

Effects of Repeated Respiratory Syncytial Virus Infections on Pulmonary Dendritic Cells in a Murine Model of Allergic Asthma

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Key Words

Airway inflammation • Asthma • Dendritic cells •
Dermatophagoides farinae • Interleukin-12 • Respiratory
syncytial virus

Abstract

Background: Primary and secondary respiratory syncytial virus (RSV) infection differentially regulates preexisting allergic airway inflammation. **Objectives:** The present study was designed to determine the effects of primary and secondary low-grade RSV infections on pulmonary dendritic cell (DC) functions. **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⁺ pulmonary DCs were isolated from these mice and then transferred to naïve mice followed by intranasal *Derf* challenge. Furthermore, either anti-IL-12 monoclonal antibody (α IL-12 mAb) or anti-IL-10 (α 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 en-

hancing both Th1 and Th2 responses. During RSV infection, α IL-12 but not α IL-10 mAb treatment blocked these immunological effects. **Conclusion:** Via IL-12, DCs may play a critical role in shifting the immune response in this experimental model of repeated respiratory viral infection in allergic asthma.

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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 asthma exacerbation [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- γ , 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 incom-

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1018–2438/08/1473–0197\$24.50/0

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plete 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 the frequency of infection and viral load. Epidemiologic studies indicate 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 using sensitive techniques such as polymerase chain reaction. 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- γ 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 DC 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 pulmonary DC functions.

Methods

Mice

Female BALB/c mice, 4–6 weeks 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., USA) 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 5 or 6 days after infection. The supernatant was centrifuged at 2,000 rpm for 10 min at 4°C to remove cellular debris. The virus concentration 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 (2×10^3 plaque-forming units in 50 μ l). RSV infection was confirmed by reverse transcriptase polymerase chain reaction 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 (fig. 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. The lipopolysaccharide concentration in the Derf preparations was <0.96 EU/mg Derf (limulus amoebocyte lysate test; E-Toxate; Sigma-Aldrich, St. Louis, Mo., USA). These mice were then challenged intranasally with 50 μ l of phosphate-buffered saline (PBS; pControls, sControls, pRSV and sRSV groups) or 50 μ g/ μ l of Derf allergen (pDf, sDf, pDf-RSV and sDf-RSV groups) daily for 3 consecutive days: once on days 14–16 (pControl, pDf and pDf-RSV groups) or twice on 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 (pControls, sControls, pDf and sDf groups) or infected with live low-grade RSV (2×10^3 plaque-forming units/mouse; pRSV, sRSV, pDf-RSV and sDf-RSV groups) on days 17 and/or 32. The lung was harvested 4 days after primary and secondary infections (day 21 and 36, respectively).

Isolation of Pulmonary DCs

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, N.Y., USA) supplemented with 1% fetal bovine serum and 0.1% penicillin-streptomycin (Gibco; hereafter referred to as cRPMI) supplemented with 1.5 mg/ml collagenase A (Boehringer-

er-Mannheim, 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- μ m mesh, and resuspended and washed in cRPMI. Cells were further purified over a Ficoll gradient by suspending the cells in 10 ml of 1.075 g/ml high-density Ficoll (Amersham Biosciences, Uppsala, Sweden) and centrifuging at 1,500 rpm for 10 min. Low-density cells were collected, resuspended, washed in cRPMI and then incubated with CD11c MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions. In accordance with a recent report utilizing a similar procedure [35], purity, in terms of CD11c expression estimated by flow cytometry, was >95% and, within the CD11c-positive population, approximately 80% expressed MHC class II (data not shown). 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% CO₂ overnight. In preliminary experiments, the viability of DCs decreased. Thus GM-CSF, a critical growth factor for DC, was added to the culture supernatants [36]. Cell culture supernatants were analyzed for IL-10 and IL-12 by enzyme-linked immunosorbent assay (ELISA) (Quantikine; R & D Systems, Minneapolis, Minn., 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 3×10^5 cells in 100 μ l/mouse. Nine days after receipt of the DCs, the mice were intranasally challenged with *Derf* allergen daily for 5 consecutive days. Allergen challenge consisted of 0.5 mg of *Derf* allergen in PBS or an equivalent volume of the PBS diluent without allergen (fig. 1c). Lung was harvested 14 days after receipt of the DCs and was subjected to either pathological examination or mononuclear cell (MNC) isolation. For pathology, sections of formalin-fixed lung tissue were stained with hematoxylin and eosin (HE). Pulmonary pathology was assessed semi-quantitatively 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 (2×10^5 /200 μ l/well) were cultured in the absence or presence of 100 mg/ml of *Derf* in 96-well plates for 48 h. The concentrations of IFN- γ and IL-5 in the supernatants were determined by ELISA (Quantikine), using the procedure described in the instruction manual.

Treatment of Mice with α IL-12 and α IL-10 Neutralizing Antibody

In a different set of experiments, four groups of BALB/c mice (pDf, sDf, pDf-RSV and sDf-RSV) were intranasally challenged with either 45 μ g/mouse of anti-mouse IL-12 p70 mAb (eBioscience, San Diego, Calif., USA) or 55 μ g/mouse of anti-mouse IL-10 mAb (eBioscience) on the day of viral infection (days 17 and/or

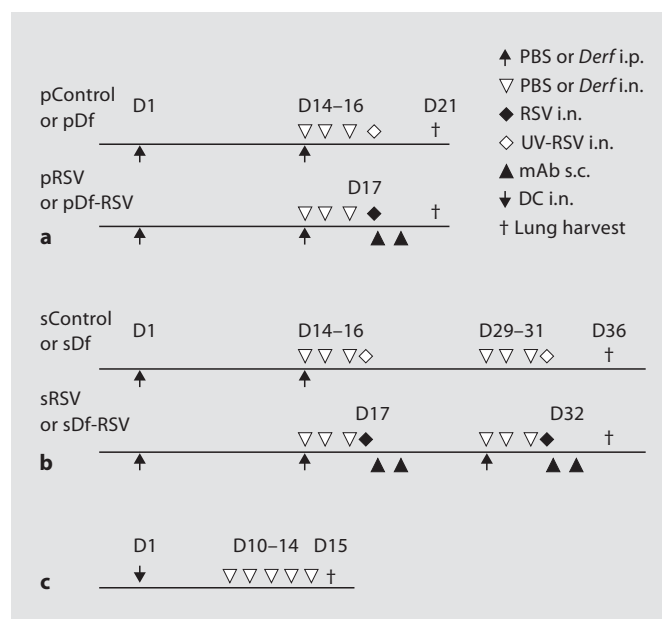


Fig. 1. Experimental protocol. Eight groups of donor mice were studied: pControl, pDf, pRSV and pDf-RSV (**a**) and sControl, sDf, sRSV and sDf-RSV (**b**; for further information, see 'RSV Infection and Allergen Sensitization' and 'Treatment of Mice with α IL-12 and α IL-10 Neutralizing Antibody'). **c** Pulmonary DCs from donor mice were intranasally inoculated into naïve mice on day 1. The mice were further challenged by *Derf* allergen for 5 consecutive days (days 10–14). Lung was harvested on day 15 for either pathological examination or MNC isolation. D = Day.

32) and on the following day (days 18 and/or 33; fig. 1a, 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 cytokine profiles were also examined.

Statistical Analysis

Results are expressed as means $\pm$ SEM. Data were evaluated using repeated-measures ANOVA with a Bonferroni multiple comparison test. A p value <0.05 was considered significant.

Results

Effects of Primary and Secondary Low-Grade RSV Infections on Allergic Asthma

As previously described [19], primary low-grade RSV infection attenuated allergen-induced airway inflammation concomitant with significant IFN- γ production in the airways, while secondary low-grade RSV infection

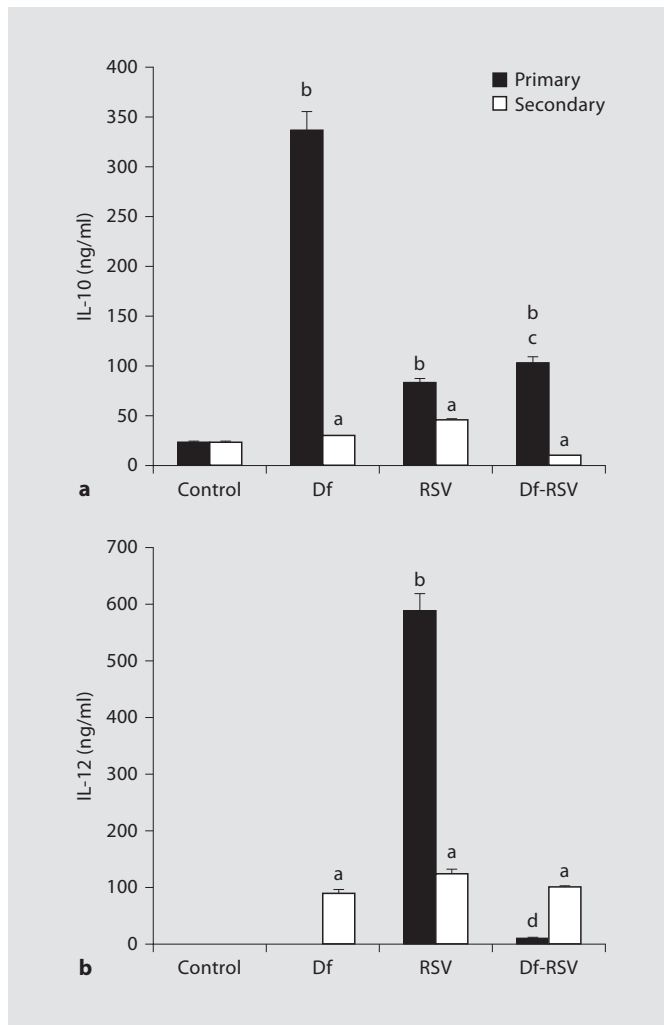


Fig. 2. *Derf*-specific cytokine production by isolated pulmonary DCs from recipients. Pulmonary DCs were isolated from eight groups of BALB/c mice. These DCs were cultured in the presence of *Derf* allergen, and concentrations of IL-10 (a) and IL-12 (b) were determined by ELISA. Each bar represents the mean (n = 8 for each group) $\pm$ SEM. ^a p < 0.05, ^b p < 0.01, vs. control, ^c p < 0.01 vs. Df, ^d p < 0.01 vs. RSV.

increased both IL-5 and IFN- γ 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⁺ cells were immunomagnetically isolated from lung tissue and their production of IL-10 and IL-12 in response to *Derf* allergen was determined (fig. 2). Production of these cytokine was insignificant in the absence of *Derf* allergen. The predominant cytokine

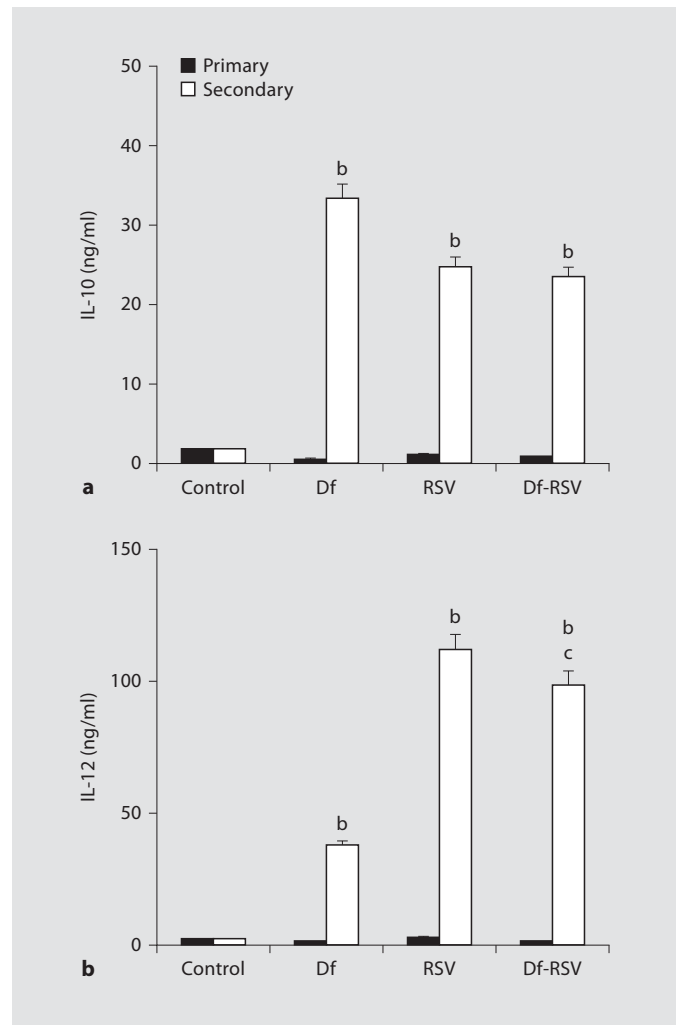
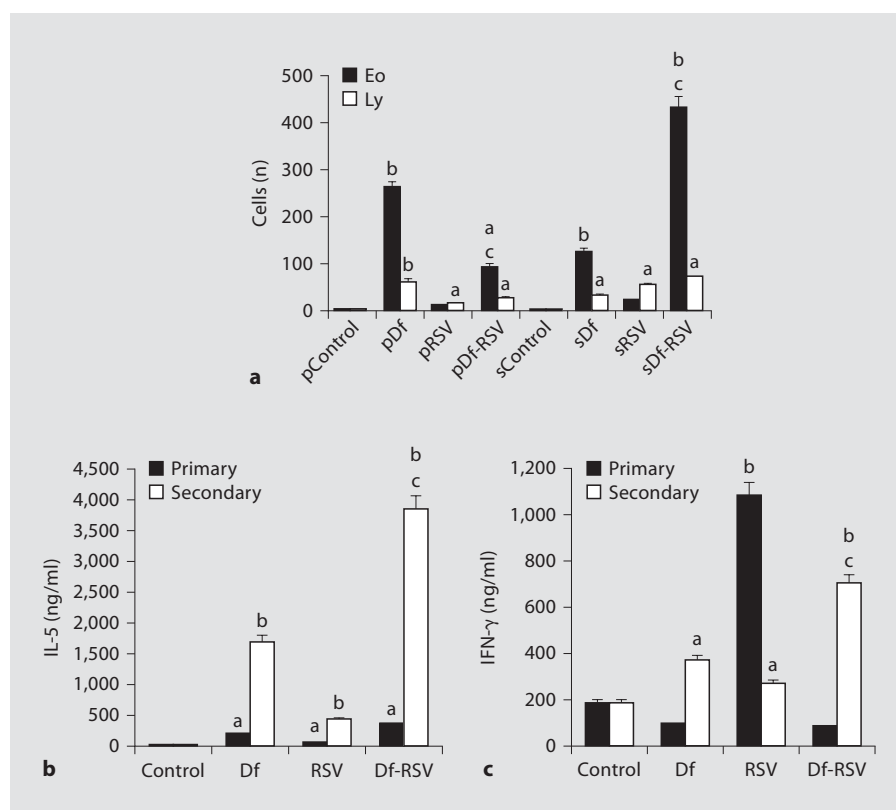


Fig. 3. Cytokine concentrations in donor lung homogenate. Lung tissues were prepared from eight groups of BALB/c mice. Concentrations of IL-10 (a) and IL-12 (b) in lung homogenate were determined by ELISA. Each bar represents the mean (n = 8 for each group) $\pm$ SEM. ^b p < 0.05 vs. control, ^c p < 0.01 vs. Df.

produced by pDf-DCs was IL-10, while that of pRSV-DCs was IL-12. IL-10 and IL-12 production by 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 sensitiza-

Fig. 4. Pathology (a) and cytokine profiles (b, c) 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 HE 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 (b) and IFN- γ (c) were determined by ELISA. Each bar represents the mean ($n = 8$ for each group) $\pm$ SEM. ^a $p < 0.05$, ^b $p < 0.01$, vs. control, ^c $p < 0.01$ vs. Df and RSV.



tion. Similar to primary RSV infection, secondary RSV infection in sDf-RSV-DCs significantly inhibited *Derf*-induced IL-10 production. In contrast, IL-12 production was comparable in cultures of sDf-RSV-DCs, sDf-DCs and sRSV-DCs. To determine the cytokine profile in lung tissue, concentrations of two immunoregulatory cytokines, IL-10 and IL-12, were determined in lung homogenates of donor mice (fig. 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.

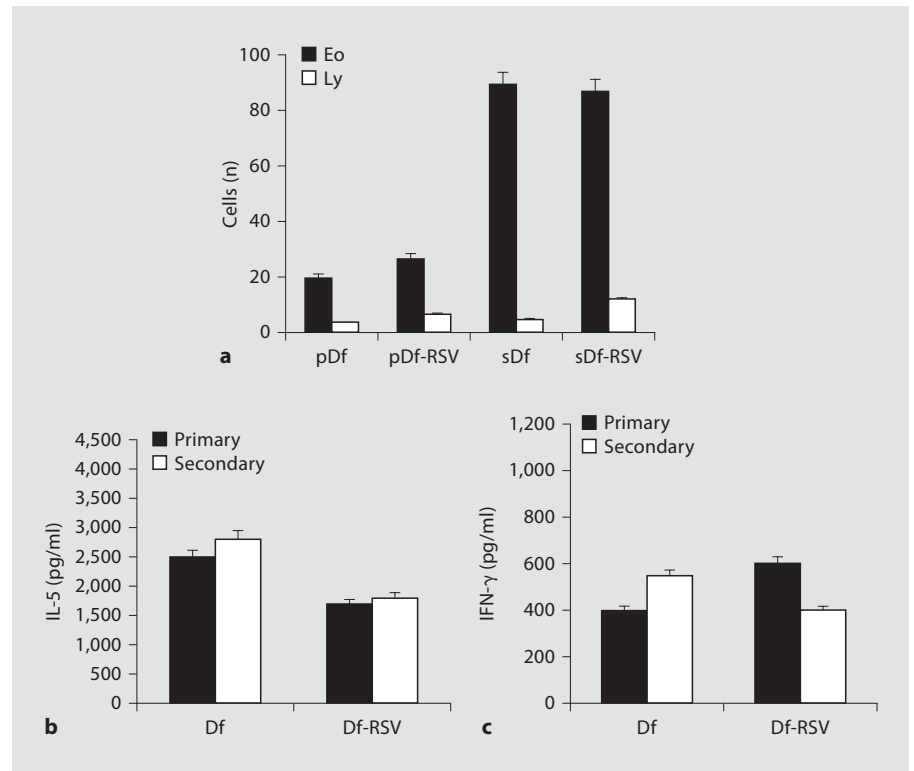
Pulmonary DC Function in vivo

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 intranasally challenged with *Derf* allergen. Semiquantitative analysis of lung pathology showed that eosinophilic infiltration

was significantly attenuated in recipients of intranasal pDf-RSV-DCs when compared to recipients of pDf-DCs. In contrast, eosinophilic infiltration was significantly augmented in recipients of sDf-RSV-DCs when compared to recipients of sDf-DCs (fig. 4a). Analysis of the profile of *Derf*-allergen-specific cytokines in the lungs of recipient mice revealed no increase in IL-5 and IFN- γ 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 significantly increased in recipients of sDf-RSV-DCs compared to recipients of sDf-DCs, suggesting activation of both Th1 and Th2 responses (fig. 4b).

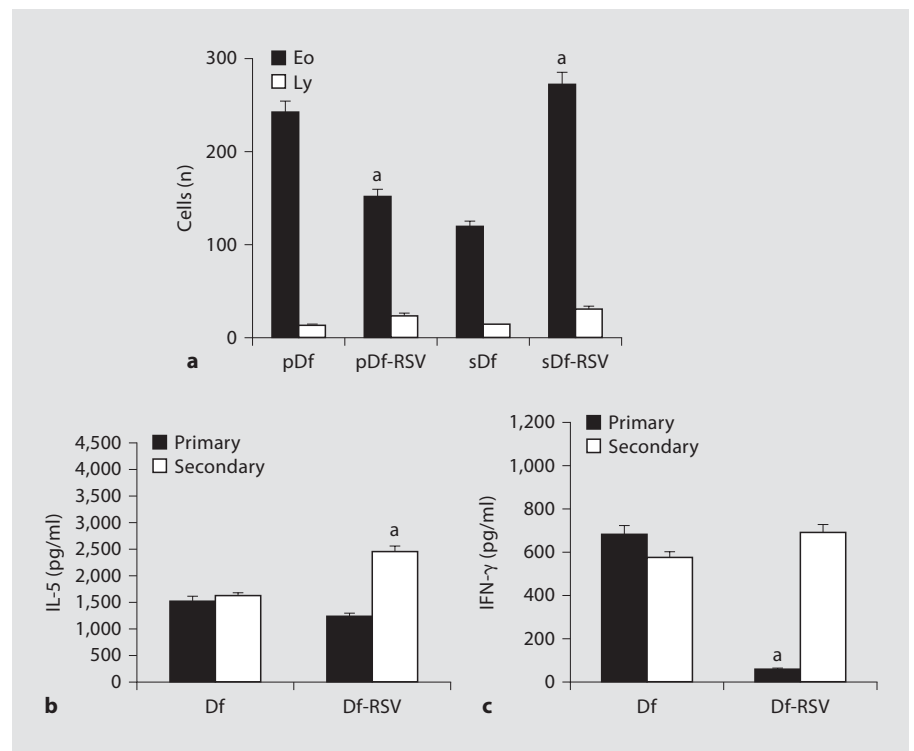
Effect of α IL-12 and α IL-10 mAb in vivo

Since IL-12 was significantly increased in the lung homogenates of sDf-RSV-DCs donor mice compared to those of sDf-DCs donor mice, α IL-12 mAb 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



5

Fig. 5, 6. Pathology (a) and cytokine profile (b, c) in lung tissues of recipients during IL-12 (5) and IL-10 (6) neutralization. Lung tissues were collected from four groups of BALB/c mouse recipients of intranasally administered donor pulmonary DCs after intranasal α IL-12 mAb (5) or α IL-10 mAb (6) administration during primary and secondary RSV infection. Lung tissue sections were stained with HE 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 (b) and IFN- γ (c) were determined by ELISA. Each bar represents the mean ($n = 8$ for each group) $\pm$ SEM. ^a $p < 0.05$ vs. Df.



6

(fig. 5a) and *Derf*-allergen-specific IL-5 and IFN- γ produced by pulmonary MNCs (fig. 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 (fig. 6a) and in cytokine profiles (fig. 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 preexisting allergic airway inflammation [18, 19, 37, 38]. 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-DC 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 responses 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 an IL-10-dominant cytokine response in DCs, while primary RSV infection induced an IL-12-dominant cytokine response. Primary RSV infection attenuated 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 the 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 DC-T cell interaction is one of the critical determinants of the Th pheno-

type: a higher IL-12 level induces Th1 cells and a higher IL-10 level induces Th2 cells [39–44]. 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 preexisting 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 [45–47].

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

Considering the findings of the present study and a previous report [33] suggesting that IL-12 may play a critical role in shifting the immune response, we next injected α 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 ex-

acerbating 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 [48] or the in vivo model of experimental colitis [49].

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 1 h after, antigen challenge [50]. In the present study, α 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, α IL-10 mAbs did not have any effect on the interaction between allergic sensitization and RSV infection in the present protocol.

A critical limitation of the present study is the purity of pulmonary DCs. The non-CD11c⁺ cells obtained by our method included approximately 10% Mac1⁺ macrophages and a few CD4⁺ cells [unpubl. observations]. Although DCs in lung tissue characteristically express CD11c, recent research has demonstrated the potential of some lung macrophages and CD8 α ⁺ lymphocytes to also express CD11c [51, 52]. Thus, the pulmonary CD11c⁺ cells used in the present study represent a mixture of DCs and other cells. Due to the limited number of CD11c⁺

cells in the lung, we had chosen not to further discriminate DCs and other cells in the present study. Further isolation procedures could also affect the viability or function of DCs. Nevertheless, the present study indicated the critical and at least partial involvement of lung DCs in the interaction between RSV infection and mite allergen sensitization. The development of DC isolation methods with higher quantity and quality will be definitely required to confirm the present result.

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 a 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. α IL-12 mAb treatment after allergen sensitization and during RSV infection significantly blocked these responses. A critical role of DCs in shifting the immune response through IL-12 signaling was suggested in this experimental model of respiratory viral infection in allergic asthma.

Acknowledgment

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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