\pm$ 6.2**	28.6 $\pm$ 9.7	15.8 $\pm$ 1.2
RSV + OVA	—	6.8 $\pm$ 0.5	15.1 $\pm$ 3.1	67.9 $\pm$ 8.5	11.3 $\pm$ 4.8
	+	8.0 $\pm$ 0.8***	30.7 $\pm$ 1.7***	44.7 $\pm$ 5.3	15.2 $\pm$ 2.0
OVA + RSV	—	44.2 $\pm$ 1.4	37.0 $\pm$ 4.6	42.0 $\pm$ 4.4	10.2 $\pm$ 2.1
	+	46.9 $\pm$ 17.3	39.7 $\pm$ 3.5	35.9 $\pm$ 3.8	13.6 $\pm$ 0.6

$\gamma\delta$ T cells were isolated from the lung parenchyma of OVA-sensitized and challenged mice by using a MoFlo cell sorter; 2×10^4 CD3⁺ TCR $\gamma\delta^+$ T cells were transferred intravenously into the corresponding mice within 1 hr before the first airway challenge. Cellular infiltration in the lungs of mice was detected 2 days after the last nebulized OVA exposure. Data are means $\pm$ SD of results for each group of five mice tested. Representative data from three independent experiments are shown.

*Significant difference ($P < 0.01$, by Mann–Whitney U -test), compared with non-transferred Mock group.

**Significant difference ($P < 0.01$, by Mann–Whitney U -test), compared with non-transferred OVA group.

***Significant difference ($P < 0.01$, by Mann–Whitney U -test), compared with non-transferred RSV + OVA group.

redistribution of $\gamma\delta$ T cell subsets, and then, alters allergen-induced airway inflammation. Indeed, adoptive transfer of $\gamma\delta$ T cells from OVA-sensitized and challenged mice increased the numbers and percentages of inflammatory cells, mainly eosinophils and neutrophils, not only in the lungs of normal mice but also in the lungs of RSV + OVA mice (Table I). On the other hand, adoptive transfer of $\gamma\delta$ T cells from RSV-infected mice diminished cellular infiltration in the lungs of allergic mice (not shown). These results suggest that: (i) under the present experimental condition, $\gamma\delta$ T cells may play a proinflammatory role in the development of OVA-induced allergic airway inflammation; (ii) RSV infection may alter the distribution of $\gamma\delta$ T cell subsets from Th2-dominance to Th1-dominance in the lungs of mice.

The $\gamma\delta$ T cells capable of modulating allergen-induced airway inflammation belong to two subsets distinguished by their TCRs: a $V\gamma 1^+$ subset that can enhance airway hyperresponsiveness as well as levels of Th2 cytokines in the airways and eosinophilic infiltrates in the lungs [Pereira et al., 1995; Hahn et al., 2004], and a $V\gamma 4^+$ subset that can be induced to suppress airway hyperresponsiveness [Lahn et al., 2002; Hahn et al., 2003, 2004]. Both $\gamma\delta$ T cells are found in spleen and lung [Hahn et al., 2004; Wands et al., 2005]. Following airway challenge, selectively depleting either subset in the lungs (using aerosolized, inhaled anti-TCR Abs) alters AHR in the predicted fashion, that is depletion of $V\gamma 1^+$ cells decreases and depletion of $V\gamma 4^+$ cell increases airway hyperresponsiveness [Lahn et al., 2002, 2004]. By using mice that immunized with recombinant vaccine virus expressing RSV F protein and challenged with live RSV, Dodd et al. [2009] reported that $V\gamma 4^+$ subset were recruited to the lungs and produced IFN- γ in a time-dependent manner. Although in the present study, it is not clear which subset of $\gamma\delta$ T cells was affected significantly by RSV infection. However, by analyzing these results that RSV infection decreased the number of $\gamma\delta$ T cells

in the lungs and inhibited type 2 cytokine expression in $\gamma\delta$ T cells, together with the result of adoptive transfer, it is possible that under the defined experimental condition, Th2-type $\gamma\delta$ T cells ($V\gamma 1^+$ cell?) may be the major subset affected largely by RSV infection.

The results support the suggestion of an unknown $\gamma\delta$ T cell-dependent mechanism in the regulation of RSV-affected, allergen-induced allergic airway inflammation.

REFERENCES

- Barends M, Van Oosten M, De Rond CG, Dormans JA, Osterhaus AD, Neijens HJ, Kimman TG. 2004. Timing of infection and prior immunization with respiratory syncytial virus (RSV) in RSV-enhanced allergic inflammation. *J Infect Dis* 189:1866–1872.
- Chu HW, Honour JM, Rawlinson CA, Harbeck RJ, Martin RJ. 2003. Effects of respiratory mycoplasma pneumoniae infection on allergen-induced bronchial hyperresponsiveness and lung inflammation in mice. *Infect Immun* 71:1520–1526.
- Dodd J, Riffault S, Kodituwakku JS, Hayday AC, Openshaw PJ. 2009. Pulmonary V gamma 4+ gamma delta T cells have proinflammatory and antiviral effects in viral lung disease. *J Immunol* 182:1174–1181.
- González PA, Prado CE, Leiva ED, Carreño LJ, Bueno SM, Riedel CA, Kalergis AM. 2008. Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. *Proc Natl Acad Sci USA* 105:14999–15004.
- González PA, Bueno SM, Carreño LJ, Riedel CA, Kalergis AM. 2012. Respiratory syncytial virus infection and immunity. *Rev Med Virol* 22:230–244.
- Gupta S, Shamsundar R, Shet A, Chawan R, Srinivasa H. 2011. Prevalence of respiratory syncytial virus infection among hospitalized children presenting with acute lower respiratory tract infections. *Indian J Pediatr* 78:1495–1497.
- Hahn YS, Taube C, Jin N, Takeda K, Park JW, Wands JM, Aydin-tug MK, Roark CL, Lahn M, O'Brien RL, Gelfand EW, Born WK. 2003. V gamma 4+ gamma delta T cells regulate airway hyperreactivity to methacholine in ovalbumin-sensitized and challenged mice. *J Immunol* 171:3170–3178.
- Hahn YS, Taube C, Jin N, Sharp L, Wands JM, Aydin-tug MK, Lahn M, Huber SA, O'Brien RL, Gelfand EW, Born WK. 2004. Different potentials of gamma delta T cell subsets in regulating airway responsiveness: V gamma 1+ cells, but not V gamma 4+ cells, promote airway hyperreactivity, Th2 cytokines, and airway inflammation. *J Immunol* 172:2894–2902.
- Hamelmann E, Oshiba A, Paluh J, Bradley K, Loader J, Potter TA, Larsen GL, Gelfand EW. 1996. Requirement for CD8+ T cells in

- the development of airway hyperresponsiveness in a murine model of airway sensitization. *J Exp Med* 183:1719–1729.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 156:766–775.
- Hassantoufighi A, Oglesbee M, Richter BW, Prince GA, Hemming V, Niewiesk S, Eichelberger MC. 2007. Respiratory syncytial virus replication is prolonged by a concomitant allergic response. *Clin Exp Immunol* 148:218–229.
- Krug N, Erpenbeck VJ, Balke K, Petschallies J, Tschernig T, Hohlfeld JM, Fabel H. 2001. Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+), and gammadelta T cells in people with asthma after segmental allergen challenge. *Am J Respir Cell Mol Biol* 25:125–131.
- Lahn M, Kanehiro A, Takeda K, Joetham A, Schwarze J, Köhler G, O'Brien R, Gelfand EW, Born W. 1999. Negative regulation of airway responsiveness that is dependent on gammadelta T cells and independent of alphabeta T cells. *Nat Med* 5:1150–1156.
- Lahn M, Kanehiro A, Takeda K, Terry J, Hahn YS, Aydinug MK, Konowal A, Ikuta K, O'Brien RL, Gelfand EW, Born WK. 2002. MHC class I-dependent Vgamma4+ pulmonary T cells regulate alpha beta T cell-independent airway responsiveness. *Proc Natl Acad Sci USA* 99:8850–8855.
- Lahn M, Kanehiro A, Hahn YS, Wands JM, Aydinug MK, O'Brien RL, Gelfand EW, Born WK. 2004. Aerosolized anti-T-cell-receptor antibodies are effective against airway inflammation and hyper-reactivity. *Int Arch Allergy Immunol* 134:49–55.
- Liu B, Kimura Y. 2007. Respiratory syncytial virus protects against the subsequent development of Japanese Cedar Pollen-induced allergic responses. *J Med Virol* 79:1600–1605.
- Munir S, Hillyer P, Le Nouën C, Buchholz UJ, Rabin RL, Collins PL, Bukreyev A. 2011. Respiratory syncytial virus interferon antagonist NS1 protein suppresses and skews the human T lymphocyte response. *PLoS Pathog* 7:e1001336.
- Pawankar RU, Okuda M, Suzuki K, Okumura K, Ra C. 1996. Phenotypic and molecular characteristics of nasal mucosal gamma delta T cells in allergic and infectious rhinitis. *Am J Respir Crit Care Med* 153:1655–1665.
- Peebles RS Jr, Sheller JR, Collins RD, Jarzecka AK, Mitchell DB, Parker RA, Graham BS. 2001. Respiratory syncytial virus infection does not increase allergen-induced type 2 cytokine production, yet increases airway hyperresponsiveness in mice. *J Med Virol* 63:178–188.
- Pereira P, Gerber D, Huang SY, Tonegawa S. 1995. Ontogenic development and tissue distribution of V gamma 1-expressing gamma/delta T lymphocytes in normal mice. *J Exp Med* 182:1921–1930.
- Schramm CM, Puddington L, Yiamouyiannis CA, Lingenheld EG, Whiteley HE, Wolyniec WW, Noonan TC, Thrall RS. 2000. Proinflammatory roles of T-cell receptor (TCR) gammadelta and TCR alphabeta lymphocytes in a murine model of asthma. *Am J Respir Cell Mol Biol* 22:218–225.
- Spinozzi F, Agea E, Bistoni O, Forenza N, Monaco A, Bassotti G, Nicoletti I, Riccardi C, Grignani F, Bertotto A. 1996. Increased allergen-specific, steroid-sensitive gamma delta T cells in bronchoalveolar lavage fluid from patients with asthma. *Ann Intern Med* 124:223–227.
- Wands JM, Roark CL, Aydinug MK, Jin N, Hahn YS, Cook L, Yin X, Dal Porto J, Lahn M, Hyde DM, Gelfand EW, Mason RJ, O'Brien RL, Born WK. 2005. Distribution and leukocyte contacts of gammadelta T cells in the lung. *J Leukoc Biol* 78:1086–1096.
- Yamamoto N, Suzuki S, Suzuki Y, Shirai A, Nakazawa M, Suzuki M, Takamasu T, Nagushima Y, Minami M, Ishigatsubo Y. 2001. Immune response induced by airway sensitization after influenza A virus infection depends on timing of antigen exposure in mice. *J Virol* 75:499–505.
- Zuany-Amorim C, Ruffié C, Hailé S, Vargaftig BB, Pereira P, Pretolani M. 1998. Requirement for gammadelta T cells in allergic airway inflammation. *Science* 280:1265–1267.