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Author(s): Xiong-bin Jiang, Mao Huang, Xue-fan Cui, Yi Zhu, Kai-sheng Yin, and Kun Yao

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Respiratory syncytial virus infection differentiates airway dysfunction in the central and peripheral airways in OVA-sensitized mice

Xiong-bin Jiang,¹ Mao Huang,¹ Xue-fan Cui,¹ Yi Zhu,¹ Kai-sheng Yin,¹ and Kun Yao²

¹Department of Respiratory Disease, the First Affiliated Hospital of Nanjing Medical University, Nanjing, P.R. China

²Department of Microbiology and Immunology, Nanjing Medical University, Nanjing, P.R. China

ABSTRACT

Much evidence suggests that respiratory syncytial virus (RSV) infection prolongs airway hyperresponsiveness (AHR) and exacerbates asthma by enhancing airway inflammation. However, the characteristic of airway inflammation and kinetics of airway dysfunction occurred in the central and peripheral airways were not fully delineated. The objective of this study was to investigate the effect of RSV on the allergic airway inflammation in different size airways and to elucidate its possible mechanism. Using a murine model of prior ovalbumin (OVA) sensitization and subsequent RSV challenge, lung resistance (R_L), and dynamic compliance (Cdyn) was conducted by barometric whole-body plethysmography. Histological examinations were carried out. Differential cells count in bronchoalveolar lavage (BAL) fluid, serum anti-OVA IgE, and IgG1 were measured. Cytokine mRNA expression in lung tissue were determined. RSV triggered a significant increase in R_L and reduction in Cdyn, as well as greatly prolonged the recovery of Cdyn more than that of R_L in OVA-sensitized mice. Also, RSV resulted in more severe peripheral airway inflammation which exhibit as goblet cell hyperplasia and CD8+ T cell infiltration. Furthermore, the number of lymphocytes, neutrophils and macrophages in BAL fluid, serum anti-OVA IgE and IgG1 were remarkably increased. Additionally, mice increased relative expression of cytokines IL-4, IL-13, and IFN- γ , but not IL-5, IL-17, and IL-17F. These findings demonstrated that RSV could selectively affect pathologic processes that contribute to altered airway function in the central and peripheral airways in OVA-sensitized mice. These processes may be involved in goblet cell hyperplasia and CD8+ T cell infiltration in peripheral airways.

KEYWORDS CD8+ T cell, dynamic compliance, goblet cell, peripheral airway, respiratory syncytial virus

The pathophysiology of atopic asthma has traditionally been attributed to an inflammatory process that occurs predominantly in the central airways and increases in airway hyperresponsiveness (AHR) mainly owing to large airway inflammation [1]. Small airways have been dubbed the “quiet zone” and its role in asthma had usually been neglected. However, increased evidences have emerged in the last few years suggesting that the airway inflammation that characterizes asthma occur not only in the central airways but extend to the distal lung and the lung parenchyma [2, 3].

Respiratory syncytial virus (RSV) is the most common viral cause of severe lower respiratory tract disease in childhood and also plays a critical role in asthma exacerbations [4, 5]. However, the mechanisms by which RSV give rise to asthma exacerbations and RSV exposure in the setting of allergy induces airway dysfunction, especially in the peripheral airways, are not fully elucidated [6]. A study by Peebles et al. [7] showed in mice that RSV exposure during sensitization with OVA prolonged the period of AHR when compared with OVA-sensitized mice alone and enhanced airway inflammation both in the large airways and small airways. In addition, Mejias and coworkers [8] reported that mice inoculated with live RSV showed a persistent progression of the severity of pneumonia and mucus production in the acute phase and illustrated increased periodic acid-Schiff (PAS)-positive hypertrophic cells in both

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Address correspondence to Xiong-bin Jiang, Department of Respiratory Disease, the First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing, Jiangsu, China 212009. E-mail: xiongbingjiang@126.com

central and peripheral airways. Thus, it was reasonable to speculate that peripheral airway inflammation after RSV exposure in OVA-sensitized mice may also play an important role in the development of airway dysfunction, but the characteristic of airway inflammation and the kinetics of the response which reflects the peripheral airway inflammation have not been reported.

The aim of current study was to compare the difference of airway dysfunction occurred in the central and peripheral airways and to determine whether RSV enhances peripheral airway inflammation during respiratory allergy that could explain the RSV-enhanced allergic response.

METHODS

Cells and Virus

HEp-2 cells (ATCC, Rockville, Md., USA) were maintained in Eagle's minimal essential media (EMEM) supplemented with glutamine, amphotericin, gentamicin, penicillin G, and 10% fetal bovine serum. The A2 strain of RSV (Long strain, type A, ATCC) was provided by the department of microbiology and immunology of Nanjing Medical University and was propagated in a HEp-2 cell monolayer grown at 37°C and a 5% CO₂-enriched atmosphere in minimal essential medium supplemented with 2% heat inactivated fetal bovine serum. At maximal cytopathic effect, the cells were harvested and disrupted by sonication in the same culture medium. The suspension was clarified by centrifugation at 10,000 g for 15 minutes at 10°C, and the resulting supernatant was layered on top of a sucrose gradient and further centrifuged at 100,000 g for 2 hours at 10°C. The pellet, containing purified virus, was resuspended in 10 mM phosphate-buffered saline (PBS; pH 7.4; Takara, Dalian, China) containing 15% sucrose and stored in aliquots at -70°C. The virus titer in the inoculum was determined by plaque assay on the same day as the inoculation of BALB/c mice.

Animals and Experiment Design

Pathogen-free 8-week-old female BALB/c mice were purchased from experimental animal center of Shanghai of the scientific institute of China, Shanghai, China (Grade: SPF, No: SCXKL 2003-0003). All mice were maintained under specific pathogen free conditions in the animal facility of our university and treated in accordance with the guidelines of the Institutional Animal Care and Use Committee and the study has been approved by the local Institutional Board.

Mice were injected intraperitoneally (ip) with 0.1 mL (10 mg) of chicken OVA, grade V (Sigma Chemical Co., St. Louis, MO), complexed with 2 mg of Al(OH)₃. For seven consecutive days (days 11–17), sensitized mice underwent inhalational challenge within a plastic chamber with 1% OVA (w/v) diluted in PBS for 40 minutes every day. To investigate the influence of RSV exposure on OVA allergy, OVA-allergic mice were inoculated by intranasal instillation of 25 μ L of sterilized PBS containing 2×10^5 plaque-forming units of purified RSV according to schedule as outlined in Figure 1 (OVA/RSV). These mice were compared with OVA-allergic mice sham inoculated with an uninfected Hep-2 cell lysate (OVA), RSV infected mice (PBS-sensitized and RSV-infected, RSV), no allergic mice (sensitized and challenged with PBS, PBS). At different time points after last challenge, lung resistance (R_L) and dynamic compliance (C_{dyn}) were recorded.

Determination of R_L and C_{dyn}

Anesthetized, tracheostomized mice were mechanically ventilated and lung function was assessed as following method: the tail vein was cannulated for intravenous injection of acetylcholine (Ach, Sigma, USA). Mice were placed in a plethysmograph, a four-way connector was attached to the tracheostomy tube, with two ports connected to the inspiratory and expiratory sides of two ventilators. Ventilation was achieved at a rate of 150 breaths per minute with a 5–6 mL/kg and a positive endexpiratory pressure

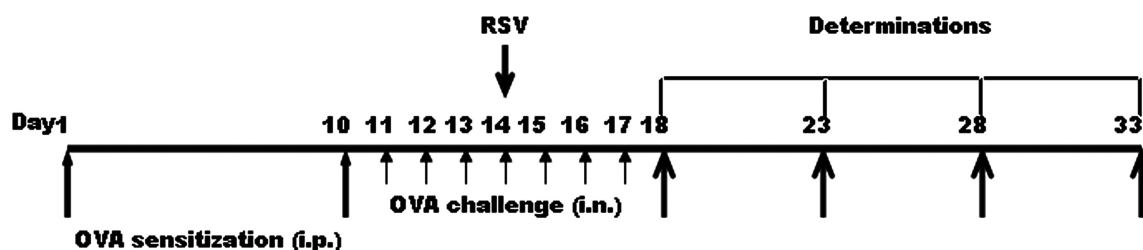


FIGURE 1. Time line of experimental protocol.

of 2 cmH₂O by the ventilator (Beijing XinAoCheng science and technology Co. limited, China). Transpulmonary pressure was detected and changes in lung volume were measured. Flow was calculated by digital differentiation of the volume signal, and R_L and C_{dyn} were continuously computed by fitting flow, volume, and pressure to an equation of motion (AniRes2003 for Windows98/2000/XP). Ach was dissolved in normal saline and administered intravenously at a starting dose of 0.5 mg/mL. Three-fold increasing concentrations of Ach were administered at 5 minutes intervals and only after transpulmonary pressure and tidal volume returned to baseline. After each Ach challenge, the data were continuously collected for 1 to 5 minutes and maximum values of R_L and minimum values of C_{dyn} were taken to evaluate changes in these functional parameters.

Preparation of BAL Fluid

The trachea was cannulated and the left bronchi were tied for histological examination. Then, the right air lumen was washed three times with 0.5 mL calcium- and magnesium-free PBS containing 0.1% BSA (Sigma, St Louis, Mo, USA) and 0.05 mM EDTA-2Na (recovery >85%). The number of nucleated cells was counted in a Burkner chamber. Cytospin slides were stained with Türk solution (Merck, Darmstadt, Germany) and differentiated by standard hematological procedures.

Enzyme Immunoassays

OVA-specific IgE in serum was measured using a commercial kit (MD Biosciences, MN) according to the manufacturer's instructions. OVA-specific IgG1 in serum was measured as described as follow: appropriate wells were coated with either anti-IgG1 (BD

Pharmingen) or OVA (2 µg/well). After blocking, wells were incubated with serial dilutions of serum or standards (mouse IgG1), followed by detection with streptavidin HRP anti-IgG1. Plates were developed and read at 450 nm using a BioRad 680 Microplate reader. Samples were measured in duplicate and the sensitivity of detection was 3 ng/mL.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total cellular RNA was extracted and purified from lung tissue with Trizol reagent (GibcoBRL, Life Technologies) according to the manufacturer's instructions. Ten microliters of total lung RNA was reverse transcribed, and cytokine PCRs (IFN-γ, IL-4, IL-5, IL-13, IL-17, IL-17F) of the cDNA were performed. To prevent interassay variation, PCRs of all samples from one experiment were done simultaneously and were loaded on the same agarose gel for quantification. All results were corrected for mRNA amplified by use of the mouse control amplifier set (Clontech), according to the manufacturer's protocol. The PCR product was analyzed by detecting the band intensities on the agarose gel by use of the Molecular analyst software system (Bio-Rad Laboratories). Primers used for amplification are listed as following primer set (Table 1). All primers were synthesized by Shanghai Sangon Biological Engineering & Technology Service Co Ltd (Shanghai, China).

Histological Studies

The left lungs were fixed in 10% formalin, and embedded in paraffin blocks. Tissue sections, 3 µm thick, were affixed to microscope slides, and deparaffinized. Tissue sections were stained with hematoxylin and eosin (H&E) for routine histologic

TABLE 1 Primers Used for RT-PCR

Gene		Sequences of primers	bp
IL-4	Forward	5'-TAG TTG TCA TCC TGC TCT T-3'	404
	Reverse	5'-CTA CGA GTA ATC CAT TTG C-3'	
IL-5	Forward	5'-AAG GAT GCT TCT GCA CTT GA-3'	365
	Reverse	5'-ACA CCA AGG AAC TCT TGC A-3'	
IL-13	Forward	5'-GCC AGC CCA CAG TTC TAC AGC-3'	182
	Reverse	5'-GTG ATG TTG CTC AGC TCC TCA-3'	
IFN-γ	Forward	5'-GCC AGC CCA CAG TTC TAC AGC-3'	309
	Reverse	5'-CGA CTC CTT TTC CGC TTC CT-3'	
IL-17	Forward	5'-TTTAACCTCCCTTGGCGCAAAA-3'	165
	Reverse	5'-CTTTCCCTCCGCATTGACAC-3'	
IL-17F	Forward	5'-TCCCCTGGAGGATAACACTG-3'	316
	Reverse	5'-GGTGCAGCCAACCTTTAGGA-3'	
β-actin	Forward	5'-CCC AGA GCA AGA GAG GTA TC-3'	267
	Reverse	5'-GAC CAG AGG CAT ACA GGG AC-3'	

studies and periodic acid–Schiff (PAS) for identification and quantification of mucus containing goblet cells. CD8+ T cell distribution in lung sections were identified by immunohistochemical (IHC) staining as described in our previously study [9]. Morphometric analysis was carried out respectively at $\times 200$ (H&E), $\times 400$ (PAS), and $\times 400$ (IHC) magnification under light microscopy. Airway types were defined as follows: alveolar duct = walls consisting entirely of alveoli; respiratory bronchiole = occasional mural alveoli, low cuboidal epithelium; terminal bronchiole = low cuboidal epithelium, intact walls; bronchiole = respiratory epithelium, no cartilage; and bronchus = respiratory epithelium and cartilage. We defined terminal bronchiole (low cuboidal epithelium, intact walls) as peripheral airways, and bronchiole or bronchus (respiratory epithelium, cartilage, or no cartilage) as central airways as described elsewhere [7]. The number of goblet cells in the airway epithelium was counted in at least 20 sections by measuring the length of 100 μm epithelium defined as basement membrane and the luminal area using a soft program (Carl Zeiss Axiovision Viewer & Sample Images, Germany). Results are expressed as the percentages of goblet cells among the epithelial cells.

Statistical Analysis

Values for all measurements are expressed as mean $\pm$ SEM. Cellular composition of the BAL fluid, serum levels of anti-OVA IgE, IgG1, and lung cytokine mRNA expression were compared with a Mann–Whitney *U*-test for unpaired data. Dose–response curves to Ach were compared by repeated measures analysis of variance (ANOVA) with Fisher's PLSD performed as a post hoc analysis. Differences were considered to be significant if $P < .05$. The correlations were analyzed between the percentages of goblet cells in peripheral airways and Cdyn, R_L by Pearson's correlation coefficient. All analyses were performed with SPSS 11.0 software (SPSS Inc., Chicago, Ill., USA).

RESULTS

Time Course of Ach-induced R_L and Cdyn

On day 18, OVA-sensitized mice after RSV exposure (OVA/RSV group) demonstrated an increase in lung resistance compared with OVA-allergic mice alone (OVA group) ($P < .05$), and developed a dose-dependent Ach-induced bronchospasm. Conversely, both OVA/RSV group and RSV group mice performed definitely lower Cdyn than that in OVA group

($P < .05$), which also exhibit as dose-dependant manner. By day 23, R_L in OVA group was not greater than that in PBS group. In contrast, OVA/RSV group had greater R_L compared with any other groups ($P < .05$). Similarly, There was no difference in Cdyn between the OVA group and PBS group ($P > .05$), but OVA/RSV group and RSV group had still prominently lower Cdyn than that in OVA group ($P < .05$). By day 28, R_L in OVA/RSV group had decreased to the levels of the other groups. In striking contrast, OVA/RSV group and RSV group still showed comparable decreases in Cdyn ($P < .05$). By day 33, R_L in OVA/RSV group returned to baseline levels and there was no difference among the four groups. However, Cdyn in OVA/RSV group and RSV group improved somewhat, but it was still lower than that in other groups ($P < .05$) (shown in Figure 2).

Lung Cellular Inflammation

As shown in Figure 3, with the exception of increased number of lymphocytes, RSV exposure also led to an increase in the number of BAL neutrophils and macrophages in OVA-sensitized mice ($P < .05$), but not eosinophils, when compared with OVA-sensitized mice alone.

Anti-OVA IgE and IgG1 Response

Serum levels of OVA-specific IgE were significantly increased in the OVA/RSV challenged group ($P < .05$ compared to the OVA group). Similarly, levels of OVA-specific IgG1, which is induced in parallel with IgE in mice, were also strikingly increased in the OVA/RSV challenged group ($P < .05$ compared to the OVA group) (Figure 4).

IL-4, IL-5, IL-13, IFN- γ , IL-17, and IL-17F mRNA Expression After RSV Infection

As shown in Figure 5, Th2 cytokine (IL-4, IL-13) mRNA expression was increased after RSV exposure in OVA-sensitized mice when compared with OVA-allergic mice, but not IL-5. Simultaneously, RSV also gave rise to an increase in IFN- γ mRNA expression. However, no increase in expression of IL-17, IL-17F mRNA was demonstrable after RSV exposure in OVA-sensitized mice ($P > .05$ compared to OVA group). Of interest, there was significantly elevated relative expression of IL-17, IL-17F after RSV infection only.

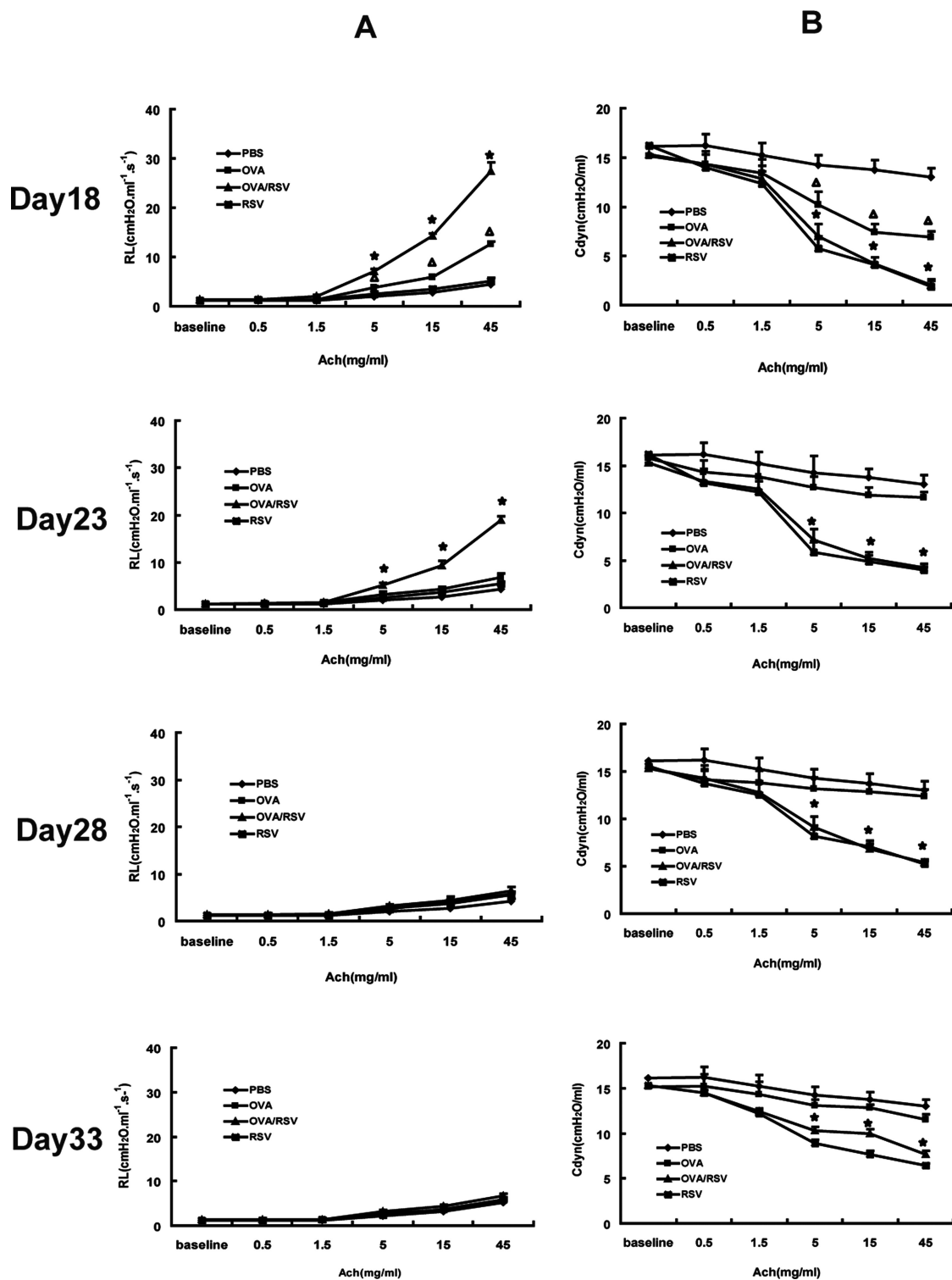


FIGURE 2. Kinetics of airway function performed on days 18, 23, 28, and 33 respectively. (A) Lung resistance is measured as cmH₂O/(mL s). (B) Dynamic compliance is in all cases assessed as cmH₂O/mL. Data points represent the mean $\pm$ SEM. * $P < .05$ compared with PBS group. * $P < .05$ compared with PBS group.

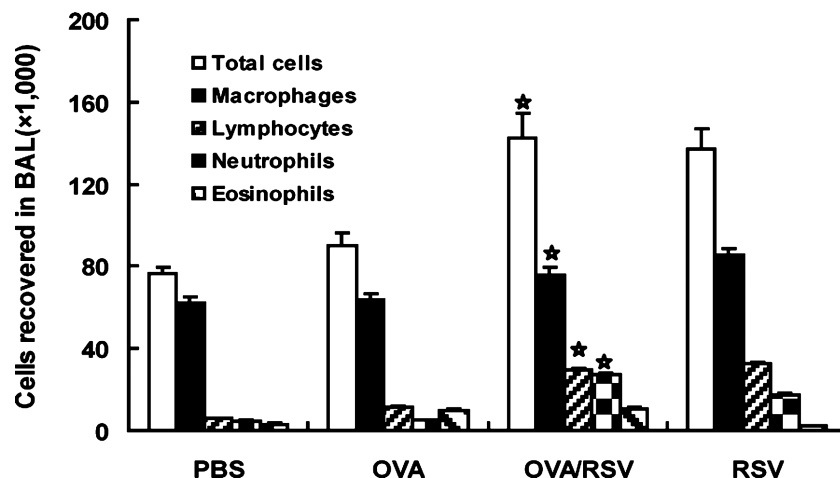


FIGURE 3. Composition of cell counts from BAL fluid measured on day 18. Each bar represents the mean $\pm$ SEM. * $P < .05$ compared with OVA group.

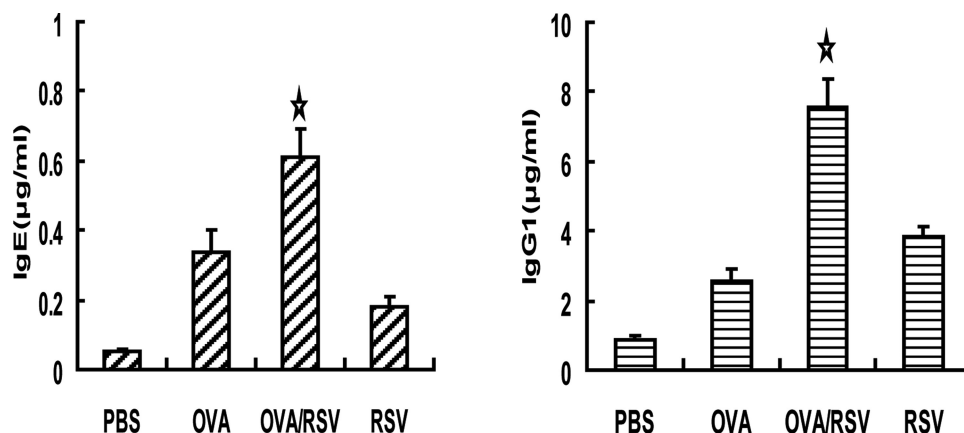


FIGURE 4. Serum levels of OVA-specific IgE and IgG1 measured on day 18. Each bar represents the mean $\pm$ SEM. * $P < .05$ compared with OVA group.

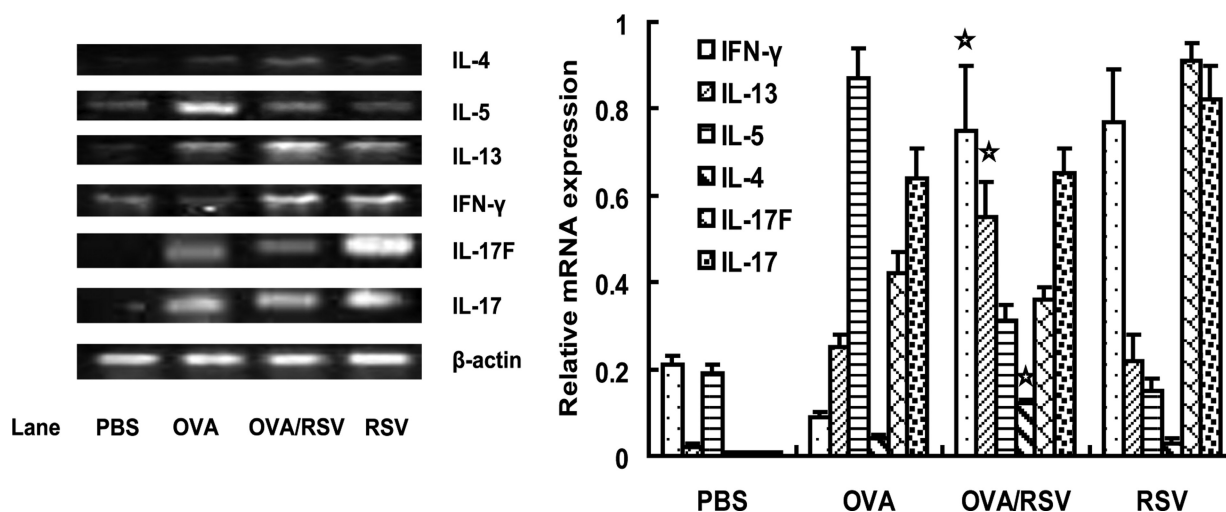


FIGURE 5. The expression of interleukin (IL)-4, IL-5, IL-13, interferon (IFN)- γ , IL-17, and IL-17F mRNA in lung tissue in different groups. Relative expression against β -actin of lung interleukin (IL)-4, IL-5, IL-13, and interferon (IFN)- γ determined on day 18 of the experimental protocol. Mean values $\pm$ SEM are depicted. * $P < .05$ compared with OVA group.

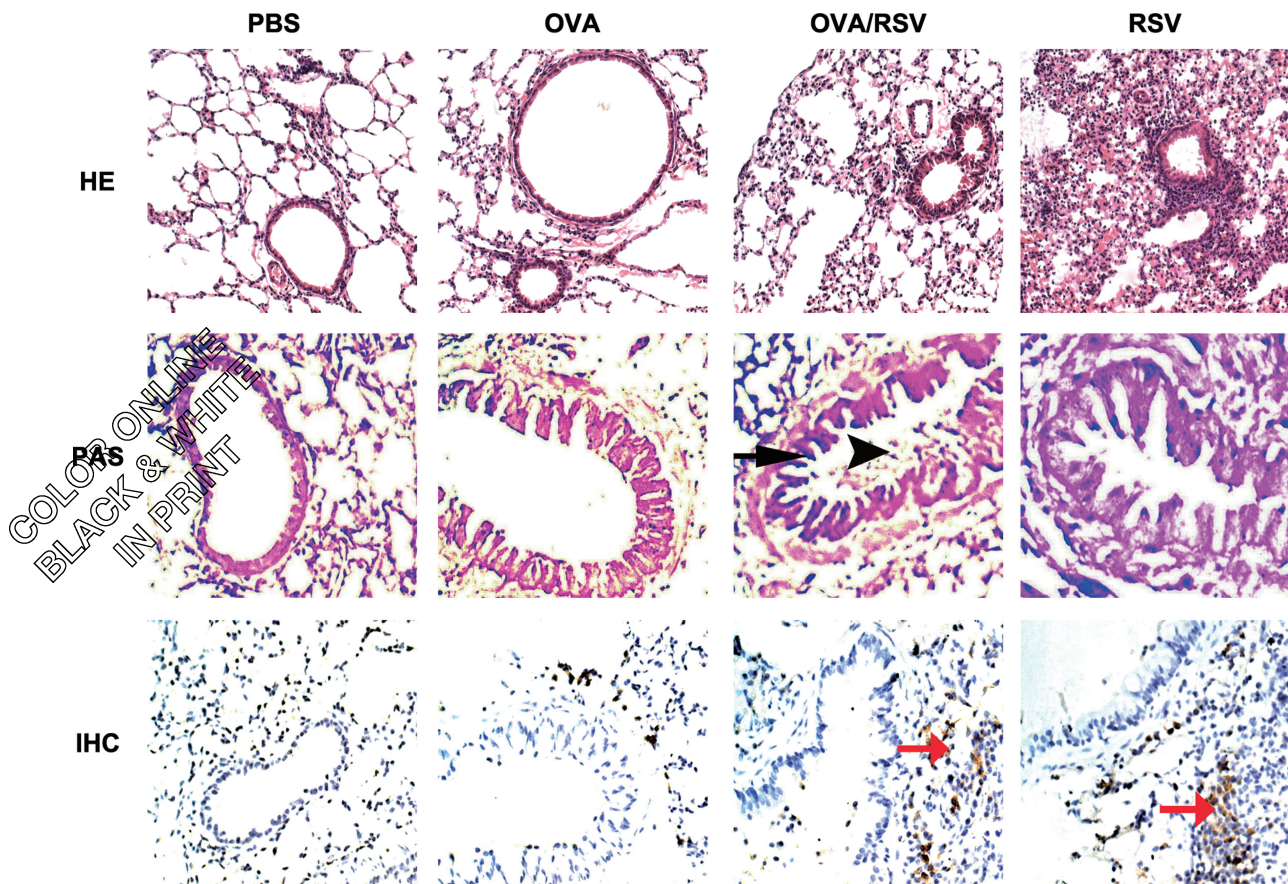


FIGURE 6. Histological analyses of lung sections stained with hematoxylin and eosin (H&E $\times 200$), periodic acid-Schiff (PAS $\times 400$), and immunohistochemistry (IHC $\times 400$) of CD8+ T cell infiltration performed on day 18 of the experimental protocol.

Histological Changes

The inflammatory characteristics were summarized in Figure 6. The peribronchial infiltrate in the OVA mice was composed primarily of eosinophils, whereas, the infiltrate in the RSV mice consisted mainly of mononuclear cells. Furthermore, RSV also resulted in hemorrhage in interstitial and alveolar, epithelial cells destruction in peripheral airways except for serious inflammation infiltration. However, the peribronchial infiltrate in the OVA/RSV mice had a number of eosinophils, mononuclear cells and granulocytes. PBS group of mice had no peribronchial inflammation (H&E staining).

The distribution and magnitude of mucus production in the airways also varied greatly in different group mice. RSV exposure in OVA-sensitized mice showed numerous PAS-positive cells (long black arrow) and intrabronchiolar mucus pool (short black arrow) in peripheral airways. On the contrary, RSV infection alone mice revealed few PAS-positive cells in peripheral airways, and intrabronchiolar mucus pool was not observed. Similarly, OVA sensitiza-

tion, without RSV exposure, was associated with the appearance of few PAS-positive cells. In no allergic mice, PAS positivity was not observed (PAS staining).

The distribution of CD8+ T cells was also different. RSV exposure in OVA-sensitized mice revealed enhanced CD8+ T cell infiltration around peripheral airways (red arrow), but RSV infection alone mice presented enhanced CD8+ T cell infiltration prone to lung tissue. In OVA-allergic mice, CD8+ T cell infiltration was almost not observed (IHC staining).

Correlations of PAS-positive Cells and Cdyn, R_L

As shown in Figure 7, the percentages of PAS-positive cells in peripheral airways negatively correlated with Cdyn ($\gamma = -0.679$, $P = .008$). In contrast, there were no correlations with the percentages of PAS-positive cells and R_L ($\gamma = 0.152$, $P = .089$).

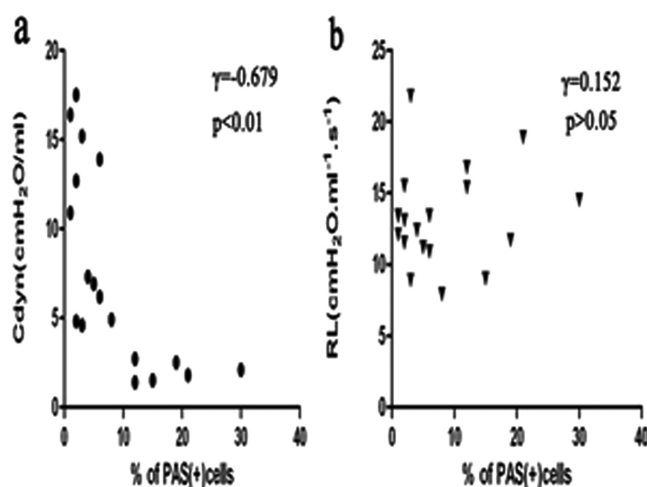


FIGURE 7. The correlation of the percentages of goblet cells among epithelial cells in peripheral airways with dynamic compliance (C_{dyn}) and lung resistance (R_L) analyzed on day 18 of the experimental protocol. The percentage of PAS-positive cells were negatively correlated with C_{dyn} ($r = -0.679$, $P < .01$), but not R_L ($r = 0.152$, $P > .05$).

DISCUSSION

Measurement of R_L and C_{dyn} not only provides the classical determination of airway responsiveness, but also provides a more detailed insight into pulmonary mechanics. It has been proposed that C_{dyn} primarily reflect narrowing of peripheral airways and the elasticity of the lung parenchyma. In contrast, R_L represent airflow obstruction of central airways [10]. RSV triggered exacerbation of asthma by inducing both central and peripheral airway inflammation. However, the characteristic of peripheral airway inflammation and the influence of RSV on the peripheral airway function were still unknown. In this study, we have defined the time-course of airway responsiveness in mice sensitized with OVA, mice with combined OVA-sensitization and RSV-challenge, and mice infected with RSV alone. RSV increased R_L and prolonged AHR in OVA-sensitized mice when compared with OVA-allergic mice alone, a finding consistent with others [7]. Conversely, RSV substantially decreased the C_{dyn} and prolonged the recovery period of C_{dyn}. Furthermore, full recovery of C_{dyn} in OVA/RSV group mice extend much longer than that of R_L, which implied RSV elicited more severe inflammation in peripheral airways than that in central airways in the setting of allergic mice. In other words, the restoration of peripheral airway inflammation was much harder than that in central airways.

To further assess the alternation in pathology underlie in physiology, the distribution of airway inflammation and mucus-producing, PAS positive cells

were evaluated. OVA-allergic mice exhibited central airway inflammation and PAS-positive cells were mainly distributed in the central airways (data not shown), which was in line with the report of others [11, 12]. However, RSV elicited enhanced airway inflammation both in the central and peripheral airways in OVA-sensitized mice. Furthermore, PAS-positive cells were predominately distributed in the peripheral airways. The alteration in distribution of PAS-positive cells and mucus production was accompanied by increased intraluminal pools of mucus. Thus, our study revealed critical difference in spatial distribution of PAS-positive cells between RSV exposure in OVA-sensitized mice and OVA-allergic mice alone.

Generally, airway goblet cell hyperplasia has been established as a pathologic characteristic of asthma. Mucus hypersecretion by hyperplastic goblet cells in airways contributes to the pathophysiology of asthma [13] and significantly correlates to patient morbidity and mortality [14]. Hashimoto [15] demonstrated that RSV infection augmented mucus production in the setting of allergic lung inflammation probably upregulates gob-5 expression, which then increases Muc5ac (murine MUC5AC homolog) production. Additionally, others have also reported that RSV induces emptying of mucin from the airway cells in OVA-sensitized mice [16]. One hypothesis is that susceptibility to mucous plugging of airways is determined by the existence and degree of goblet cell metaplasia that exists in the peripheral airways [17]. Thus, some patients may have massive overgrowth of peripheral airways by goblet cells, as seen in the airways of patients with mucous plugging who died of acute, severe asthma. It is reasonable to assume that asthmatic individuals with marked goblet cell hyperplasia, especially in the peripheral airways, may be most at risk from stimuli (e.g., viral infections) which may trigger goblet cell degranulation. Our results verified, to some extent, above-mentioned viewpoint. Moreover, the negative correlation of goblet cell ratio with lung C_{dyn} observed in our study confirmed that goblet cell hyperplasia in peripheral airways may conclusively correlated with the decrease of C_{dyn}, but not R_L. How goblet cell hyperplasia/mucus production may be linked to C_{dyn} and not to R_L is unclear at present but peripheral airway epithelial function may be a critical factor in the development of altered dynamic compliance.

RSV induced influx of a great number of neutrophils, lymphocytes, and macrophages in BAL fluid in OVA-sensitized mice, which was remarkably different from that of OVA-allergic mice. Neutrophil-derived chemokines (IL-8, (MIP)-1a, MIP-1b) and neutrophil granule contents can not only cause further inflammation, hyperresponsiveness, and damage

of the airways [18], neutrophil recruitment, by releasing elastase, could result in goblet cell hypersecretion and induce mucin MUC5AC gene expression in airway epithelium [19] and mucus production [20]. On the other hand, goblet cell hyperplasia and mucous plugging are common in patients with acute asthma and these patients also showed neutrophil recruitment into the airways [21]. Furthermore, airways of severe acute asthmatics showed great, 30-fold increases in goblet cell area in peripheral airways with marked accumulation of mucus in airway lumens [17], but not in large airways. Therefore, it was reasonable to speculate that neutrophil inflammation accompanied by RSV infection may be link to the pathophysiological alternation. Additionally, with the exception of increased number of BAL lymphocytes, a large amount of CD8+ T lymphocytes distributing around peripheral airways were also observed, but not in central airways. The prominence of CD8+ T cells in the peribronchiolar and alveolar interstitial infiltrated may be consistent with the correlation of CD8+ T cells with virus clearance. Airway CD8+ cytotoxic T cells are important effect cells for antiviral and the typical response to RSV infection in BALB/c mice includes a type-1 pattern of cytokine expression. But the T-cell response to RSV was altered by the milieu associated with the allergic inflammation produced by allergen sensitization and converted to Th2 inflammation (such as IL-4, IL-5, IL-13) [22] which was also confirmed in our previous finding [9]. This kind of RSV-specific T-cell response, in turn, potentiates the allergen-induced inflammation and enhances the release of cytokines and other mediators in peripheral airways that was probably responsible for the decline of Cdyn.

RSV did lead to a significant increase in the IL-4, IL-13, and IFN- γ mRNA expression in OVA-sensitized mice than that in OVA-allergic mice. IL-4 has been demonstrated in the immunologic cascade, resulting in allergen-driven AHR [23]. Furthermore, Temann et al. reported that the Th2 cytokine IL-4 plays a central role in the induction of mucin glycoprotein gene expression and mucin release in mouse respiratory epithelium [24]. IL-13 is the major effect cytokine of goblet cell hyperplasia, airway hyperreactivity [25]. Apart from this, it has also been shown that Th2 cytokine IL-13 can activate neutrophils and lead to neutrophil recruitment to the airways [26], which implied that IL-13 may be correlated with decline of Cdyn. It is amazing that IFN- γ mRNA expression also increased. Generally, Th1 cytokine IFN- γ overcount with Th2-type airway inflammation in allergic asthma. However, data from the current study in mice suggest that the Th1- and Th2-type cytokine responses are not mutually exclusive and that

there is an overall enhancement of proinflammatory cytokine production [27]. Whether IFN- γ played a role in the reduction of Cdyn was under further exploration. It was reported that IL-17, produced by a subset of CD4 helper T cells (Th17 cells), can stimulate Muc5ac expression in primary human tracheobronchial epithelial cells [28]. Of note, our data showing that RSV exposure in OVA-sensitized mice does not increase IL-17, IL-17F mRNA expression, which is contrary to a report of Peebles [6]. Possible explanations for these differences may link to the dose of virus given. In our study, mice were infected with 1×10^5 plaque-forming units of RSV intranasally. On the contrary, theirs with 1×10^7 plaque-forming units of RSV. It is reasonable to speculate that the increased mucus expression in the OVA/RSV group was not associated with increased lung IL-17, IL-17F in our model.

Interestingly, RSV infection alone also caused significant decline in Cdyn, but had little effect on the R_L . As we know RSV replicates best at 37°C and therefore preferentially infects respiratory epithelium of the lower airways. Furthermore, apart from destruction of peripheral airway epithelium, RSV infection alone resulted in lesions located predominantly in avoletr, which exhibit the character of reduction in lung elastic recoi and collapsion of avoler. In this regard, we hypothesized that the disparity in the mechanism of decreased Cdyn in RSV group versus OVA/RSV group mice may be due main to its impact on interstitial and alveolar, but not goblet cell hyperplasia in distal airways.

In summary, our findings demonstrate that RSV differentiated airway dysfunction in the central and peripheral airways in OVA-sensitized mice which exhibit as disassociation of Cdyn and R_L . The data also highlighted that goblet cell hyperplasia and CD8+ T lymphocyte infiltration around peripheral airways may be link to the decline of Cdyn. Clearly, such events observed will be a clinically important consideration. As AHR measured solely as changes in R_L may not totally define airway function, the accurate detection of distal airway dysfunction is important in RSV-induced asthma. Although the clinical significance of the reduced Cdyn which represent the peripheral airway dysfunction is not yet well-known, it is possible that poorly controlled inflammation in peripheral airways, which are not penetrated by conventional inhaled steroids, may contribute to accelerated decline in lung function and airway remodeling—the hallmarks of asthma. The further identification of the requirements for alteration of specific components of lung function has important implications for the treatment of the complex syndrome of asthma, especially virus-related asthma.

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

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