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# Effects of Respiratory Syncytial Virus Infection on Dendritic Cells and Cysteinyl Leukotrienes in Lung Tissues of a Murine Model of Asthma

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## ABSTRACT

**Background:** Pulmonary dendritic cells (DCs) play critical roles in both allergy and in viral infection. Levels of cysteinyl leukotrienes (cysLTs) increase after allergen sensitization and viral infection and can modulate the migration and functions of DCs. The present study examines the effects of respiratory syncytial virus (RSV) infection on numbers of DCs and cysLT concentrations in lung tissues of mice sensitized with mite allergen.
**Methods:** We examined Control, *Dermatophagoides farinae* allergen sensitized (Df), RSV infected (RSV) and Df allergen sensitized and RSV infected (Df-RSV) Balb/c mice. We then determined the number of CD11c-positive DCs and the LT concentration in lung tissues of the mice and examined lung pathology and cytokine profiles in thoracic lymph nodes.
**Results:** Infection with RSV significantly enhanced allergic airway inflammation in Df mice with concomitant increases in Th1 and Th2 immunity. The number of DCs and the cysLT concentrations were significantly increased in the lungs of Df and RSV mice and more so in Df-RSV, than in Df mice.
**Conclusions:** The present findings suggest that RSV infection increases the number of DCs and the cysLT concentrations in lung tissues of asthma patients, both of which could result in enhanced allergic airway inflammation.

## KEY WORDS

asthma, cysteinyl leukotrienes, dendritic cell, *Dermatophagoides farinae*, respiratory syncytial virus

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## INTRODUCTION

Bronchial asthma is a representative chronic pulmonary inflammatory disease characterized by infiltration with inflammatory cells such as eosinophils, lymphocytes and mast cells.[1] Acute exacerbation of asthma symptoms is potentially life threatening and results in significant costs.[2] Viral respiratory tract infections are the most frequent causes of asthma exacerbations in both adults and children.[3,4] In addition to sensitization with mite allergens, viral respiratory tract infection is also considered a critical risk factor for the development of asthma.[5] Yet according to the so-called hygiene hypothesis, repeated respiratory tract infection during childhood could prevent the development of asthma.[6] Thus the interaction between viral respiratory tract infections and allergic asthma remains uncertain. Among respiratory viruses, respiratory syncytial virus (RSV) has attracted attention because several epidemiological studies have suggested that infants infected with RSV subsequently develop asthma later in childhood.[7]

Dendritic cells (DCs) are specialized antigen presenting cells in the airway that play critical roles in

the pathogenesis of both asthma and viral respiratory infections.[8,9] Cysteinyl leukotrienes (cysLTs) are critically involved in the pathogenesis of asthma and they are produced in the airway during respiratory infection where they are involved in protection against respiratory pathogens.[10] Additionally, cysLTs could affect DC migration and function.[11,12] Based on these findings, the present study focuses upon DCs as a cellular component and on cysLTs as liquid mediators to determine the relationship between viral respiratory tract infection and allergic asthma.

## METHODS

### MICE
Specific pathogen-free female Balb/c mice aged 4–6 weeks (Charles River Laboratories, Yokohama, Japan) were housed under pathogen-free conditions at the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine. The Nagasaki University School of Medicine Committee on Animal Research reviewed and approved all experimental procedures. Each experiment was repeated at least three times.

### RSV PREPARATION AND INOCULATION
The A2 strain of human RSV (American Type Culture Collection [ATCC], Rockville, MD, USA) was propagated in monolayers of HEp-2 cells (ATCC). A large stock of the virus was prepared by infecting HEp-2 cells with the ATCC stock virus, and then collecting the medium 5 or 6 days later. Cellular debris was removed by centrifugation at 2,000 rpm for 10 minutes at 4℃. The concentration of the virus was adjusted as assessed by quantitative plaque-forming assays. Clarified supernatant was collected and stored at –70℃. Mice were infected under light ether (Wako, Osaka, Japan) anesthesia by intranasal RSV inoculation [5 × $10^5$ plaque forming units (PFU)/50 $\mu$l]. Infection was confirmed by reverse transcriptase polymerase chain reaction to detect RSV N protein mRNA in the lung tissues of the mice as described.[13] Controls were sham-infected in a similar manner with RSV that had been inactivated by exposing medium from infected cells to ultraviolet (UV) light for 15 minutes on ice.

### EXPERIMENTAL PROTOCOL
Control, *Dermatophagoides farinae* sensitized (Df), RSV infected (RSV), and Df sensitized plus RSV infected (Df-RSV), Balb/c mice were immunized twice intraperitoneally on days 1 and 14 with 0.5 mg/mouse of Df allergen (LG-5339, Cosmo Bio, Tokyo, Japan) precipitated in aluminum hydroxide. The mice were then intranasally challenged with 50 $\mu$l of PBS (Control and RSV groups) or with 50 $\mu$g/$\mu$l of Df allergen (Df and Df-RSV groups) for 3 consecutive days (days 14–16) and sham infected with UV inactivated RSV (Control and Df groups) or with live RSV (RSV and Df-RSV groups) on day 17. Lungs were harvested 4 days after RSV infection (day 21).

## SEMIQUANTITATION OF PULMONARY INFLAMMATION AND DCS
Lung sections stained with hematoxylin and eosin (HE) were coded and evaluated at least twice in a blinded fashion by three observers as described.[14] Sections were also immunohistochemically stained with murine anti CD11c mAbs (PharMingen, San Diego, CA, USA) and CD11c positive pulmonary DCs were also counted. The numbers of eosinophils, lymphocytes and DCs were determined in 10 peribronchovascular areas per section under an oil immersion lens. The results are expressed as mean numbers of cells from each group.

### Analysis of Cytokine Production
Single cell suspensions were prepared from thoracic lymph nodes (2.0 × $10^5$/200 $\mu$l) of all groups of mice and cultured in the presence of 100 $\mu$g/ml of Df allergen as described.[15] The production of IL-5 and IFN-γ in 48-hour culture supernatants was determined by ELISA (Quantikine, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

### LT CONCENTRATIONS IN LUNG HOMOGENATES
To measure the concentrations of cysLTs and LTB4, lungs were homogenized in 3 ml of lysis buffer (0.5% Triton X-100, 150 mM NaCl, 15 mM Tris, 1 mM CaCl2, and 1 mM MgCl2, pH 7.4). The homogenates were incubated on ice for 30 minutes, and then centrifuged at 3,000 rpm for 10 minutes. Supernatants were collected and passed through a filter (0.2 $\mu$m pore size). The concentrations of cysLTs and LTB4 in lung homogenates were assayed by enzyme immunoassays (EIA, Cayman Chemical Company, Ann Arbor, MI, USA). The results are expressed as absolute amounts of LTs per wet weight of the lung.

### Statistical Analysis
Results are expressed as means ± standard error of the mean (SEM). Data were evaluated using the repeated-measures ANOVA with a Bonferroni multiple comparison test. A *P*-value of <0.05 was considered to indicate a significant difference.

## RESULTS

### RSV INFECTION ENHANCED Df-INDUCED ALLERGIC AIRWAY INFLAMMATION
Figure 1A shows representative lung pathology of the four groups of mice. Sensitization with Df allergen resulted in eosinophilic infiltration and goblet cell metaplasia, while RSV infection caused mononuclear cellular infiltration. Infection with RSV enhanced Df-induced pulmonary inflammation. Semi-quantitative analysis of lung tissue revealed that Df sensitization significantly increased the number of eosinophils and

A



B

(cells)
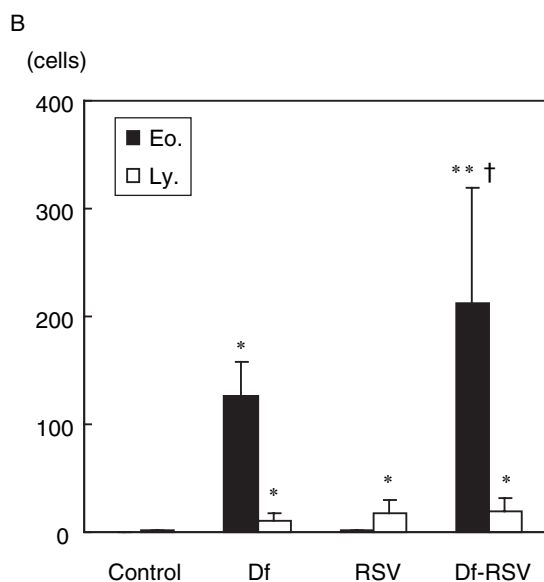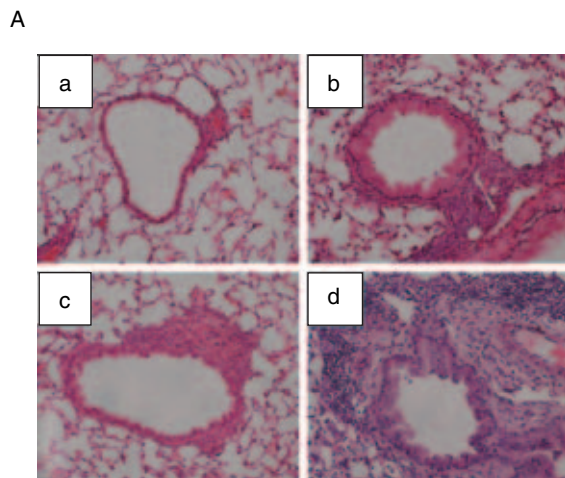


**Fig. 1** Pulmonary pathology. (**A**). Representative photomicrographs show lung tissues stained with HE from 4 groups of Balb/c mice (*n* = 8 per group). **a.** Control, **b.** Df, **c.** RSV, **d.** Df-RSV. (**B**). Semi-quantitative analysis of pulmonary pathology. Bars represent mean ± SEM (*n* = 8 per group). **, $p < 0.01$ and *, $p < 0.05$ *vs*. Control; †, $p < 0.05$ *vs*. Df.

lymphocytes, whereas RSV infection significantly increased that of lymphocytes (Fig. 1B). In addition, RSV infection significantly increased eosinophils in Df-RSV mice compared with Df mice.

## INFECTION WITH RSV MODULATED CYTOKINE PROFILES IN LUNG TISSUES OF Df SENSITIZED MICE

Analysis of cytokine profiles in draining lymph nodes of the lungs revealed that Df sensitization elicited IL-5 dominant Th2-like responses, whereas RSV infection induced IFN-γ dominant Th1-like responses (Fig. 2). Primary RSV infection significantly enhanced both IL-

(pg/ml)



(pg/ml)
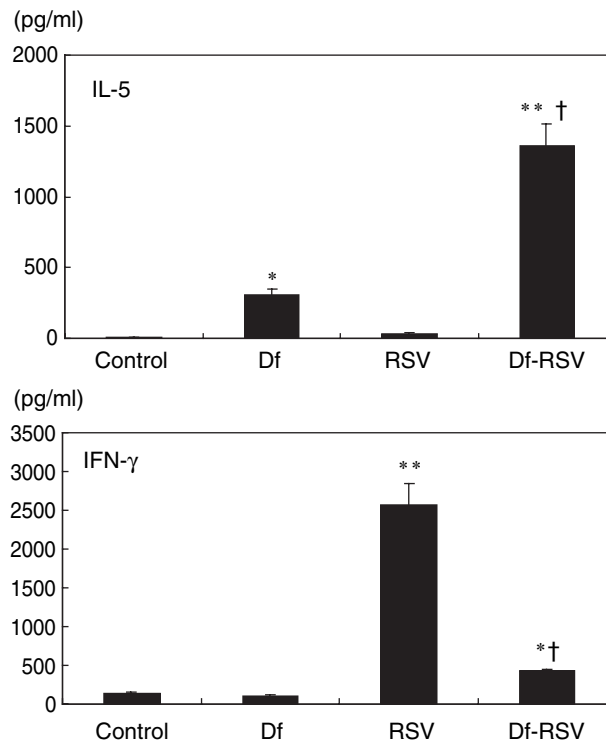


**Fig. 2** Cytokine production from Df-stimulated thoracic lymph nodes. Single cells from thoracic lymph nodes of 4 groups of Balb/c mice were stimulated with Df allergen. Results are shown as means ± SEM (*n* = 8 per group) of IL-5 and IFN-γ in culture supernatants. **, $p < 0.01$ and *, $p < 0.05$ *vs*. Control; †, $p < 0.05$ *vs*. Df.

5 and IFN-γ production in Df-RSV mice compared with mice sensitized with Df alone.

## INCREASES IN NUMBER OF PULMONARY DCs IN RSV-INFECTED AND/OR Df-SENSITIZED MICE

Figure 3 shows the immunohistochemical results for murine CD11c in lung tissues of the four groups of mice. Both Df sensitization and RSV infection significantly increased the number of pulmonary CD11c positive DCs compared with the control. Compared with mice sensitized with Df alone, RSV infection significantly increased the number of pulmonary DCs in the lung tissues of DF-RSV mice.

## Df SENSITIZATION AND/OR RSV INFECTION INCREASED CONCENTRATION OF LUNG cysLT, BUT NOT LTB₄

Both Df sensitization and RSV infection significantly increased the amount of cysLTs in lung homogenates of all four groups of mice and RSV infection further increased the cysLT concentration in Df-RSV mice (Fig. 4). In contrast, the LTB4 concentrations in all four groups of mice did not significantly differ.
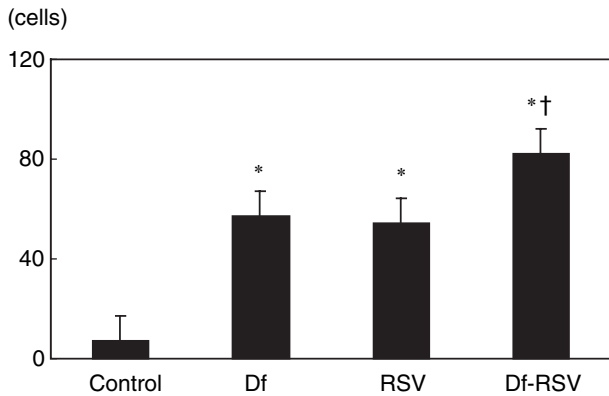
(cells)



**Fig. 3** Semi-quantitative analysis of pulmonary CD11c positive dendritic cells. Numbers of CD11c positive pulmonary dendritic cells were compared in 4 groups of Balb/c mice. Results are shown as means $\pm$ SEM ($n = 8$ per group) of CD11c positive pulmonary dendritic cells. *, $p < 0.05$ *vs*. Control; †, $p < 0.05$ *vs*. Df.

## DISCUSSION

In agreement with our previous findings,[16] primary RSV infection significantly enhanced mite allergen-induced allergic airway inflammation associated with both Th1 and Th2 responses. Infection with RSV significantly increased the number of pulmonary DCs and the cysLT concentration, both of which could be involved in the pathogenesis of virus-induced asthma exacerbation.

Pulmonary dendritic cells are airway antigen presenting cells. Immature DCs take up and process antigen, a process that initiates DC maturation. Mature DCs then migrate to regional lymph nodes and present antigen naïve T cells and initiate primary T cell responses to either Th1 or Th2. Thus DCs are essential in the development and maintenance of pulmonary inflammatory responses including infection and allergy. In fact, deleting CD11c-positive DCs causes allergen-induced allergic airway inflammation to deteriorate in a murine model.[17] Inhaled allergen rapidly shifts DCs from the bloodstream to the airway mucosa in human asthma patients.[18] In accordance with that finding, the present study demonstrated that challenge with mite allergen significantly increased the number of DCs in lung tissue of a murine model of allergic asthma. Thus, increases in pulmonary DCs potentially enhance subsequent allergen challenge and/or respiratory viral infection.

As well as allergen challenge, RSV infection also causes DC mobilization to the airway mucosa in children,[19] and increases the number of pulmonary DCs and their capacity for antigen processing and presentation in mice.[20] Similarly, the present study also demonstrated that RSV infection significantly increased the number of DCs *per se* and even further

(pg/ml/g lung)



(pg/ml/g lung)
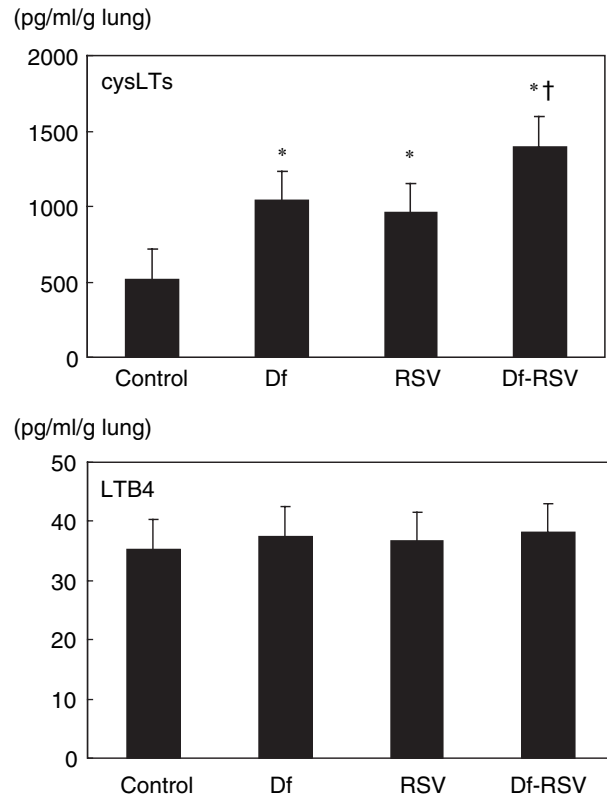


**Fig. 4** Concentrations of leukotrienes in lung tissues. Concentrations of cysLTs and LTB4 in lung homogenates of 4 groups of Balb/c mice determined by EIA. Results are shown as means $\pm$ SEM ($n = 8$ per group) of cysLTs and LTB4. *, $p < 0.05$ *vs*. Control; †, $p < 0.05$ *vs*. Df.

increased the number in mice sensitized with mite allergen.

Several biological properties of CysLTs are associated with immediate allergic responses including bronchoconstriction, mucous hypersecretion, attracting proinflammatory cells including eosinophils and increasing capillary permeability. Viral infection frequently increases cysLT concentrations in asthma patients.[21,22] Antagonists to CysLT receptors subdue symptoms in children with RSV-bronchiolitis and prevent virus-induced acute exacerbation of asthma in children and in a murine model.[23-25] The present study found that RSV infection, like mite allergen sensitization, significantly increased the amount of cysLTs in lung tissues. Infection with RSV significantly and additionally increased cysLTs in Df mice. Collectively, cysLTs could be a representative mediator in the interaction between viral infection and asthma.

We previously reported that DCs derived from murine bone marrow and from human monocytes express cysLT receptors.[26,27] CysLTs cause antigen-presenting Langerhans cells in the skin to migrate to accessory lymph nodes[11] and enhance the ability of

DCs to induce Th2-like responses.[12] Although the present study did not directly demonstrate this interaction, RSV infection probably increases pulmonary concentrations of cysLTs, which cause DC activation and result in enhanced allergic airway inflammation. Taken together, the present findings suggest that cysLTs are potent therapeutic targets for the treatment and prevention of virus-induced exacerbation of asthma. The clinical application of anti-LT therapy to treat virus-induced asthma exacerbation should be promising.

## ACKNOWLEDGEMENTS

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