

Adoptive transfer of dendritic cells isolated from helminth-infected mice enhanced T regulatory cell responses in airway allergic inflammation

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SUMMARY

Our and others' previous studies have shown that Schistosoma japonicum (SJ) infection can inhibit allergic reactions. Moreover, we found that adoptive transfer of dendritic cells (DCs) from inhibited mice showed a similar inhibitory effect on allergy, suggesting a critical role of DCs in SJ-infected mediated inhibition of allergy. In this study, we further examined the mechanism by which DCs contribute to inhibition of allergy. Our results showed that DCs from SJ-infected mice (SJDCs) produced significantly higher levels of IL-10 compared to those from naive control mice (NDCs). Adoptive transfer of SJDCs, unlike NDCs, significantly increased CD4⁺CD25⁺Foxp3⁺ T cells and CD4⁺CD25⁺IL-10⁺ T cells regulatory T-cell responses in vivo. This was correlated with significantly reduced production of IL-4 and IL-5 by CD4⁺ T cells, eotaxin in lung tissues and reduced airway allergic inflammation in the SJDC recipients following allergen sensitization and challenge. These data suggest that helminth infection may induce tolerogenic DCs that can inhibit the development of airway allergic inflammation through enhancing T regulatory cell responses.

Keywords asthma, dendritic cell, hygiene hypothesis, regulatory T cell, Schistosoma japonicum

Abbreviations: NDC, dendritic cell from naive mice; SJ, Schistosoma japonicum; SJDC, dendritic cell from SJ-infected mice

INTRODUCTION

In most developed countries, the prevalence of allergic diseases such as asthma has increased significantly over the past few decades, which appears to be associated with a general decline of infectious diseases in these areas (1,2). A number of epidemiological and clinical studies had shown an inverse correlation between atopic diseases and helminth infections (1,3–5). Both allergy and helminth infections are characterized by elevated levels of Immunoglobulin E (IgE), tissue eosinophilia and Th2 responses (1,6). Allergic Th2 response is an abnormality in human, whereas Th2 responses to helminth infections are considered an important protective mechanism against these parasitic infections (6,7). More recently, the immunomodulatory effect of helminth infection has been identified (8). We and many other researchers have demonstrated that parasite infections can inhibit the Th2 response and subsequent allergic inflammatory diseases (9–13). However, the mechanism underlying this inhibition remains unclear.

Dendritic cells (DCs) are crucial for the initiation of adaptive immune responses because of their ability to activate naive T cells (14,15). Dendritic cells express co-stimulatory molecules and produce cytokines, such as IL-10 and IL-12, thus influencing the activation, differentiation and function of antigen-specific T cells (15–20). In addition to

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activating effector T cells, DCs are recently recognized to be strong inducers of T regulatory (Treg) cells (17–19). The function of DCs is influenced by the state of maturation, cytokine production and the expression of co-stimulatory molecules. Although many studies have shown the association of immature DCs with Treg development, some recent studies indicated that mature DCs, expressing high levels of costimulatory molecules, actively induced the development of Treg cells that had potent inhibitory/regulatory activity (20,21). Although many factors are likely involved, IL-10 production by DCs appears more critical for the DCs to induce Treg.

The induction and expansion of Treg by helminth infections has been widely reported (22–26). CD4⁺CD25⁺ Treg cells arise normally in naive mice and constitute 5–10% of the peripheral CD4⁺T cells. Many types of Treg have been reported. In particular, natural Tregs are generated in the thymus, and they spontaneously express the transcription factor Foxp3 and high levels of CD25. Foxp3 is considered essential for the development and function of Treg (27–30). Inducible Tregs are generated by peripheral activation, particularly in the presence of IL-10 or TGF- β . Tregs inhibit immune responses requiring direct contact with their targets and/or the production of inhibitory cytokines, including IL-10 and TGF- β (31,32). Diverse populations of regulatory T cells (Treg) play an important role in regulating Th2 responses to allergens (33).

In a recent study, we reported that prior *Schistosoma japonicum* (SJ) infection can inhibit airway allergic inflammation in a mouse model, which is correlated with reduced production of Th2 cytokines (13). More interestingly, we found that adoptive transfer of DCs from the infected mice can also inhibit allergic inflammation, which is associated with enhanced IL-10 production in the recipients. These data suggest that DCs play a critical role in mediating the inhibitory effect of helminth infection on allergy. However, the mechanism by which DCs from infected mice inhibit allergy remains unclear. Because the adoptive transfer of DCs from *Schistosoma japonicum*-infected mice (SJDC) enhanced IL-10 production in recipient mice (13), we hypothesized that the DCs from infected mice enhanced Treg function in the recipients leading to inhibition of allergic Th2 responses. In this study, we examined the cytokine production of the DCs from infected and naive mice and the development of Treg in the recipients of different DCs. The results showed that adoptive transfer of SJDC led to enhanced Treg responses with high Foxp3 expression and IL-10 production, which was associated with significant reduction of Th2 cytokines by CD4⁺ T cells and airway inflammation following allergen sensitization and challenge.

MATERIALS AND METHODS

Animals and parasites

Female BALB/C mice (8–10 week old) were purchased from Chinese Military Medical Scientific Institute (Beijing, China). The mice were maintained at the animal housing facility at Tianjin Medical University (Tianjin, China). The mice were used according to the guidelines for experimental use of animals by Tianjin Medical University. *Schistosoma japonicum* was obtained from Institute of Prevention and Cure of Parasitic Diseases (Jiangsu, China).

Reagents and antibodies

Ovalbumin (OVA), phorbol 12-myristate 13-acetate (PMA), ionomycin calcium salt from *Streptomyces conglobatus* (ionomycin) and concanavalin A from *Canavalia ensiformis* (ConA) were obtained from Sigma-Aldrich (St Louis, MD, USA). The kit and conjugated mAbs for intracellular cytokine staining, including Mouse Regulatory T cell Staining kit (with phycoerythrin (PE) anti-Foxp3, fluorescein isothiocyanate (FITC) anti-CD4 and allophycocyanin (APC) anti-CD25), FITC anti-mouse CD4 (L3T4, clone RM4-5), APC anti-mouse CD25 (clone PC61-5), PE anti-mouse IL-4 (clone 11B11), PE anti-mouse/human IL-5 (clone TRFK5), PE anti-mouse IFN- γ (clone XMG1-2), PE anti-mouse IL-10 (clone JES5-16E3), FITC anti-mouse CD69 (clone H1-2F3), functional grade purified anti-mouse CD3 (clone 17A2), functional grade purified anti-mouse CD28 (clone 37-51) and appropriately labelled isotype-matched control Abs, were purchased from eBioscience (San Diego, CA, USA). CD11c Micro-Beads were purchased from Miltenyi Biotec (Bergish Gladbach, Germany). Anti-mouse Eotaxin/CCL11 Ab was purchased from R&D System (Minneapolis, MN, USA). Mouse IL-10 and IL-12 ELISA kits were purchased from Invitrogen Co. (Carlsbad, CA, USA). Complete RPMI 1640 medium containing 10% heat-inactivated foetal bovine serum, 1% L-glutamine, 25 μ g/mL gentamycin and 5×10^{-5} M 2-mercaptoethanol (Kodak, Rochester, NY, USA) was used for cell culture.

Infection of mice and adoptive transfer of DCs

Mice were infected subcutaneously with 23 ± 3 cercariae of SJ as described (13). Successful infection was confirmed by identifying parasitic eggs in faecal samples at 5 weeks after inoculation (13). Eight weeks post-infection, spleens were aseptically collected, and DCs were isolated using a MACS (Miltenyi Biotec) CD11c⁺ column according to manufacturer's instructions. Dendritic cells from naive

mice were isolated using the same protocol. The purity of the isolated CD11c+ DC was >90% based on flow cytometry. The isolated CD11c+ cells from infected mice are designated as dendritic cells from SJ-infected mice (SJDCs). The CD11c+ cells isolated from age- and gender-matched naive mice were designated as DCs from naive mice (NDCs). For adoptive transfer, isolated CD11c+ cells were first washed in protein-free phosphate-buffered saline (PBS), and 1×10^6 DCs were injected to naive mice by tail vein as described (13,34).

DC cytokine analysis

To test the spontaneous production of cytokines by the freshly isolated DCs, the isolated DCs were cultured with complete medium using 96-well culture plates at 5×10^5 cells/well in the presence of 0.1 mg/mL OVA for 72 h. The levels of IL-12 and IL-10 in culture supernatants were measured by ELISA as described (34).

Allergen sensitization and challenge

Mice were sensitized intraperitoneally (i.p.) with 100 µg of OVA in 2 mg of AIK (SO₄)₂ adjuvant for two times at a 14-day interval. One week after the second sensitization, mice were challenged intranasally (i.n.) with 50 µg of OVA in 50 µL of PBS for seven consecutive days. For DC recipients, the sensitization was performed 2 h after adoptive transfer of DCs. Mice with or without DC transfer were sensitized and challenged with OVA to examine the effect of the DCs on the development of allergic airway inflammation and allergen-driven immune responses. Mice were killed at 7 days after last OVA challenge, and different tissue samples were collected for further analysis.

BALF collection and eosinophil cell counting

The mouse tracheas were cannulated, and the lungs were washed four times with 0.5 mL of sterile phosphate-buffered saline (PBS, pH7) to collect bronchoalveolar lavage fluids (BALF). The total number of cells in the BALF was first counted. The BALF were then centrifuged, and the supernatants were collected for measurement of cytokines; the cell pellets were resuspended to prepare slides for differential cell counting. The slides were air-dried and then stained with Wright and Giemsa stains. The percentage of eosinophils was calculated by counting 200 cells in each slide.

Histopathological analysis

Lungs were collected, fixed in 10% formalin, embedded, sectioned and stained by haematoxylin and eosin (H&E).

Slides were examined for pathological changes under light microscopy. Pulmonary inflammation and tissue damages were scored according to Underwood's standards. Eotaxin/CCL11 expression in the lung tissues was examined by immunohistochemistry using anti-mouse Eotaxin/CCL11 Ab (R&D System) according to the manufacturer's instructions. Staining of CCL11 on the slides was analysed using NIS-ELEMENTS BR 3.0 software.

Intracellular cytokine detection by flow cytometry

Single spleen cell suspensions, after removing RBC, were prepared at $2-3 \times 10^6$ cells/mL. For analysing the intracellular expression of IL-4 and IFN- γ , cells were stimulated with 25 ng/mL PMA and 1 µg/mL ionomycin in the presence of 10 µg/mL brefeldin A for 4–6 h at 37°C in a humidified atmosphere of 5% CO₂ in air. For analysing the intracellular expression of IL-5 and IL-10, cells were stimulated with 3 µg/mL ConA for 2 days at 37°C followed by incubation with anti-CD3 mAb (10 µg/mL) plus soluble anti-CD28 (2 µg/mL) in the presence of 10 µg/mL brefeldin A for 6 h. Brefeldin A was used to block intracellular transport mechanisms, thereby producing an accumulation of cytokines in the Golgi apparatus. After the stimulations, whole cells were washed and incubated for 10 min with Fc receptor blocker to prevent nonspecific binding. Subsequently, the samples were separately incubated with FITC anti-mouse CD4 Ab (L3T4), and APC anti-mouse CD25 and the isotype control Abs for 20 min in dark at room temperature. After a washing step using ice-cold PBS, the plasma membrane of the cells was permeabilized by permeabilizing solution (eBioscience) for another 20 min in dark at 4°C. The cells were then washed once by ice-cold PBS and incubated with PE anti-mouse IL-4, PE anti-mouse IFN- γ , PE anti-mouse/human IL-5, PE anti-mouse/human IL-10 and isotype control Abs, separately, for 30 min in dark at room temperature. Finally, cells were washed with ice-cold PBS and resuspended in 0.5 mL PBS. Data acquisition (10 000 events) was performed using FACS ARIA, and data analysis was performed using FACS DIVA software (BD Biosciences, San Jose, CA, USA).

Flow cytometric analysis of Foxp3 expression

To analyse the expression of transcription factor Foxp3, Mouse Regulatory T cell Staining Kit was used in accordance with the manufacturer's instructions. Briefly, freshly isolated spleen cells were pre-incubated with FcR Block to prevent nonspecific binding, and cell surface staining was then performed using FITC anti-mouse CD4 (L3T4), APC anti-mouse CD25 and their isotype control Abs for 20 min at room temperature in dark. Cells were washed twice in

ice-cold staining buffer (eBioscience) and resuspended in Fixation/Permeabilization working Solution (eBioscience) for 15 min at 4°C in dark. Intracellular staining for Foxp3 was performed using PE anti-Foxp3 Ab for 20 min at 4°C in dark. Cells from individual mice were analysed independently, analysed without pooling. Data acquisition (10 000 events) was performed using FACS ARIA and the FACS DIVA software (BD Biosciences).

Statistical analysis

All data were analysed by one-way ANOVA. A value of $P < 0.05$ was considered statistically significant. The results are expressed as mean $\pm$ SD.

RESULTS

SJDC produced significantly higher levels of IL-10 than NDC

We have reported that adoptive transfer of SJDCs can inhibit airway allergic reactions induced by OVA sensitization and challenge in mice (13). Because cytokine production is closely related to the function of DCs, we further tested the IL-10 and IL-12 production profile of DCs by culture of freshly isolated DCs from mice with (SJDCs) or without (NDCs) SJ infection, respectively. We found that SJDCs, after a 3-day period culture in the presence of OVA, produced significantly higher levels of IL-10 than NDCs (Figure 1). In contrast, the levels of IL-12 in the culture supernatants were comparable between SJDCs and NDCs. The results demonstrate that SJ infection can change the profile of DCs and, in particular, increase IL-10 production by the DCs, suggesting its potential to induce regulatory T cells.

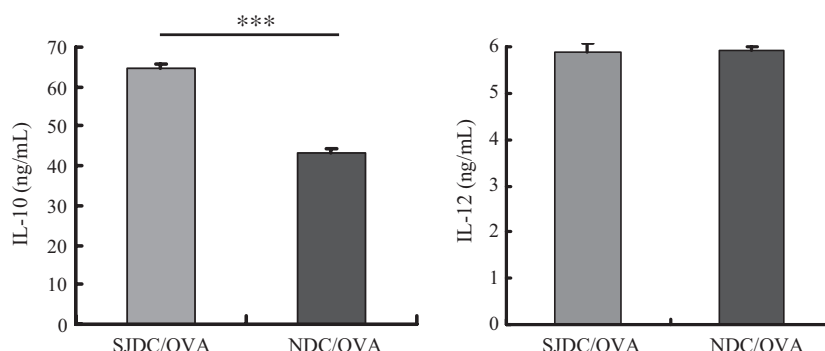


Figure 1 Dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) produced significantly higher levels of IL-10 than DCs from naive mice (NDCs). Eight weeks post-infection, SJDCs and control NDCs were cultured with complete culture medium with OVA for 72 h. Pooled DCs of five mice in each group were set up at five replicate wells. IL-10 and IL-12 levels in the culture supernatants were tested using ELISA. Data are presented as Mean $\pm$ SD. *** $P < 0.001$. Data from one representative experiment of two independent experiments with similar results are shown.

Adoptive transfer of SJDC induced CD4+CD25+Foxp3+ cells following allergen sensitization and challenge

To directly examine the capacity of SJDCs in inducing regulatory T cells, SJDCs and NDCs isolated from age- and gender-matched naive mice were adoptively transferred to naive mice by tail vein (1×10^6 cells/mouse), respectively. Two hours later, the mice were sensitized i.p. with OVA (in alum) for two times at a 2-week interval. One week after the second sensitization, the mice were challenged i.n. with OVA for seven consecutive days. Twenty-four hours after last challenge, the levels of CD4+CD25+Foxp3+ Treg cells in the spleens of different groups were analysed by flow cytometry using three-colour staining. As shown in Figure 2, when the isolated spleen cells were gated on CD4+ cells, the percentage of double-positive cells co-expressing CD25 and Foxp3 in the recipients of SJDCs were significantly higher than that in NDC recipients (8.4% vs. 3.9%, $P < 0.01$) and those without DCs transfer (8.4% vs. 3.7%, $P < 0.01$). The transfer of NDCs had no significant effect on CD4+CD25+Foxp3+ cells (3.7% vs. 3.9%, $P > 0.05$). The data clearly indicate the expansion of CD4+CD25+Foxp3+ Treg in the SJDC recipients following allergen sensitization and challenge.

Because IL-10 is a critical cytokine by which regulatory T cells play their role in immune regulation, we further tested the IL-10-producing CD4+CD25+ T cells in the mice that received different types of adoptively transferred DCs using intracellular staining. As shown in Figure 3, the mice that received SJDCs and OVA sensitization/challenge (SJDC/OVA) showed significantly higher percentage of IL-10-producing CD4+CD25+ T cells than those that

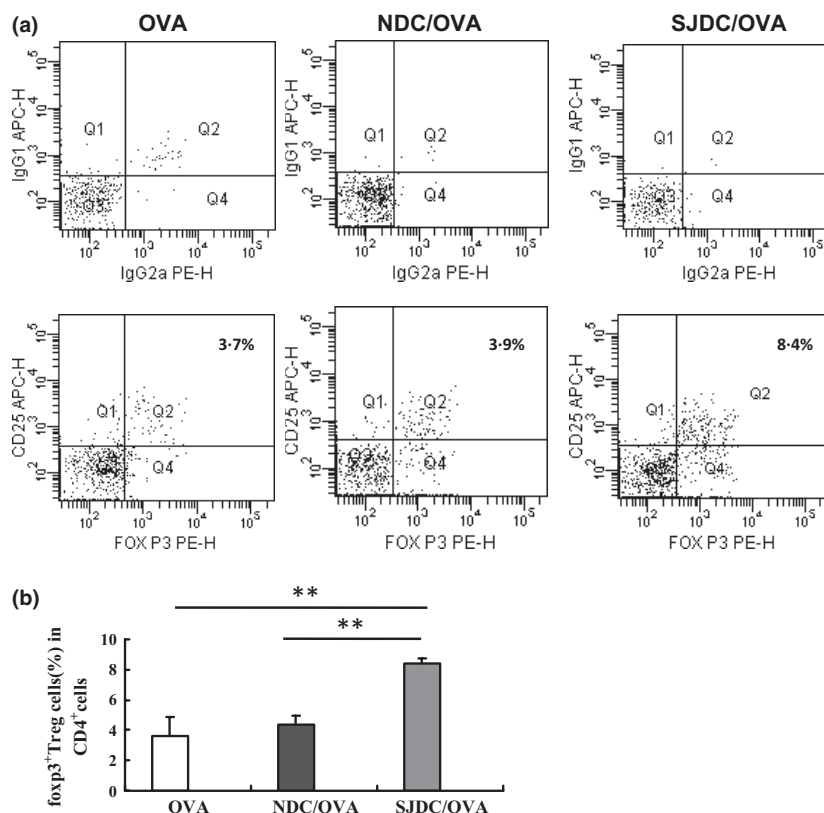


Figure 2 The adoptive transfer of dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) enhances CD4+CD25+Foxp3+ Treg responses following allergen sensitization and challenge. SJDCs and DCs from naive mice (NDCs) were adoptively transferred to naive mice by tail vein, and the recipient mice and the control mice without cell transfer were then sequentially sensitized i.p. and challenged i.n. with ovalbumin (OVA) as described in the Materials and methods. The mice were killed 24 h after the last challenge with OVA. The percentages of CD4+CD25+Foxp3+ Treg cells in the spleen were tested by three-colour staining using flow cytometry. (a) Representative dot plots of different groups. CD4+ cells were gated and analysed for the specific markers with isotype controls (upper panel) being used in each individual mouse, so the specific staining was obtained by subtraction of the isotype control staining in the cells from the same mouse. (b) Summary of the frequency of CD4+CD25+Foxp3+ cells (Mean $\pm$ SD) in different groups (five mice in each group were individually analysed). Differences in CD4+CD25+Foxp3+ frequency among the three groups were evaluated using one-way ANOVA. ** $P < 0.01$. The experiments were performed twice, and similar results were obtained.

received NDCs with the same OVA treatment (NDC/OVA) (5.6% vs. 2.9%, $P < 0.001$). The transfer of the NDCs has no significant effect on IL-10-producing CD4+CD25+ T cells. The data demonstrate that SJDCs have the capacity to enhance IL-10-producing regulatory T cells following allergen exposure.

Adoptive transfer of SJDC inhibits allergen-induced Th2 cells without significant effect on Th1 cells

In our previous study, we performed bulk splenic cell culture analysis of cytokine production by the recipients SJDCs and NDCs following OVA sensitization and challenge and found a significant reduction in IL-4 and IL-5 production in SJDC recipients (13). To further

examine whether the adoptive transfer of SJDCs indeed influence cytokine production by CD4+ T cells, we here performed intracellular cytokine analysis on CD4+ T cells obtained from the mice that received different types of DCs with OVA sensitization and challenge. The results showed that the percentages of IL-4-producing and IL-5-producing CD4+ T cells were significantly reduced in the recipients of SJDCs compared to those that received NDCs or without DC transfer (Figure 4). The transfer of NDCs had no significant effect on IL-4-producing or IL-5-producing CD4+ T cells. The levels of IFN- γ -producing cells were comparable among the different groups. The results suggest that SJDC transfer can modulate Th2 responses induced by allergen sensitization and challenge.

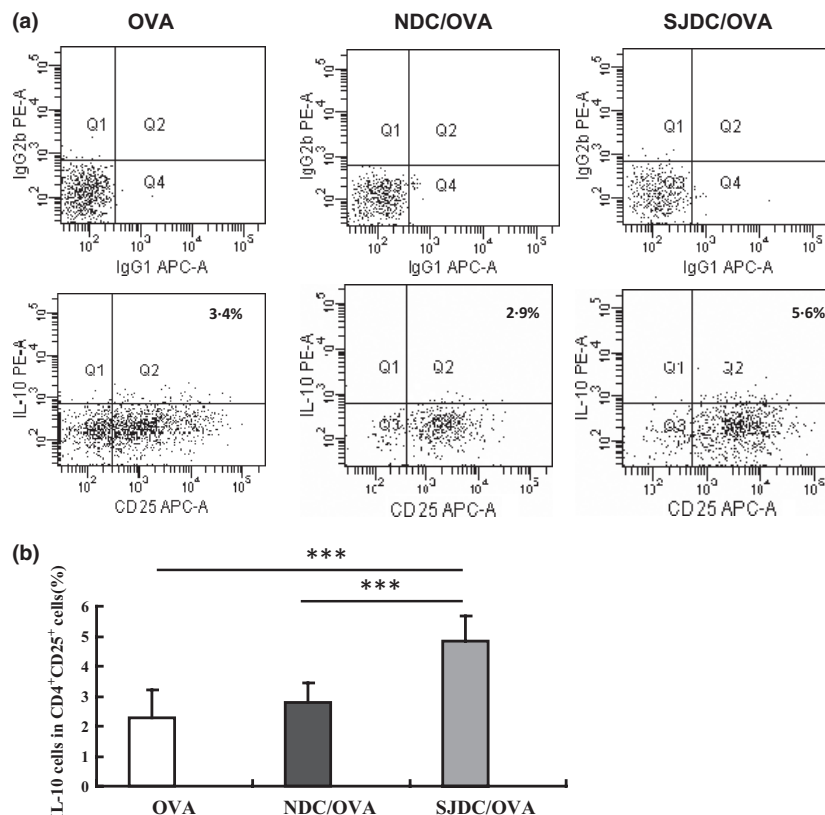


Figure 3 The adoptive transfer of dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) enhanced CD4+CD25+IL-10+ cells following allergen sensitization and challenge. The single spleen cells obtained from the different groups of mice described in the legend to Figure 2 were measured for the intracellular IL-10 expression by CD4+CD25+ using flow cytometric analysis as described in Materials and methods. (a) Representative dot plots of different groups. CD4+ cells were gated and analysed for the specific markers with isotype controls (upper panel) being used in each individual mouse, so the specific staining was obtained by subtraction of the isotype control staining in the cells from the same mouse. (b) Summary of the frequency (Mean $\pm$ SD) of CD4+CD25+IL-10+ cells in different groups (five mice in each group were individually analysed). Differences in CD4+CD25+IL-10+ frequency among the three groups were evaluated using one-way ANOVA. *** $P < 0.001$. The experiments were performed twice, and similar results were obtained.

Adoptive transfer of DCs from SJ infected mice inhibits lung eotaxin production and eosinophilia induced by OVA exposure

We have reported that the adoptive transfer of SJDCs can significantly reduce airway eosinophilic inflammation induced by OVA sensitization and challenge (13). The repeated analysis of airway inflammation in this study showed similar results, confirming the inhibitory effect of SJDCs on allergic inflammation (Figure 5). Because the recruitment of eosinophils is also regulated by local chemokines especially eotaxin, also called CCL11 (35–37), we further examined eotaxin expression by bronchiole epithelial cells in the recipients of different DCs following OVA sensitization and challenge. As shown in Figure 6, the level of eotaxin expression, measured by the average OD of eotaxin-positive regions, in the recipients of SJDCs was significantly lower than those that received NDCs or

without DC transfer. Together, the results suggest that the adoptive transfer of SJDCs cannot only suppress Th2 cell responses as shown earlier, but also inhibit eosinophilic chemokine production in the local tissues, thus inhibiting the development of airway allergic inflammation through multiple mechanisms.

DISCUSSION

We and others previously reported that SJ infection was capable of inhibiting airway eosinophilic inflammation and mucus production induced by allergen (13,38,39). We also showed that DCs play a critical role in this inhibition process (13). In this study, we further demonstrated that the DCs from SJ-infected mice, after adoptive transfer, induced cells with a CD4+CD25+Foxp3+ phenotype. Intracellular cytokine analysis showed that SJDCs induced IL-10-producing CD4+CD25+ cells. Therefore, the DCs

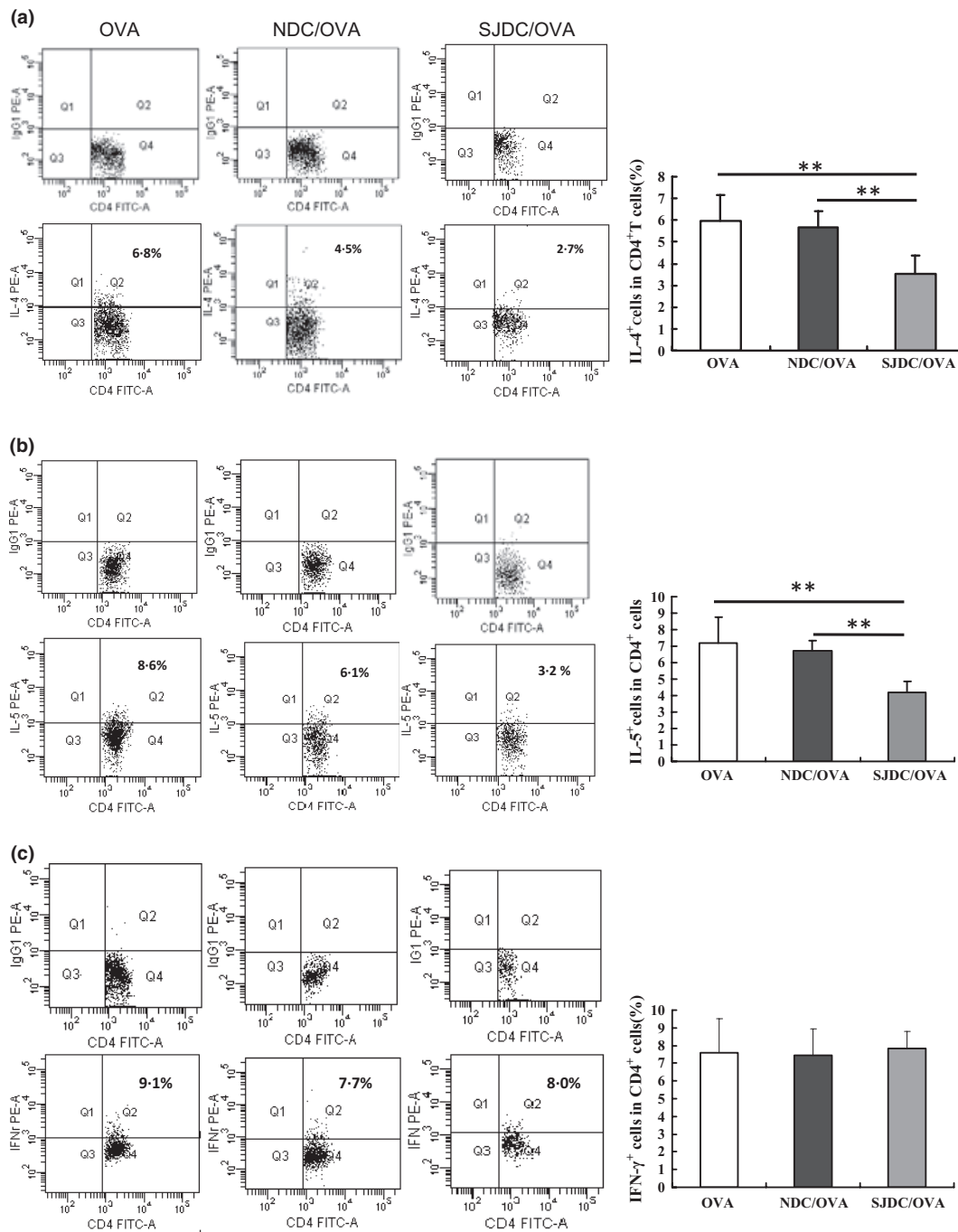


Figure 4 The adoptive transfer of dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) reduced IL-4-producing and IL-5-producing CD4⁺ T cells. The single spleen cells obtained from the different groups of mice described in the legend to Figure 2 were measured for intracellular expression of IL-4, IL-5 and IFN-γ by CD4⁺ cells using two-colour staining and flow cytometric analysis. The left panels show representative flow cytometric plots of CD4⁺IL-4⁺ (a), CD4⁺IL-5⁺ (b) and CD4⁺IFN-γ⁺ (c) cells of the different groups, and the right panels show the summaries of frequencies (mean ± SD) of the corresponding groups (five mice in each group were individually analysed). CD4⁺ cells were gated and analysed for the specific markers with isotype controls being used in each individual mouse (upper panels of a, b and c), so the specific staining was obtained by subtraction of the isotype control staining in the cells from the same mouse. Differences in CD4⁺IL-4, CD4⁺IL-5 and CD4⁺IFN-γ frequency among the three groups were evaluated using one-way ANOVA. ***P* < 0.01. One representative experiment of two experiments with similar results is shown.

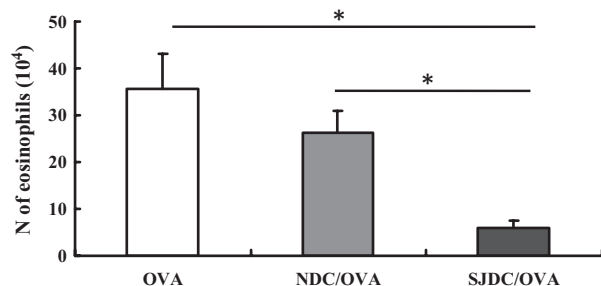


Figure 5 Effect of adoptive transfer of dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) on airway eosinophilic inflammation. The mice treated with DC transfer and OVA sensitization and challenge as described in the legends to Figure 2. Twenty-four hours after the last challenge with ovalbumin (OVA), the mice were sacrificed and the pulmonary cellular infiltration was examined by differential cell counts of bronchoalveolar lavage fluids (BALFs). The figure shows the amounts (mean $\pm$ SD) of eosinophils in total BALF cells. Five mice in each group were individually analysed. *** $P < 0.05$. One representative experiment of two experiments with similar results is shown.

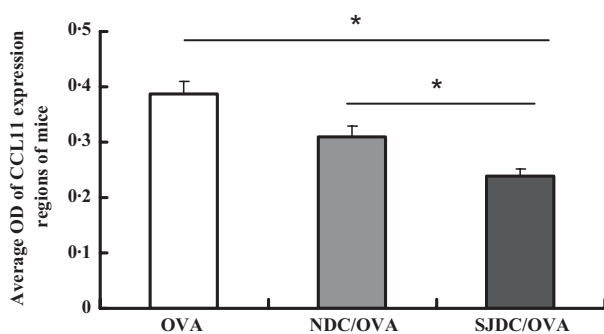


Figure 6 The adoptive transfer of dendritic cells isolated from *Schistosoma japonicum*-infected mice (SJDCs) significantly inhibits eotaxin (CCL11) production in the lung. The mice treated with various DC transfer and ovalbumin (OVA) sensitization and challenge as described in the legends to Figure 2. The mice were killed 24 h after the last challenge with OVA. Lung tissues were fixed, and sections were stained with anti-CCL11 antibody as described in Materials and methods. Average optical density (AOD) of CCL11-positive expression was determined. Compared with mice in groups OVA and NDC/OVA, the AOD of CCL-11 in group SJDC/OVA was significantly lower ($P < 0.05$). No significant difference was found between OVA and NDC/OVA groups.

from SJ-infected mice have strong capacity to induce regulatory T cells. Moreover, we found that the Treg-inducing capacity of SJDCs was associated with higher IL-10 production by these DCs. In correlation with the higher Treg activity in SJDC recipients, Th2 cytokine (IL-4 and IL-5) production by CD4⁺ T cells induced by allergen (OVA) sensitization and challenge was significantly reduced in these mice, shown by intracellular cytokine analysis. In

contrast, IFN- γ production by CD4⁺ T cells did not change with the transfer of SJDCs or control NDCs. This is also consistent with the cytokine patterns of the DCs in that SJDCs produced higher IL-10 than NDC but the IL-12 production of the two types of DCs was comparable. Although the reason for the comparable IL-12 production remains unclear, it is inline with the failure of the transfer of SJDCs in altering IFN- γ responses, not suggesting an important role of immune deviation in the inhibition of allergic reactions in this model. The results were consistent with our previously reported results obtained from bulk culture of splenocytes and analysis of BALF (13). In addition, the adoptive transfer of SJDCs reduced eotaxin production in the lung tissues, consistent with the reduction of eosinophilia in the airways. Overall, the results in this study suggest that SJ infection can influence DC function, leading to the development of tolerogenic DCs that produce IL-10 and induce Treg, thus subsequently suppressing allergic reactions induced by allergen.

The demonstration of the capacity of DCs from SJ-infected mice to induce CD4⁺CD25⁺Foxp3⁺ regulatory T cells is very helpful for our understanding on the mechanism by which helminth infections modulate allergic responses. Numerous reported studies have shown that parasitic especially helminth infections can inhibit the development of allergic diseases in humans and experimental animals (9–13,22,25). It was also demonstrated that the inhibitory effect was associated with increased IL-10 production and the development of IL-10-producing Treg in some studies (38–41). However, it was unclear how the Treg was induced. Our present data have filled this gap by showing a critical role of IL-10-producing DCs induced by helminth infection in enhancing CD4⁺CD25⁺Foxp3⁺ Treg responses in the setting of allergic reactions. In theory, DCs can modulate T-cell responses through at least three mechanisms, including induction of Treg, T-cell anergy and switch of Th1/Th2 balance (17,18). Because our data showed that the adoptive transfer of SJDCs had no significant effect on Th1 cytokine (IFN- γ) responses, the switch of Th1/Th2 balance is unlikely the major mechanism for the observed inhibition of allergy. The significant increase in Treg in the recipients of SJDCs suggests that induction of Treg is likely the major mechanism by which the DCs influenced by SJ infections modulate allergen-driven Th2 responses. Notably, many studies in other model systems have demonstrated the linkage between IL-10-producing DCs and Treg (42,43). Diminished IL-10 production by DCs was also found in atopic children (44). In addition to its capacity to induce Treg, DCs modulated by SJ infection may also have direct inhibitory effect on allergen-driven

effector Th2 cells by inducing cell anergy. Further study on this aspect will be helpful for comprehensive elucidation of the mechanism(s) by which DCs from infected mice inhibit allergic responses.

In summary, we demonstrated that helminth infection increased IL-10 production by DCs and that the adoptive transfer of the DCs from helminth-infected mice inhibited the development of allergy, which was associated with enhanced IL-10-producing CD4+CD25+Foxp3+ T regulatory cells response. The data provide evidence for the key role played by DCs in bringing the linkage between the long-time documented inhibitory effect of helminth infection on allergic diseases and the induction of Treg activity. Further study on the molecular mechanisms by

which the worms and/or their eggs and products modulate the function of DCs and the mechanism by which the modulated DCs induce Treg or directly influence innate and adaptive immune cells would be very helpful for developing new approaches for preventing and treating allergic diseases and other autoimmune diseases.

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