

**Effects of repeated respiratory syncytial virus infections on pulmonary dendritic  
cells in asthma model**

**Running title:** RSV infection and dendritic cells

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**ABSTRACT**

**Background:** Primary and secondary respiratory syncytial virus (RSV) infection differentially regulates pre-existing allergic airway inflammation.

**Objectives:** The present study is designed to determine the effects of primary and secondary low-grade RSV infections on functions of pulmonary dendritic cells .

**Methods:** Eight groups of BALB/c mice were used: one group each for control primary and secondary sensitization, primary and secondary sensitization to *Dermatophagoides farinae* (*Derf*) allergen, primary and secondary infection with RSV, and primary and secondary sensitization to *Derf* plus infection with RSV. CD11c positive pulmonary dendritic cells were isolated from these mice and then transferred to naïve mice followed by intranasal *Derf* challenge. Furthermore, either anti-IL-12 monoclonal antibody ( $\alpha$ IL-12 mAb) or  $\alpha$ IL-10 mAb were injected into donor mice after *Derf* challenge and during RSV infection to determine the involvement of IL-12 and IL-10.

**Results:** Primary RSV infection failed to induce polarization in DCs since it failed to induce IL-10 and IL-12 production in *Derf*-sensitized donor lung. In contrast, secondary RSV infection significantly enhanced IL-12 production from *Derf*-sensitized donor lung, thereby enhancing both Th1 and Th2 responses.  $\alpha$ IL-12, but not  $\alpha$ IL-10 mAb treatment during RSV infection blocked these immunological effects.

**Conclusion:** Dendritic cells *via* IL-12 may play a critical role in shifting the immune response in this experimental model of repeated respiratory viral infection in allergic asthma.

**Key words:** airway inflammation, asthma, dendritic cell, respiratory syncytial virus, IL-12, *Dermatophagoides farinae*

## Introduction

Allergic asthma is a chronic inflammatory pulmonary disease characterized by airway narrowing due to eosinophilic airway inflammation, mucus hypersecretion, and airway hyperreactivity in response to inhaled allergen [1-6]. Viral respiratory tract infections are the most frequent causes of asthma exacerbation in both children and adults, and they represent one of the critical risk factors in the development of asthma [7-9]. Among respiratory viruses, respiratory syncytial virus (RSV) has attracted special attention as a cause of allergic sensitization and exacerbator of asthma [10-13]. The interaction between viral infection and allergic asthma can result in a paradoxical response, i.e., primary viral infection induces increased production of IFN- $\gamma$ , a T helper (Th) 1 cytokine, which attenuates the Th2-like response generally observed in allergic asthma. In fact, RSV reinfection is common throughout life because immunity to it is incomplete and transient [14, 15], and according to the so-called “hygiene hypothesis,” repeated infections in early childhood might prevent the development of allergic asthma [16]. Thus interaction between viral infection and immunity in asthma remains uncertain.

Factors potentially affecting the outcome of viral infection in allergic asthma include frequency of infection and viral load. Epidemiologic study indicates repeated, but not

single, episodes of respiratory infection can cause subsequent pulmonary dysfunction [17]. Using a model of RSV infection in mite allergen-sensitized mice, we found that primary and secondary RSV infections differentially regulate allergic airway inflammation [18, 19]. Viral load is also a factor in this interaction. Primary high grade RSV infection transiently enhances allergic airway inflammation, while primary low grade RSV infection attenuates allergic airway inflammation [18, 19]. Although underdiagnosed, RSV infection occurs not only in children but also in adults [20]. Clinically, viral load is lower in adults than children, with the presence of virus in respiratory samples being detectable only by use of sensitive techniques such as polymerase chain reaction (PCR). We previously developed a murine model of low-grade RSV infection resembling subclinical RSV infection in human adult subjects [19].

Dendritic cells (DCs) are the major airway antigen-presenting cells involved in the induction of the primary immune response [21]. DCs are involved in antigen uptake, processing, and presentation of antigenic fragments to T cells in the respiratory mucosa [22]. Furthermore, DCs determine the type of T cell-mediated immune response (Th1 or Th2) [23,24] and play a central role in initiating both allergy and infection [25-27]. Since respiratory viruses (including measles and RSV) directly infect DCs and modulate

their function [28-31], we previously hypothesized that RSV infection might enhance the Th2-like response induced by aeroallergen-sensitized DCs. Unexpectedly, however, bone marrow derived DCs (BM-DCs) from mice that were infected only once with RSV failed to enhance but rather attenuated aeroallergen-induced Th2-like responses [32]. At the same time, it was reported that influenza A infection induced a robust IFN- $\gamma$  response, promoting both DC-mediated Th1- and Th2-like responses [33].

Taken together, we hypothesized that primary and secondary low grade RSV infection might differently regulate pulmonary DCs function in our mite allergen sensitized murine model of allergic asthma. We therefore investigated the effects of primary and secondary low-grade RSV infections on functions of pulmonary DCs.

## **Methods**

### ***Mice***

Female BALB/c mice, 4-6 wks of age, free of specific pathogens, were purchased from Charles River Laboratories (Yokohama, Japan). They were housed under pathogen-free conditions at the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine. All experimental procedures were reviewed and approved by the Nagasaki University School of Medicine Committee on Animal Research. 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) was propagated in monolayer cultures of HEp-2 cells (ATCC). For preparation of a large stock of the virus, HEp-2 cells were infected with the ATCC stock virus, and cell supernatant was collected at 5 or 6 days post-infection. The supernatant was centrifuged at 2000 rpm for 10 min at 4°C to remove cellular debris. The concentration of virus was adjusted as assessed by quantitative plaque-forming assay [34]. The clear supernatant was collected and stored at -70°C. Mice were infected under light ether (Wako, Osaka, Japan) anesthesia by intranasal inoculation of low grade RSV ( $2 \times 10^3$  plaque forming

unit [PFU] in 50  $\mu$ l). RSV infection was confirmed by reverse transcriptase PCR for RSV N protein mRNA in the lung tissues of RSV-inoculated mice as previously described [18]. Controls were sham-infected with inactivated RSV in a similar manner. To inactivate the RSV, an aliquot of the viral suspension was irradiated with ultraviolet (UV) light for 15 min on ice.

### ***RSV infection and allergen sensitization***

Eight groups of donor mice were prepared and designated: primary Control (pControl), pDf, pRSV, pDf-RSV, secondary Control (sControl), sDf, sRSV, and sDf-RSV (Figure 1A, B). All groups of mice were immunized twice intraperitoneally on days 1 and 14 with 0.5 mg/mouse of *Dermatophagoides farinae* (*Derf*; LG-5339, Cosmo Bio, Tokyo, Japan) allergen precipitated in aluminum hydroxide. Lipopolysaccharide (LPS) concentration in the *Derf* preparations was < 0.96 EU/mg *Derf* (Limulus amoebocyte lysate test; E-Toxate; Sigma-Aldrich, St. Louis, MO). These mice were then challenged intranasally with 50  $\mu$ l of phosphate buffered saline (PBS) (p and sControls, p and sRSV groups) or 50  $\mu$ g/ $\mu$ l of *Derf* allergen (p and sDf groups, p and sDf-RSV groups) daily for three consecutive days: once at days 14-16 (pControl, pDf, and pDf-RSV groups) or twice at days 14-16 and 29-31 (sControl, sDf,

and sDf-RSV groups). These mice were also sham infected once or twice with UV inactivated RSV (p and sControls, p and sDf groups) or infected with live low grade RSV ( $2 \times 10^3$  PFU/mouse) (p and sRSV groups, p and sDf-RSV groups) on day 17 and/or 32. Lung was harvested 4 days after primary and secondary infections (day 21 and 36, respectively).

### *Isolation of pulmonary DC*

After dissecting the lung from eight groups of donor mice, the lung tissues were cut into small pieces, which were digested with continual agitation for 1 h at 37°C in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 1% fetal bovine serum (FBS) and 0.1% penicillin-streptomycin (Gibco) (hereafter referred to as cRPMI) supplemented with 1.5 mg/ml collagenase A (Boehringer-Mannheim, Germany), 0.02 mg/ml DNase I (Boehringer) and 0.75 mg/ml hyaluronidase (Sigma-Aldrich). The lung tissues were mechanically filtered through a 250- $\mu$ m mesh, and resuspended and washed in cRPMI. Cells were further purified over a Ficoll gradient by suspending the cells in 10 ml 1.075 g/ml high density Ficoll (Amersham Biosciences, Sweden) and centrifuging at 1500rpm for 10 min. Low density cells were collected, resuspended, and washed in cRPMI, and then incubated with CD11c MicroBeads (Miltenyl Biotec,

Germany) according to manufacturer's guidelines. The isolated cells had the DC phenotype, particularly MHC Class II low expression. Purity was established using CD11c-FITC antibodies and enriched populations of pulmonary DCs (> 75%) were obtained. Isolated pulmonary DCs were subjected to *in vitro* function assay or used for *in vivo* transfer.

#### ***DC function analysis in vitro***

These isolated pulmonary DCs were washed and cultured in cRPMI supplemented with GM-CSF (20 U/ml) and *Derf* allergen (1 mg/ml) at 37°C/5% CO<sub>2</sub> overnight. In preliminary experiments, the viability of DCs decreased. Thus GM-CSF, a critical growth factor for DC, was added to the culture supernatants [35]. Cell culture supernatants were analyzed for IL-10 and IL-12 by enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems Inc., Minneapolis, MN, USA), using the procedure described in the instruction manual.

#### ***DC function analysis in vivo***

Purified lung DCs from donor mice were washed and resuspended in cRPMI, and naïve BALB/c mice were intranasally inoculated with  $3 \times 10^5$  cells in 100  $\mu$  l/mouse. Nine

days after receipt of the DCs, the mice were challenged intranasally by *Derf* allergen daily for five consecutive days. Allergen challenge consisted of 0.5 mg of *Derf* allergen in PBS or an equivalent volume of the PBS diluent without allergen(Figure1C). Lung was harvested 14 days after receipt of the DCs and was subjected to either pathology examination or mononuclear cell (MNC) isolation. For pathology, section of formalin-fixed lung tissue were stained with hematoxylin and eosin (H&E). Pulmonary pathology was assessed semiquantitatively as previously described [18]. In brief, coded pulmonary sections were evaluated under oil immersion in a blinded fashion by three different observers. The numbers of eosinophils and lymphocytes were determined in 10 peribronchovascular areas per section. The results were expressed as mean cell numbers from each group. Lung MNCs were prepared from the eight groups of mice as previously described [17,19]. MNCs ( $2 \times 10^5/200 \mu\text{l/well}$ ) were cultured in the absence or presence of 100 mg/ml of *Derf* in 96-well plates for 48 hrs. The concentrations of IFN- $\gamma$  and IL-5 in the supernatants were determined by ELISA (Quantikine, R&D Systems Inc.), using the procedure described in the instruction manual.

#### ***Treatment of mice with $\alpha$ IL-12 and $\alpha$ IL-10 neutralizing Ab***

In a different set of experiments, four groups of BALB/c mice (p and sDf, p and

sDf-RSV) were intranasally given either 45 µg/mouse of anti-mouse IL-12 p70 mAb (eBioscience, San Diego, CA, USA) or 55 µg/mouse of anti-mouse IL-10 mAb (eBioscience) on the day(day 17 and/or 32) and day after(day 18 and/or 33) viral infection (Fig.1A and B). Isotype Abs (eBioscience) were also used and it was confirmed that these had no significant effects. As mentioned above, pulmonary DCs of these mice were isolated and inoculated into naïve mice that were subsequently intranasally challenged with *Derf* allergen. Lung was harvested 14 days after receipt of the DCs. Both lung pathology and pulmonary cytokines profile were also examined.

***Statistical analysis.***

Results are expressed as the mean  $\pm$  standard error of mean (SEM). Data were evaluated using repeated-measures ANOVA with a Bonferroni multiple comparison test. A *P*-value of  $<0.05$  was considered significant.

## Results

### Effects of primary and secondary low-grade RSV infections in allergic asthma

As previously described elsewhere [19], primary low-grade RSV infection attenuated allergen-induced airway inflammation concomitant with significant IFN- $\gamma$  production in the airway, while secondary low-grade RSV infection increased both IL-5 and IFN- $\gamma$  production and resulted in exacerbation of allergen-induced allergic airway inflammation (data not shown).

### Pulmonary DC function *in vitro*

To determine the immunoregulatory profile of pulmonary DCs, CD11c<sup>+</sup> cells were immunomagnetically isolated from lung tissue and their production of IL-10 and IL-12 in response to *Derf* allergen was determined (Figure 2). Production of these cytokine was insignificant in the absence of *Derf* allergen. The predominant cytokine produced by primary *Derf* sensitized (pDf)-DCs was IL-10, while that of primary RSV infected (pRSV)-DCs was IL-12. IL-10 and IL-12 production by primary *Derf*-sensitized plus RSV-infected (pDf-RSV)-DCs was significantly inhibited compared to production of these cytokines by pDf-DCs or pRSV-DCs, suggesting these DCs were less able to cause a shift in T cell responses. IL-10 production was significantly lower in response to

secondary than primary *Derf* sensitization of DCs in the presence or absence of RSV infection. Secondary RSV infection also significantly lowered IL-12 production, whereas secondary *Derf* sensitization significantly increased IL-12 production compared to primary sensitization. Similar to primary RSV infection, secondary RSV infection in secondary *Derf* sensitized plus RSV infected (sDf-RSV)-DCs significantly inhibited *Derf*-induced IL-10 production. In contrast, IL-12 production was comparable in cultures of sDf-RSV-DCs, secondary *Derf* sensitized (sDf)-DCs, and secondary RSV infected (sRSV)-DCs. To determine the cytokine profile in lung tissues, concentrations of two immunoregulatory cytokines, IL-10 and IL-12, were determined in lung homogenates of donor mice (Figure 3). IL-10 and IL-12 concentrations were significantly increased following secondary *Derf* sensitization and RSV infection but not following primary *Derf* sensitization and/or RSV infection. In comparison with secondary *Derf* sensitization alone, secondary RSV infection significantly increased IL-12 but not IL-10 concentration in the lung homogenates of sDf-RSV-DC donors.

### **Pulmonary DC function *in vivo***

To assess the *in vivo* relevance of alterations in pulmonary DC function by RSV infection, DCs from the eight groups of donor mice were adoptively transferred to naïve

BALB/c recipients and then the latter were challenged intranasally with *Derf* allergen. Semi-quantitative analysis of lung pathology showed that eosinophilic infiltration was significantly attenuated in recipients of intranasal pDf-RSV-DCs when compared to recipient of pDf-DCs. In contrast, eosinophilic infiltration was significantly augmented in recipients of sDf-RSV-DCs when compared to recipients of sDf-DCs (Figure 4A). Analysis of the profile of *Derf* allergen-specific cytokines in the lungs of recipient mice revealed no increase in IL-5 and IFN- $\gamma$  levels in recipients of pDf-RSV-DCs above levels found in recipients of pDf-DCs. By contrast and consistent with the lung pathology findings, these levels increased significantly in recipients of sDf-RSV-DCs compared to recipients of sDf-DCs, suggesting the activations of both Th1 and Th2 responses (Figure 4B).

#### **Effect of $\alpha$ IL-12 and $\alpha$ IL-10 mAb *in vivo***

Since IL-12 was significantly increased in the lung homogenates of sDf-RSV-DCs donor mice compared to those of sDf-DCs donor mice,  $\alpha$ IL-12mAb was intranasally inoculated into donor mice to determine the effect of IL-12 on pulmonary DC following interaction between aeroallergen sensitization and RSV infection. Neutralization of IL-12 within pulmonary DCs during RSV infection reduced eosinophilic inflammation

in lung tissue (Figure 5A) and *Derf*-allergen specific IL-5 and IFN- $\gamma$  produced by pulmonary MNCs (Figure 5B) to levels that were comparable between recipients of Df-DCs and Df-RSV-DCs following both primary and secondary RSV infections. In marked contrast to this finding, neutralization of IL-10 within pulmonary DCs during RSV infection had no effect, and significant differences persisted in lung pathology (Figure 6A) and in cytokines profiles (Figure 6B) between recipients of Df-DCs and Df-RSV-DCs.

## Discussion

DCs are airway antigen-presenting cells that initiate immunity to foreign antigens such as harmless mites and pathogenic respiratory virus. Depending on viral load, RSV can significantly affect the status of pre-existing allergic airway inflammation [18, 19, 36, 37]. In particular, exacerbation of allergic airway inflammation by repeated but not single RSV infections has been shown in a murine model [18, 19]. Nonetheless, the underlying mechanisms remain unknown. Using a murine BM-DCs transfer system, we previously found that direct and primary RSV infection of BM-DCs resulted in Th1-like responses [32]. This system, however, could not be used to investigate the effect of repeated infection, since RSV infected BM-DCs do not remain viable for very long. Thus, the *in vitro* cytokine response of murine pulmonary DCs immunomagnetically isolated from mite-allergen sensitized mice following primary and secondary RSV infections were assessed in the present study. Subsequently, the airways of naïve mice were inoculated with these DCs to examine their *in vivo* effects.

Analysis of the *in vitro* pulmonary DC function clearly demonstrated that primary *Derf* allergen sensitization induced in DCs an IL-10 dominant cytokine response, while primary RSV infection induced an IL-12 dominant cytokine response. Primary RSV infection attenuated the IL-10 but not IL-12 production in Df-DCs, and

primary *Derf* allergen sensitization attenuated IL-12 but not IL-10 production in RSV-DCs, resulting in neither the Th1 nor Th2 phenotype in primary RSV infected Df-DCs. In contrast to the primary infection, secondary RSV infection did not significantly attenuate either IL-10 or IL-12 production in Df-DCs. Maintaining similar levels of IL-12 and IL-10 production by pulmonary DCs could result in both Th1 and Th2 enhancement as previously described in influenza A viral infection [33]. The local cytokine profile around sites of DCs-T cell interaction is one of the critical determinants of Th phenotype: higher IL-12 level induces Th1 cells and higher IL-10 level induces Th2 cells [38-43]. Although the cellular source of IL-10 and IL-12 remains undetermined, concentration measurements in donor lung tissues revealed that primary RSV infection failed to enhance IL-10 and IL-12 levels in lung homogenates of Df mice. In marked contrast to primary infection, secondary RSV infection significantly increased IL-12, but not IL-10 concentrations in the lung homogenates of Df mice, suggesting secondary RSV infection induced a Th1 response in *Derf*-allergen sensitized mice. In its original and simplified form, the Th1-Th2 theory supposes that enhancement of the Th1 response suppresses the Th2 response. However, in the present study, enhanced Th1 response caused by secondary RSV infection in pre-existing Th2-dominant allergic airway inflammation exacerbates the Th2 response and

associated allergic airway inflammation. Although Th2 cells are definitely involved in the pathogenesis of allergic asthma, many investigators reported that Th1 cells by themselves or in conjunction with Th2 cells are potentially involved [44-46].

To determine whether *in vitro* properties of RSV-infected pulmonary DCs were associated with *in vivo* immunity, these DCs were intranasally inoculated into the airway of naïve mice. *In vivo* properties proved to be consistent with *in vitro* properties of pulmonary DCs: DCs from primary RSV-Df mice induced less inflammation compared to DCs from primary Df mice, while DCs from secondary RSV-Df mice exacerbated allergic airway inflammation in comparison with DCs from secondary Df mice. Pulmonary cytokine profiles of recipient mice indicated that primary RSV infection failed to alter IL-5 and IFN- $\gamma$  production (in recipients of DCs from primary RSV-Df mice *versus* recipients of DCs from primary Df mice), while secondary RSV infection significantly enhanced IL-5 and IFN- $\gamma$  production (in recipients of DCs from secondary RSV-Df mice *versus* recipients of DCs from secondary Df mice), suggesting the activation of both Th1 and Th2 responses.

Considering the suggestion from the findings of the present study and previous report [33] that IL-12 plays a critical role in shifting the immune response, we next injected  $\alpha$ IL-12 mAb into the same mouse models. Neutralization of intrinsic IL-12, a

representative Th1-inducing cytokine, significantly abrogated secondary RSV-induced exacerbation of allergic airway inflammation and both Th1 and Th2 immunity in mite-allergen sensitized mice. These results confirmed that Th1 immunity not only prevented Th2 immunity but also could exacerbate Th2 immunity in secondary RSV infected mice with *Derf*-allergen induced allergic airway inflammation at the DC level. The IL-12-mediated mechanism involved in exacerbating secondary RSV-induced allergic airway inflammation is yet to be determined. Possibly, IL-12 preserves the effects of regulatory Th1 and Th2 cells established during RSV infection and/or allergen challenge as reported for *in vitro* Th1 responses [47] or the *in vivo* model of experimental colitis [48].

IL-10 is a regulatory cytokine that has been suggested for treatment of asthma because of its immunosuppressive and anti-inflammatory properties. Nonetheless, the biological effects of IL-10 can vary depending on the surrounding cytokine and cellular milieu and timing of expression during the immune response. In a murine model of allergic asthma, IL-10 suppresses the development of allergic airway inflammation when given at the time of, but not 1h after, antigen challenge [49]. In the present study,  $\alpha$ IL-10 mAbs were given on the day of RSV infection and after *Derf* allergen challenge, and thus might show no effects on Df mice. Similarly,  $\alpha$ IL-10 mAbs did not have any

effect on the interaction between allergic sensitization and RSV infection in the present protocol.

A critical limitation in the present study is the purity of pulmonary DCs. The non-CD11c<sup>+</sup> cells obtained by our method included approximately 10% Mac1<sup>+</sup> macrophages and a few CD4<sup>+</sup> cells (unpublished observations). Although DCs in lung tissue characteristically express CD11c, recent research has demonstrated the potential of some lung macrophages and CD8 $\alpha$  positive lymphocytes to also express CD11c [50, 51]. In particular, the latter could be critically involved in the RSV-induced pathology in the lung[51]. Although contamination by CD8 $\alpha$  positive lymphocytes cannot be excluded, most CD11c positive cells were DCs in the present study since the expressions of CD8 $\alpha$  in CD11c positive cells were sparse and were not enhanced by RSV infection (mean fluorescence intensity [MFI]:  $6.8 \pm 3.5$  in control vs  $6.1 \pm 5.9$  in RSV group) in contrast to MHC class II expression (MFI:  $4.7 \pm 4.1$  in control vs  $124.3 \pm 55.9$  in RSV group) by flow cytometry analysis (our preliminary unpublished observation).

In conclusion, we demonstrated in the present study that primary low grade RSV infection after *Derf* allergen sensitization poorly induced IL-10 and IL-12 production in donor lung, thus failing to stimulate DC polarization, and thereby neither

a Th1 nor Th2 response. In contrast, secondary RSV infection selectively enhanced IL-12 production in donor lung, which activated pulmonary DCs. Activated pulmonary DCs produced IL-12 and enhanced not only Th2 but also Th1 responses.  $\alpha$ IL-12 mAb treatment after allergen sensitization and during RSV infection significantly blocked these responses. A critical role of dendritic cells in shifting the immune response through IL-12 signaling was suggested in this experimental model of respiratory viral infection in allergic asthma.

### **Acknowledgements**

This study was supported by a Grant-in-Aid for Scientific Research (No. 17607009) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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## Figure legends

### Figure 1. Experimental protocol

Eight groups of donor mice were designated; **(A)** primary Control (pControl), pDf, pRSV, and pDf-RSV and **(B)** secondary Control (sControl), sDf, sRSV, and sDf-RSV were prepared. All mice were immunized twice intraperitoneally on days 1 and 14 with Df allergen in alum and then challenged intranasally with PBS (p and sControls, p and sRSV groups) or *Derf* allergen (p and sDf groups, p and sDf-RSV groups) on three consecutive days: The pControl, pDf, and pDf-RSV groups were challenged only once at days 14-16 whereas the sControl, sDf, sDf-RSV groups were both at days 14-16 and 29-31. Four groups (p and sControls, p and sDf) were sham infected with UV-RSV and four (p and sRSV groups, p and sDf-RSV groups) were infected with RSV once or twice on day 17 and/or 32. In a different set of experiments, mice were intranasally given either  $\alpha$ IL-12 p70 mAb or  $\alpha$ IL-10 mAb on the day and day following viral infection (days 17, 18 and/or days 32, 33). For pulmonary DCs isolation, lung was harvested 4 days after primary and secondary infection(days 21 and 36, respectively).

**(C)** Pulmonary DCs from donor mice were intranasally inoculated into naïve mice on day' 1. The mice were further challenged by *Derf* allergen for five consecutive days (days' 10-14). Lung was harvested on days' 15 for either pathology examination or

mononuclear cell isolation.

**Figure 2. *Derf*-specific cytokine production by isolated pulmonary DCs from recipients.**

Pulmonary DCs were isolated from eight groups of BALB/c mice. These DCs were cultured in the presence of *Derf* allergen, and concentrations of IL-10 and IL-12 were determined by ELISA. Each bar represents the mean (n=8 for each group)  $\pm$  SEM.

\*\*p<0.01 vs. Control, \*p<0.05 vs. Control, † p<0.01 vs. Df,  $\neq$ p<0.01 vs. RSV.

**Figure 3. Cytokine concentrations in donor lung homogenate.**

Lung tissues were prepared from eight groups of BALB/c mice. Concentrations of IL-10 and IL-12 in lung homogenate were determined by ELISA. Each bar represents the mean (n=8 for each group)  $\pm$  SEM. \*\*p<0.05 vs. control, † p<0.01 vs. Df.

**Figure 4. Pathology (A) and cytokine profile (B) in lung tissues of recipients.**

Lung tissues were collected from eight groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs. Lung tissue sections were stained with H&E and infiltrations of eosinophils (Eo) and lymphocytes (Ly) in the

peribronchovascular area were semiquantitatively analyzed (A). Pulmonary mononuclear cells (MNCs) were isolated from eight groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs. These MNCs were cultured in the presence of *Derf* allergen, and concentrations of IL-5 and IFN- $\gamma$  were determined by ELISA (B). Each bar represents the mean (n=8 for each group)  $\pm$  SEM. \*\* p<0.01 and \* p<0.05 vs. control, † p<0.01 vs. Df and RSV.

**Figure 5. Pathology (A) and cytokine profile (B) in lung tissues of recipients during IL-12 neutralization.**

Lung tissues were collected from four groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs after intranasal  $\alpha$ IL-12 mAb administration during primary and secondary RSV infection. Lung tissue sections were stained with H&E and infiltrations of eosinophils (Eo) and lymphocytes (Ly) in the peribronchovascular area were semiquantitatively analyzed (A). Pulmonary MNCs were isolated from eight groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs. These MNCs were cultured in the presence of *Derf* allergen, and concentrations of IL-5 and IFN- $\gamma$  were determined by ELISA (B). Each bar represents the mean (n=8 for each group)  $\pm$  SEM.

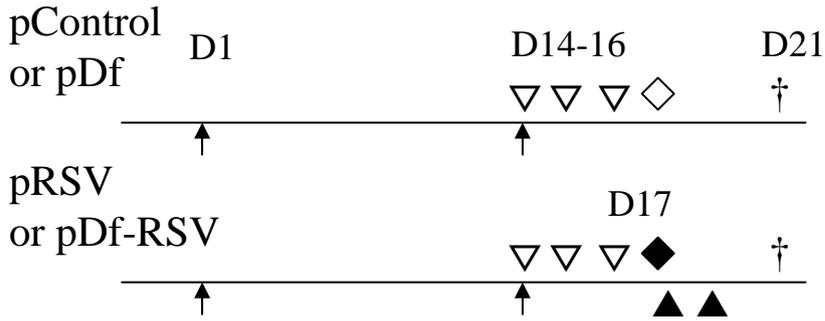
**Figure 6. Pathology (A) and cytokine profile (B) in lung tissues of recipients during IL-10 neutralization.**

Lung tissues were collected from four groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs after intranasal  $\alpha$ IL-10 mAb administration during primary and secondary RSV infection. Lung tissue sections were stained with H&E and infiltrations of eosinophils (Eo) and lymphocytes (Ly) in the peribronchovascular area were semiquantitatively analyzed (A). Pulmonary MNCs were isolated from eight groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs. These MNCs were cultured in the presence of *Derf* allergen, and concentrations of IL-5 and IFN- $\gamma$  were determined by ELISA (B). Each bar represents the mean (n=8 for each group)  $\pm$  SEM. \* p<0.05 vs. Df.

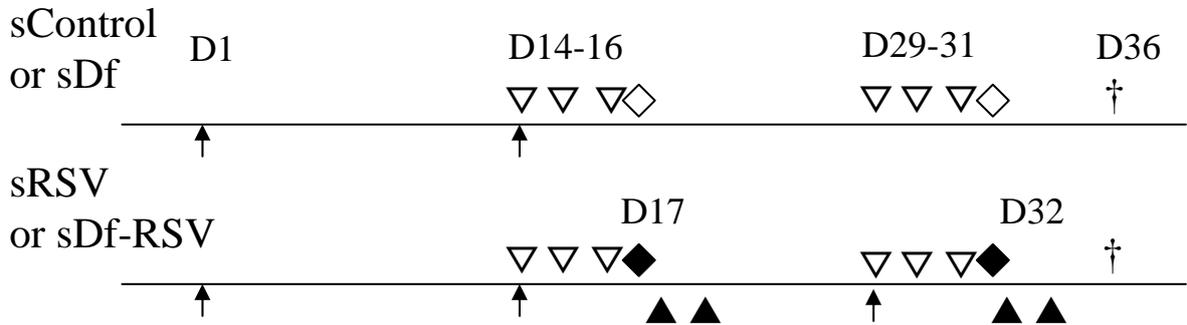
Fig. 1

↑:PBS or *Derf* i.p. , ∇:PBS or *Derf* i.n. , ◆:RSV i.n. ,  
 ◇:UV-RSV i.n., ▲:mAb s.c., ↓:DC i.n., †:lung harvest

A.



B.



C.



Fig. 2

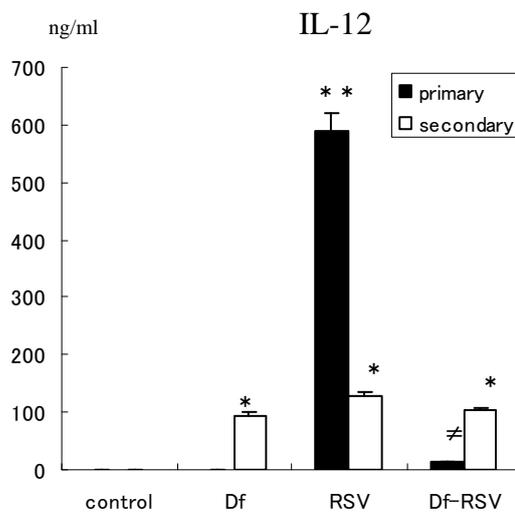
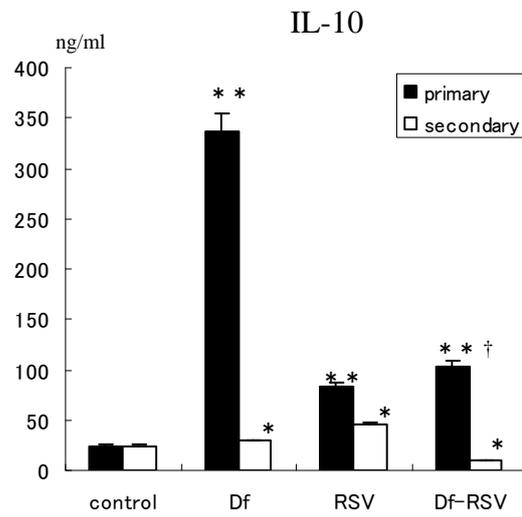
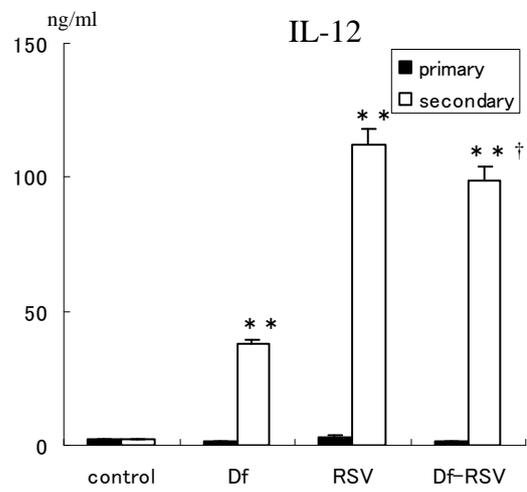
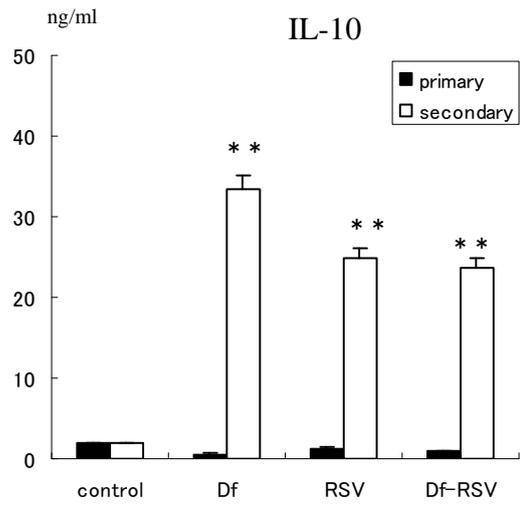
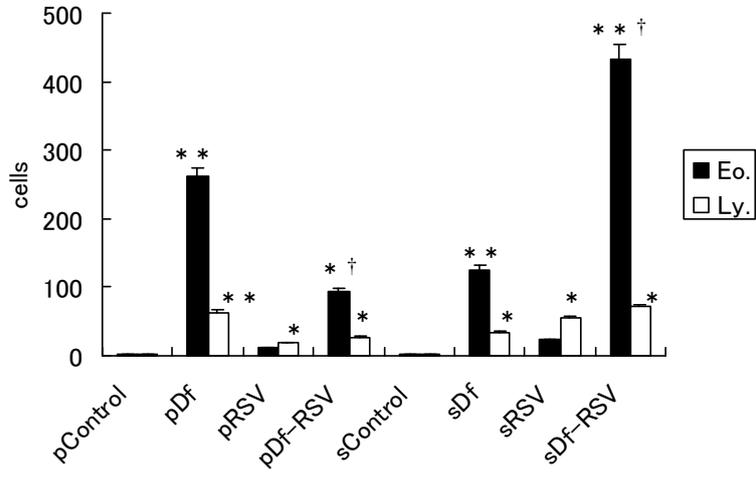


Fig. 3



**Fig. 4**

A.



B.

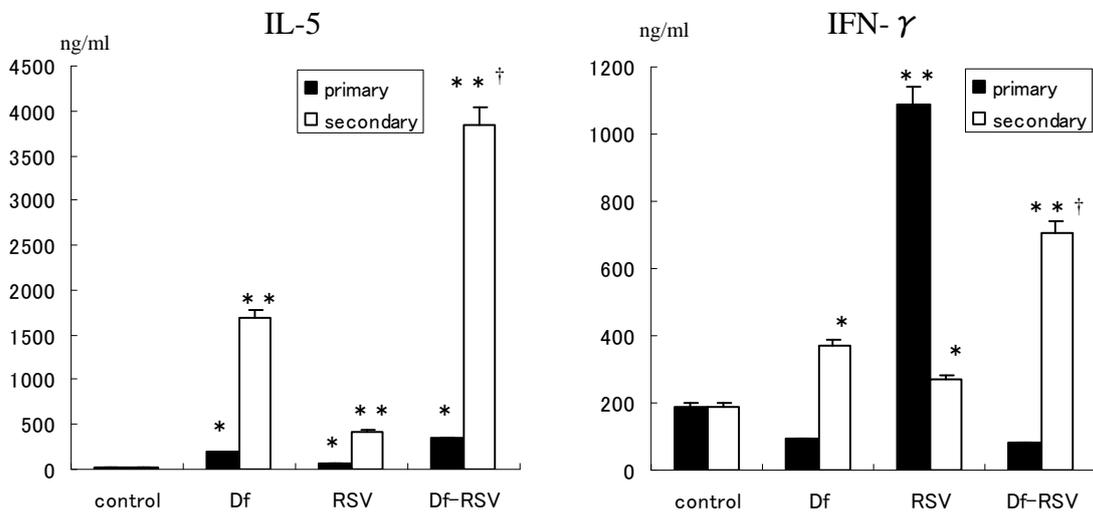
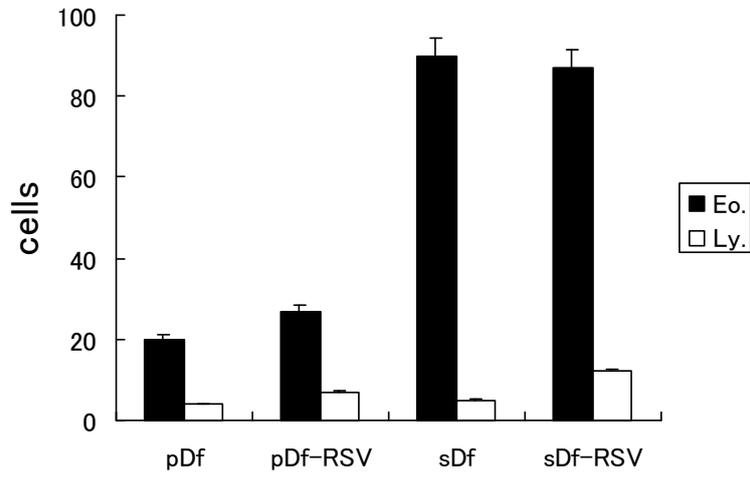


Fig. 5

A.



B.

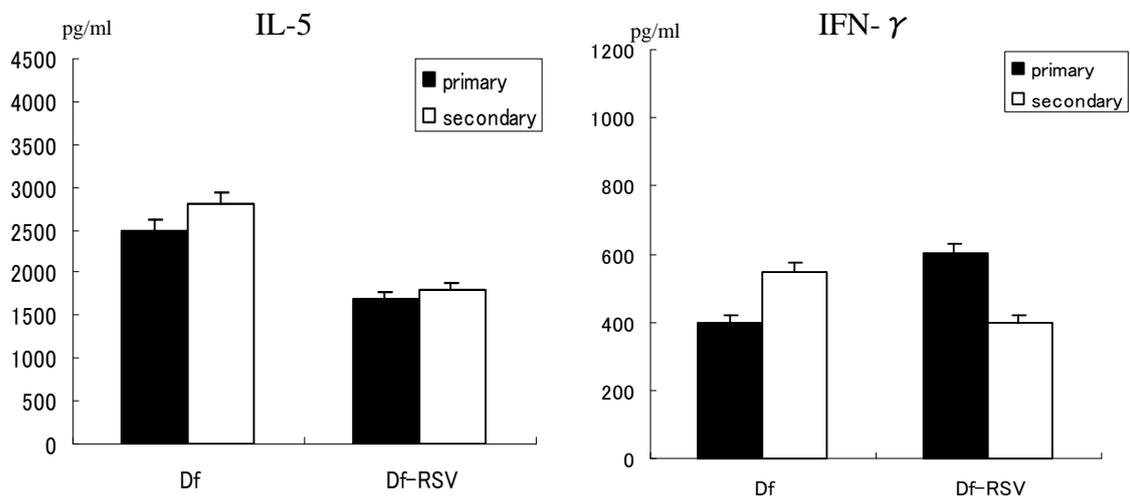
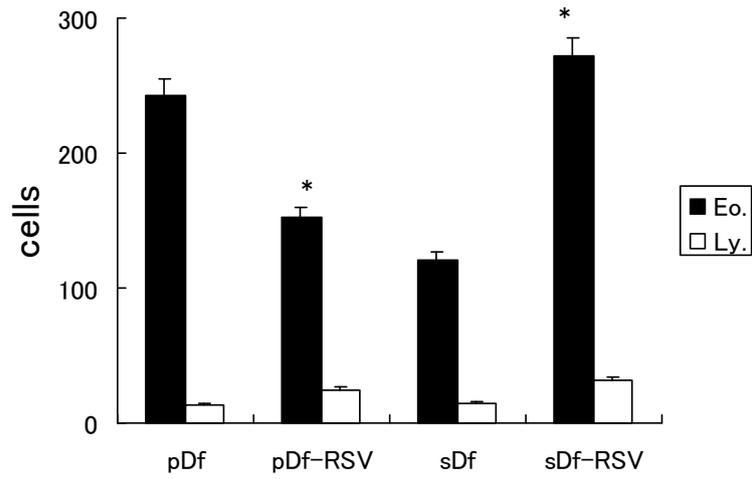


Fig. 6

A.



B.

