

Helminth infection inhibits airway allergic reaction and dendritic cells are involved in the modulation process

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SUMMARY

Several previous studies have demonstrated that some helminth infections can inhibit allergic reactions, but the examination on the effect of live *Schistosoma japonicum* (SJ) infection on allergic inflammation remains limited. The aim of this study was to examine the effect and mechanism of chronic SJ infection on airway allergic inflammation in a murine model. The data showed that chronic SJ infection suppressed airway eosinophilia, mucus production and antigen-specific IgE responses induced by ovalbumin (OVA) sensitization and challenge. Cytokine production analysis showed that chronic SJ infection reduced allergen-driven interleukin (IL)-4 and IL-5 production, but had no significant effect on IFN- γ production. More importantly, we found that the adoptive transfer of dendritic cells (DCs) from SJ-infected mice dramatically decreased airway allergic inflammation in the recipients, which was associated with significant decrease of IL-4/IL-5 production and increase of IL-10 production. The results suggest that SJ infection may inhibit the development of allergy and that DCs may be involved in the process of helminth infection-mediated modulation of allergic inflammation.

Keywords allergy, dendritic cells, hygiene hypothesis, *Schistosoma japonicum*

INTRODUCTION

Allergy and asthma are common human diseases with significantly increased prevalence over the past decades, especially in developed areas (1,2). On the basis of the apparent association of the general decline of infectious burdens and the dramatic increase of allergy and asthma, the 'hygiene hypothesis' was raised. This hypothesis suggests that exposure to certain infections may have an inhibitory effect on the development of allergy and asthma (3–6). Indeed, numerous experimental studies have demonstrated an inhibitory role of certain bacterial infections in allergic asthma through the modulation of the balance of Th1/Th2 responses (7–9). However, the influence of parasitic infection on the development of allergy and asthma has been less well-studied, although some epidemiological and clinical studies have suggested that chronic helminth infection may also inhibit allergic asthma (5,10–15). Recently, some experimental studies showed a protective effect of certain chronic helminth infections, including *Schistosoma* and *Strongyloides*, on the development of airway inflammation (16–28). So far, *Schistosoma mansoni* (SM) is the major *Schistosoma* species on which most studies have focused (12,16,17,20,23).

Schistosoma japonicum (SJ) is a *Schistosoma* species that is epidemic in certain areas of China and Southeast Asia (29–33). Although both SJ and SM are major species of the genus *Schistosoma*, significant differences exist between these two species with regard to epidemiology, parasitology, life cycle and host (34,35). *Schistosoma japonicum* is mainly found in Asia, whereas SM is prevalent throughout Africa, the Middle East and some parts of South America. Adult worms of SJ are the largest schistosomes infecting humans and the tegumental surface of both the genders is smooth. For SM, the male tegument has tubercles on the dorsal sur-

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Abbreviations: NDC, dendritic cell from naïve mice; SJ, *Schistosoma japonicum*; SJDC, dendritic cell from SJ-infected mice; SM, *Schistosoma mansoni*

face, whereas the female tegument is smooth. More importantly, the location of the species in both definitive and intermediate hosts is different. Specifically, adult worms of *SJ* in humans are found in the inferior mesenteric vein–portal vein system, whereas *SM* is found in small mesenteric vein. In the intermediate hosts, the snail host for *SJ* belongs to the genus *Oncomelania*, whereas genus *Biomphalaria* is the suitable snail host of *SM*. Considering the multiple differences between *SJ* and *SM*, the previous findings in *SM* cannot be directly translated to *SJ*. Rather, further study on the effect of *SJ* on allergy/asthma, especially in a live infection setting, is necessary.

Dendritic cells (DCs) are the most important antigen-presenting cells, especially in primary T-cell responses. T-cell cytokine patterns are largely influenced by the function of DCs. It has been recently reported that DCs play a critical role in bacterial infection-mediated inhibition of allergic reactions (36–38). Specifically, it is found that intracellular bacterial infection can modulate the phenotype and function of DCs, which can inhibit the development of Th2-like allergen-specific CD4⁺ T cells in both *in vitro* and *in vivo* settings. However, the role played by DCs in helminth-mediated modulation of allergic reactions has not been reported yet. In this study, we examined the effect of chronic *SJ* infection on airway allergic inflammation and explored the role played by DCs in the modulating effect of *SJ* infection on the allergic reactions using a DC adoptive transfer approach. The data provide evidence that chronic *SJ* infection can inhibit allergic airway inflammation and that DCs may be involved in the modulating process through direct or indirect inhibition of the development of allergen-driven Th2-like T-cell responses.

MATERIALS AND METHODS

Animals

Eight- to ten-week-old female BALB/c mice were purchased from Chinese Military Medical Scientific Institute. The mice were bred at the animal housing facility at Tianjin Medical University. The mice were used according to the guidelines for experimental use of animals by Tianjin Medical University.

Infection and confirmation

Schistosoma japonicum was obtained from Institute of Prevention and Cure of Parasitic Diseases (Jiangsu, China). Mice were percutaneously infected with 23 ± 3 cercariae of *SJ* as described elsewhere (16). Successful infection was confirmed by identifying parasitic eggs in faecal samples at 5 weeks after inoculation. To identify the eggs, a drop

of saline was first added onto a slide followed by addition of a small amount of faeces and mixing. The smear was examined for eggs by light microscopy.

Sensitization and challenge with allergen

Eight weeks after infection, mice infected with *SJ* (confirmed by egg identification in faeces) were sensitized intraperitoneally with 100 µg of ovalbumin (OVA; Sigma-Aldrich, St Louis, MO, USA) in 2 mg of AIK(SO₄)₂ adjuvant. Two weeks later, the OVA sensitization was repeated one more time. One week after the second sensitization, mice were challenged intranasally with 50 µg of OVA in 50 µL of PBS for seven consecutive days. Control mice were treated with the same OVA sensitization and challenge, but without previous infection. Twenty-four hours after the last challenge with OVA, mice were killed for further analysis.

Isolation and adoptive transfer of DCs

Mice infected by *SJ* for 8 weeks were killed and the spleens were aseptically collected. Splenic DCs were isolated as described elsewhere (36,39). Briefly, red blood cell removed single splenocyte suspensions were labelled with CD11c⁺ microbeads at 6–12 °C for 15 min (adding 100 µL CD11c⁺ microbeads per 10⁸ cells), then the suspension was centrifuged at 300 *g* for 10 min. Cells were collected and resuspended with PBS (500 µL/10⁸ cells). Finally, DCs were isolated using a MACS CD11c (Miltenyi Biotec, Tübingen, Germany) column according to the manufacturer's instructions. Dendritic cells from naïve mice were isolated in the same way. The purity of isolated DCs was >90%. The isolated CD11c⁺ cells from infected mice are designated as dendritic cell from *SJ*-infected mice (SJDCs). The CD11c⁺ cells isolated from age- and gender-matched naïve mice were designated as DC from naïve mice (NDCs). These CD11c⁺ cells were washed with PBS without proteins and adoptively transferred to naïve mice by tail vein (1×10^6 cells/mouse). Two hours after cell transfer, the recipients and control mice were sensitized and challenged with OVA as described above to examine the effect of the DCs on the development of allergic airway inflammation and allergen-driven immune responses.

BALF collection and differential cell counting

The mouse tracheas were cannulated and the lungs were washed four times with 0.5 mL of sterile phosphate-buffered saline (PBS, pH7.4). The bronchio-alveolar lavage fluids (BALF) were then centrifuged immediately following the washing of the lungs. 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 numbers of eosinophils, neutrophils and lymphocytes in a total of 200 cells were counted in each slide.

Histopathological analysis

Lungs were collected and fixed in 10% formalin, and then embedded in paraffin, sectioned and stained using haematoxylin and eosin (H&E). Pulmonary inflammation and tissue damages were scored according to Underwood's standards (36) on the following three aspects: (i) the level of perivascular and peribronchial eosinophils infiltration (score 0–5); (ii) the level of tissue oedema (score 0–5) and (iii) the level of epithelial injury in lungs (score 0–5). The potential maximum total score (most severe pathological changes) for each mouse is 15. The data are presented as the average total score for each mouse in the experimental groups (mean $\pm$ SEM). Bronchial mucus and mucus-containing goblet cells within bronchial epithelium were analysed by staining with periodic acid-Schiff (PAS).

Cell culture

For examination of cytokine production, cells from spleen were cultured with OVA re-stimulation as previously described (8,9). Briefly, spleens were aseptically removed and single-cell suspensions were prepared and cultured at a concentration of 7.5×10^6 cells/mL (1 mL/well) with OVA (1 mg/mL) in 24-well plates at 37°C for 72 h in culture medium: RPMI 1640 (Hyclon, Beijing, China) containing 10% heat-inactivated foetal bovine serum (Gibco, Carlsbad, CA, USA), 100 U/mL penicillin and 100 g/mL streptomycin.

Determination of cytokines

Interleukin (IL)-4, IL-5 and IFN- γ in the supernatants of splenocyte culture (72 h) and BALF were measured with enzyme-linked immunosorbent assay (ELISA) kits (BioSource International, Camarillo, CA, USA). The assays were performed according to the manufacturer's protocol.

Determination of serum antibodies

The levels of OVA-specific IgE in sera were determined with an anti-OVA IgE ELISA kit (Alpha Diagnostics International, San Antonio, TX, USA) in accordance with the manufacturer's instructions. Briefly, standards and diluted samples were added to appropriate wells and incubated at room temperature for 60 min. After washing, diluted detecting antibody was added and incubated at room temperature

for 30 min, followed by development with Streptavidin-horseradish peroxidase (HRP) and HRP-substrate tetramethyl-benzidine (TMB). The reaction was stopped at 15 min and the colour reaction was measured at the absorbance of 450 nm using an ELISA reader.

Statistical analysis

The results in this study were analysed using one-way ANOVA.

RESULTS

Schistosoma japonicum infection inhibits pulmonary eosinophilia and bronchial mucus production induced by sensitization/challenge with OVA

We examined the effect of chronic *SJ* infection on an OVA-induced airway asthma-like inflammatory reaction. As both eosinophil infiltration into the lung and mucus oversecretion are the hallmarks of asthma-like reaction, a direct comparison was first made for the histopathological analysis in the lung. As shown in Figure 1, *SJ*-infected/OVA-treated mice showed markedly decreased levels of infiltrating cells, especially eosinophils, into the bronchial and pulmonary tissues compared with that in the mice with the same allergen treatment but without *SJ* infection (OVA) (Figure 1a–c). Massive infiltration of eosinophils in the bronchial submucosa, alveolar and perivascular sheaths was observed in the mice treated with OVA only (Figure 1b), whereas much less cellular infiltration was seen in *SJ*-infected mice with the same OVA treatment (Figure 1a). No infiltration of eosinophils was observed in naïve mice (Figure 1c). The analysis of infiltrating cells in the BALF showed that the *SJ*-infected/OVA-treated mice exhibited significantly less eosinophil infiltration into the lung than those with OVA treatment only. The amount of eosinophils in the *SJ*-infected/OVA-treated mice was 14.60 ± 5.01 per 200 infiltrating cells in BALF, whereas that of eosinophils in the mice treated with OVA only was 87.20 ± 2.53 ($P < 0.05$). Moreover, the OVA-treated mice with *SJ* infection had more lymphocyte infiltration (43.80 ± 6.66 per 200 infiltrating cells) in the BALF, compared with that in those with OVA treatment alone (21.40 ± 2.71) ($P < 0.05$). Moreover, mucus staining periodic acid-Schiff (PAS) showed that the mucus-containing goblet cells in the airway epithelium induced by OVA treatment were also dramatically reduced in the mice with chronic *SJ* infection (Figure 1d–f). In addition, the semi-quantitation of the general degree of cellular inflammation and tissue damage was also remarkably decreased in the infected mice (3.6 ± 1.1) than that in the uninfected mice (8.4 ± 1.1), as shown by the analysis of pathological scores of lung tissues ($P < 0.05$).

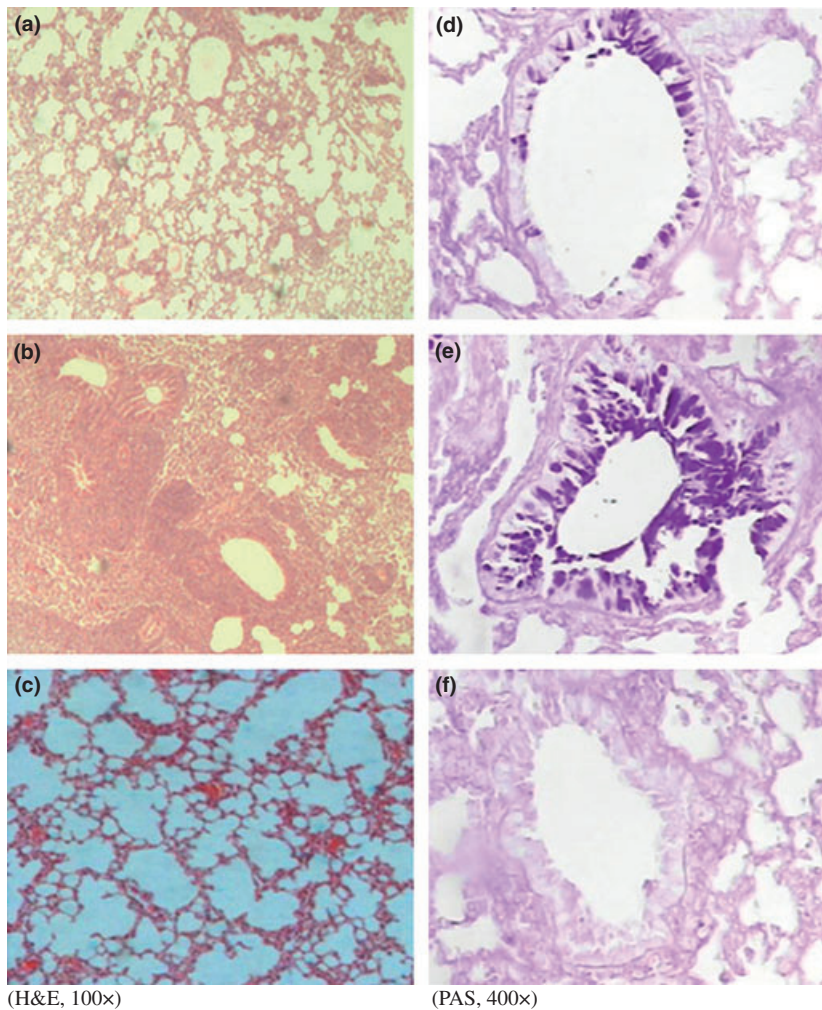


Figure 1 Chronic *Schistosoma japonicum* (*SJ*) infection reduced allergen-induced airway inflammatory reaction and mucus production. BALB/c mice were infected with 23 ± 3 cercariae of *SJ* percutaneously. Eight weeks after infection, the infected mice [*SJ*/ovalbumin (OVA)] and control mice without infection (OVA) were sensitized intraperitoneally with OVA in AlK(SO₄)₂ adjuvant twice in a 2-week interval. One week after the second sensitization, the mice were challenged intranasally with 50 µg of OVA in 50 µL PBS once daily for seven consecutive days. The mice were killed 24 h after the last challenge with OVA. Lung tissues were fixed and sections were stained either with H&E (a, b, c) for histological changes (100×), or with PAS (d, e, f) for mucus and mucus-containing goblet cells (400×). (a) and (d), OVA-treated mice with prior *SJ* infection. (b) and (e), OVA-treated mice without prior *SJ* infection. (c) and (f), naïve mice. The experiments were performed twice and similar results were obtained.

Overall, these results suggest that *SJ* infection prior to allergen sensitization and challenge can inhibit airway allergic inflammatory responses, especially eosinophilia, and mucus overproduction, which are the hallmark pathological changes in asthma-like reactions.

OVA-treated mice with prior *SJ* infection show decreased OVA-specific IgE production and allergen-driven Th2 cytokine production

As IgE and Th2 cytokine production are the basis for allergenic reactions, we examined OVA-specific IgE and cytokine responses in the serum of OVA-immunized mice, with or without *SJ* infection. As shown in Figure 2a, the levels of allergen-specific IgE of the *SJ*-infected/OVA-treated mice were remarkably lower than those of the OVA-treated mice without *SJ* infection ($P < 0.001$). In addition, the levels of IL-4 and IL-5 in the splenocyte culture supernatants of the OVA-treated mice with *SJ* infection were

dramatically lower than those of the OVA-treated mice without *SJ* infection ($P < 0.001$), although they were still higher than those of naïve mice (Figure 2b and c). IFN- γ productions were in marginal levels (6–15 pg/mL) in all groups and there were no significant difference among groups. For BALF, the IL-4 and IL-5 levels of the *SJ*-infected/OVA-treated mice were also less than those of the mice with OVA treatment only ($P < 0.001$) (Figure 3). The results indicate that *SJ* infection is capable of suppressing allergen-specific IgE responses and regulating cytokine-producing patterns of mice to allergen exposure, predominantly decreasing the levels of IL-4 and IL-5.

Adoptive transfer of DC from *SJ*-infected mice inhibits pulmonary eosinophilia and bronchial mucus production

As DCs are the most important antigen-presenting cells for directing the pattern of T-cell responses, we further examined the role played by DC in the *SJ*-mediated

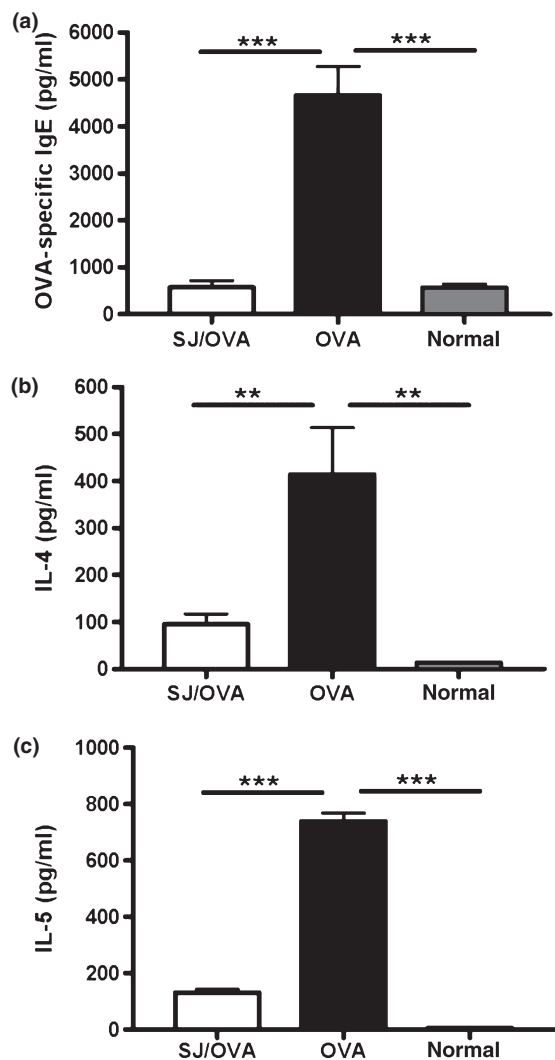


Figure 2 Prior *Schistosoma japonicum* (SJ) infection decreased ovalbumin (OVA)-specific IgE production and allergen-driven Th2 cytokine production. The mice (4–5 mice/group) were infected with cercariae of SJ and subsequently sensitized/challenged with OVA, as described in the legend of Figure 1. (a) The mice were bled 24 h after the last challenge with OVA, and OVA-specific IgE in serum samples from individual mice were determined by ELISA. Normal (naïve) mice were used as control. (b, c) The mice were killed 24 h after the last challenge with OVA and splenocytes were cultured at 7.5×10^6 cells/mL with the stimulation of OVA (1 mg/mL). Culture supernatants were harvested at 72 h and tested for interleukin (IL)-4 and IL-5 using ELISAs. The data are presented as mean $\pm$ SEM of individual mice in each group. ** $P < 0.01$; *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

inhibition of airway inflammation and Th2 responses using an adoptive transfer approach. We adoptively transferred SJDCs and NDCs respectively, to naïve recipient mice and then sensitized/challenged the recipients with

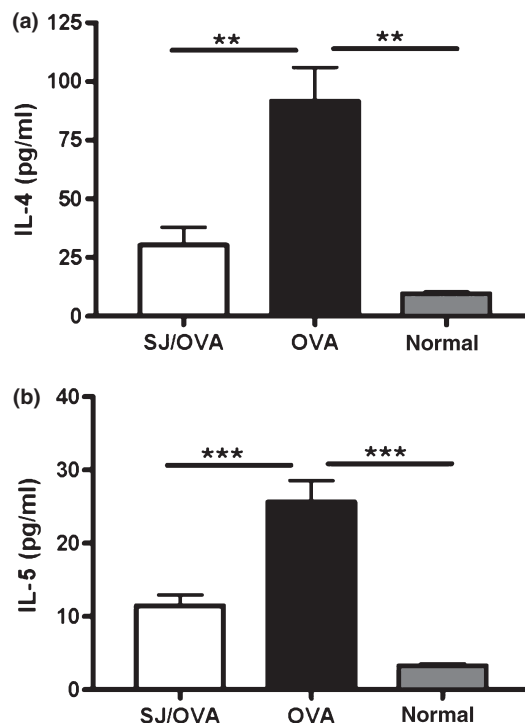


Figure 3 Effect of prior *Schistosoma japonicum* (SJ) infection on cytokine levels in the BALF. Mice were infected with cercariae of SJ and subsequently sensitized/challenged with ovalbumin (OVA), as described in the legend of Figure 1. The mice were killed 24 h after the last challenge with OVA. The mouse tracheas were cannulated and the lungs were washed with sterile PBS (pH7.4). The bronchio-alveolar lavage fluids were then centrifuged immediately following the washing of the lungs. The supernatants were collected for measurement of interleukin (IL)-4 and IL-5 using ELISAs. The data are presented as mean $\pm$ SEM of individual mice in each group. ** $P < 0.01$; *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

OVA using the same protocol as described above. These two groups were designated as SJDC/OVA and NDC/OVA groups respectively. The mice with OVA treatment (sensitization and challenge) only (designated as OVA in Figure 4) were taken as control. The data showed that inflammation in the lung tissues of SJDC/OVA mice was light, with very low levels of cellular infiltration and very few hyperplasia of goblet cells (Figure 4-a1). The structure of bronchus was normal, with only a few eosinophils in the peribronchial and perivascular areas, but no mucus in the lumen of the bronchus. In contrast, inflammation in the lung tissues of NDC/OVA mice and OVA mice was much more serious and extensive. Dramatic bronchial epithelial hyperplasia, especially goblet cells, was observed (Figure 4-b1, c2). Massive eosinophilic infiltration was found in both peribronchial and perivascular areas of these two groups of mice (Figure 4-b1, c1), with

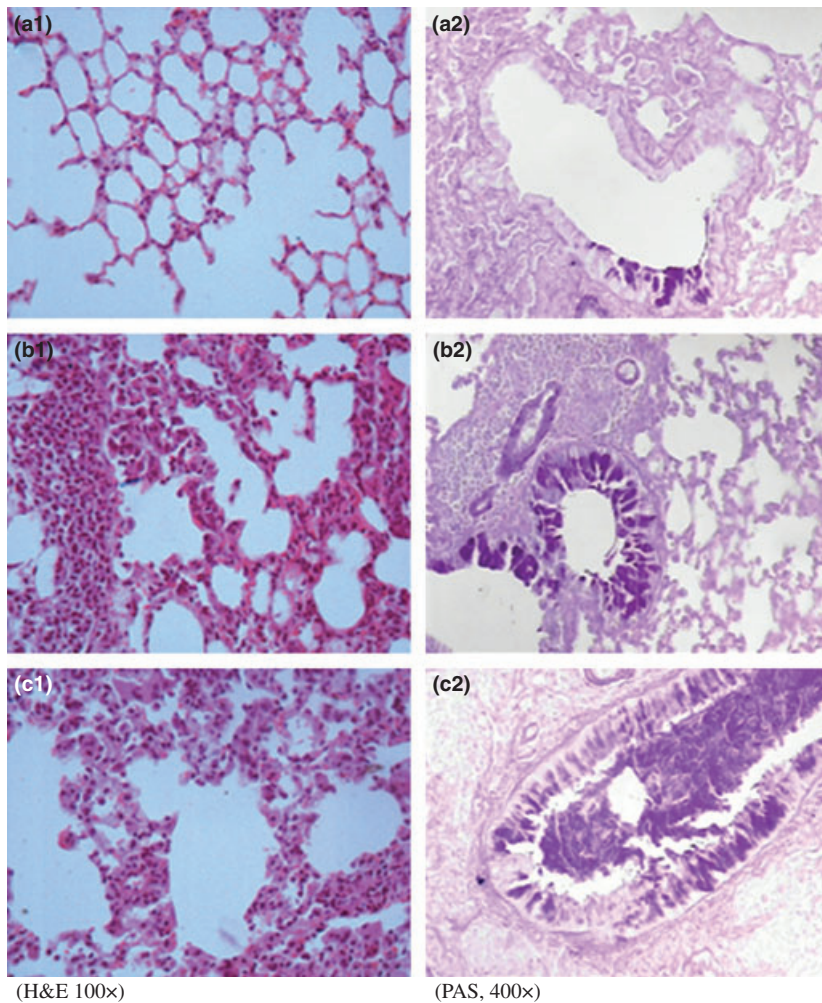


Figure 4 The effect of adoptive transfer of SJDCs on airway inflammation and bronchial mucus production. Eight weeks post-infection of *Schistosoma japonicum*, spleens were aseptically collected and dendritic cells (DCs) were isolated using a MACS CD11c column. The CD11c⁺ cells isolated from *S. japonicum*-infected mice, designated as SJDCs, and those isolated from age- and gender-matched naïve mice, designated as NDCs, were adoptively transferred to naïve mice (6–8 mice/group) by tail vein (1×10^6 cells/mouse) respectively. Two hours later, the mice were sensitized and challenged with OVA using the same protocol as described in the legend of Figure 1. The mice were killed 24 h after the last challenge with ovalbumin (OVA). Lung tissues were fixed and sections were stained either with haematoxylin and eosin (a1, b1, c1) for infiltrating cells and lung structure (100 $\times$), or with PAS (a2, b2, c2) for mucus and mucus-containing goblet cells (400 $\times$). (a1) and (a2), SJDC-transferred and OVA-treated mice. (b1) and (b2), NDC-transferred and OVA-treated mice. (c1) and (c2), OVA-treated mice. The experiments were performed twice and similar results were obtained.

large amounts of mucus observed in the lumen of bronchus, especially in OVA mice (Figure 4-c2). Consistently, statistically significantly lower numbers of eosinophils in BALF were found in SJDC/OVA mice, compared with that in control (OVA) or NDC recipient (NDC/OVA) group ($P < 0.01$) (Figure 5). The data indicate that DCs from *SJ*-infected mice are more inhibitory than those from naïve mice for the development of airway allergic inflammation in the recipient.

SJDCs significantly inhibit OVA-specific IgE production and allergen-driven Th2 cytokine production

To test whether SJDCs can modulate OVA-specific IgE production of the recipient mice, we tested OVA-specific IgE production of SJDC/OVA mice, NDC/OVA mice, OVA mice and naïve mice. As shown in Figure 6, the levels of OVA-specific IgE production of SJDC/OVA mice

were dramatically lower than those of OVA and NDC/OVA mice ($P < 0.001$), which were very close to the background level in naïve mice (not shown). Therefore, the SJDCs can inhibit not only airway allergic inflammation, but also allergen-specific IgE production of the recipient mice following allergen exposure.

To determine the modulation of SJDCs on allergen-driven cytokine production, we further examined the cytokine production in the BALF and that by the spleen cells (SPC). The results showed that the levels of IL-4 and IL-5 in SJDC/OVA mice were significantly lower than those in OVA and NDC/OVA mice in both BALF (Figure 7) and the culture supernatants of SPC (allergen driven) (Figure 8). In sharp contrast, the levels of IL-10 in the BALF of SJDC/OVA mice were significantly higher than those of OVA mice and NDC/OVA mice ($P < 0.001$) (Figure 7). Notably, the recipients of NDC (NDC/OVA) also showed certain levels of decrease in IL-4 and IL-5 production

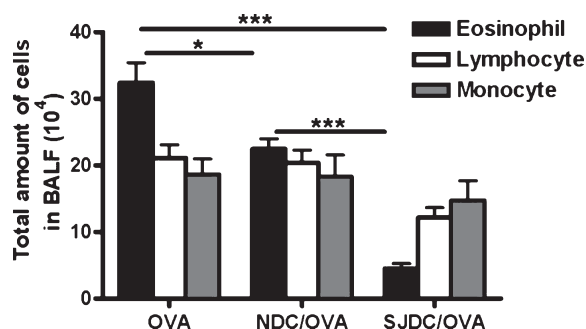


Figure 5 Effect of adoptive transfer of SJDCs on airway eosinophilic inflammation. SJDCs and NDCs were isolated and adoptively transferred to naïve mice by tail vein as described in the legend of Figure 4. Two hours later, the mice were sensitized and challenged with ovalbumin (OVA) to induce asthma as described in the legend of Figure 1. Twenty-four hours after the last challenge with OVA, all the mice were killed. Pulmonary cellular infiltration was examined by differential cell counts of bronchio-alveolar lavage fluids (BALF). The figure shows the amounts of eosinophil, lymphocyte and monocyte in total BALF cells. The data are presented as mean $\pm$ SEM of individual mice in each group. * $P < 0.05$, comparison of eosinophils between groups; *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

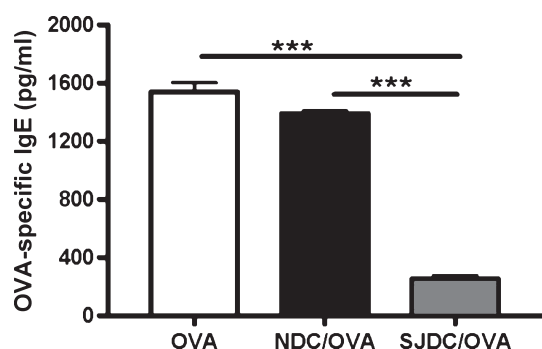


Figure 6 Effect of adoptive transfer of SJDCs on allergen-specific IgE response. The sera from the different groups of mice, as described in Figure 4, were determined for ovalbumin (OVA)-specific IgE using ELISA. The data are presented as mean $\pm$ SEM of individual mice in each group. *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

compared with that in OVA mice, but not IL-10 production (Figures 7 and 8). Taken together, the data suggest that adoptive transfer SJDC can significantly inhibit allergen-specific IgE responses and Th2-related cytokines, but increase IL-10 production.

DISCUSSION

This report shows that chronic *SJ* infection can inhibit the development of allergic airway inflammation and that DCs play a critical role in this inhibition in a mouse

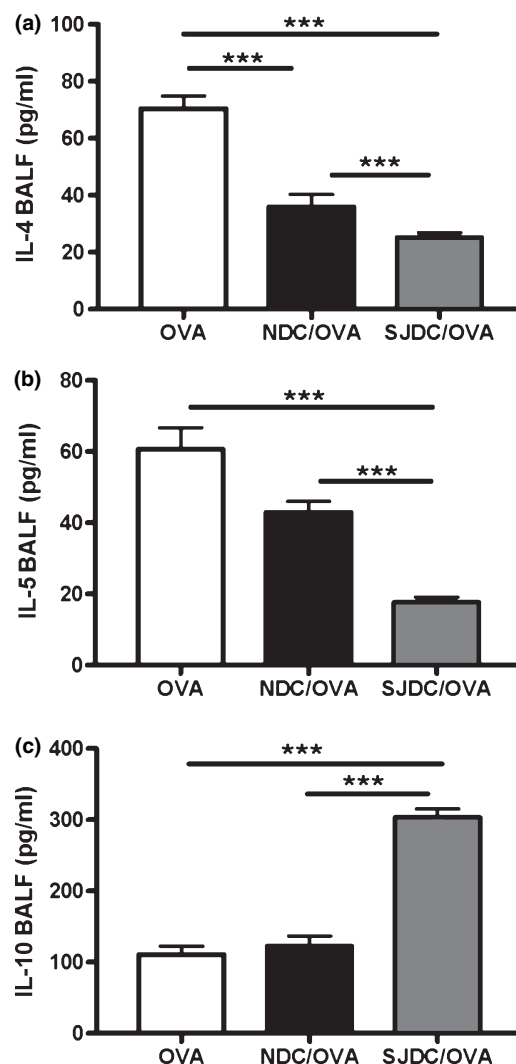


Figure 7 The effect of adoptive transfer of SJDCs on cytokine levels in bronchio-alveolar lavage fluids (BALF). The BALF supernatants collected from the different groups of mice, described in Figure 5, measured for interleukin (IL)-4, IL-5 and IL-10 using ELISAs. The data are presented as mean $\pm$ SEM. The figures show the levels of IL-4, IL-5 and IL-10 in BALF of mice in each group respectively. The data are presented as mean $\pm$ SEM of individual mice in each group. *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

model. The data showed that *SJ* infection had an inhibitory effect on OVA-induced pulmonary inflammation, especially eosinophilic infiltration, bronchial mucus secretion and allergen-specific IgE production. The inhibition of allergic airway inflammation, mucus overproduction and allergen-specific IgE responses by *SJ* infection appears to be well-correlated with the alteration of allergen-driven cytokine patterns. The results showed that the levels of IL-4 and IL-5 in both BALF and the supernatants of

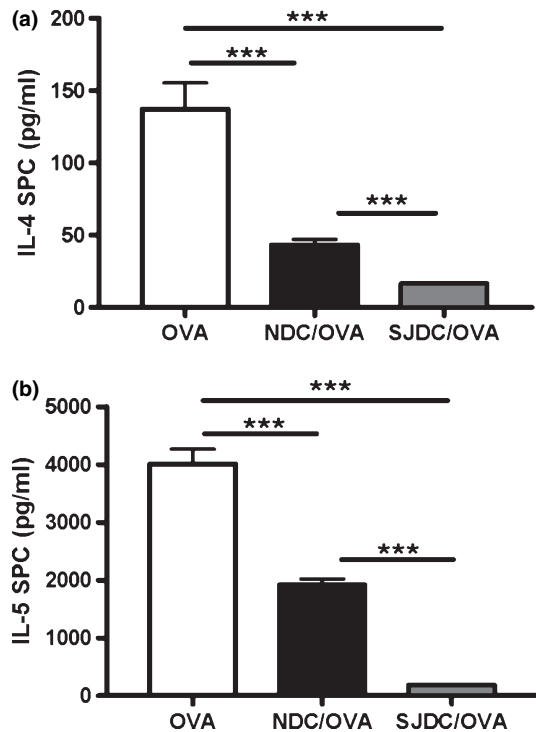


Figure 8 The effect of adoptive transfer of SJDCs on ovalbumin (OVA)-driven cytokine production by splenocytes. The splenocytes isolated from the different groups of mice described in Figure 5 were cultured at 7.5×10^6 cells/mL with the stimulation of OVA. Culture supernatants were harvested at 72 h and tested for interleukin (IL)-4 and IL-5 using ELISAs. The data are presented as mean $\pm$ SEM of individual mice in each group. *** $P < 0.001$. One representative experiment of two experiments with similar results is shown.

splenocyte culture of *SJ*-infected/OVA-treated mice were lower than those of the mice with OVA treatment only. Notably, during the preparation of this study, Mo *et al.* (39) reported that *SJ* infection reduced both IL-4 and allergen-specific IgE in a similar mouse model. The Mo *et al.*'s data largely coincide with our finding. The demonstration and confirmation of the inhibitory role of *SJ* infection on allergic responses are important because unlike *SM* infection, which has been shown to be inversely associated with allergy in epidemiology studies, *SJ*, the dominant species in Southeast Asia area, has not been studied epidemiologically for its relationship with the development of allergy and asthma. From our and Mo *et al.*'s experimental data, one might expect that although the two *Schistosoma* genera are different in many aspects in epidemiology and parasitology, the existence of *SJ* infection in Southeast Asia may, like *SM* in other areas of the world, be inhibitory for allergic reactions, thus contributing to the relatively lower prevalence of allergy and asthma in this region. It should also be pointed out that

our and Mo *et al.*'s results on *SJ* are in line, but not the same, with the report on *SM* by Mangan *et al.* (17). Specifically, although both studies showed the inhibition of airway inflammation by *Schistosoma* infection, Mangan *et al.*'s study on *SM* found no reduction in IL-4 production and allergen-specific IgE responses (17), unlike the finding of this study showing significantly reduced IL-4 and IgE in *SJ*-infected mice. It is unclear if this is because of the different *Schistosoma* species used in the studies or the different allergy models tested. Notably, the protocols for establishing the airway allergic inflammations used in the studies are different. Specifically, the protocol used in the *SM* study is more representative of an acute inflammation involving three OVA challenges, whereas our *SJ* study is more sub-chronic involving seven OVA challenges. Moreover, it has been shown that the protective effect of *SM* on allergic inflammation depends on the intensity as well as the stage of infection (40). As this study only involves the examination of allergic reaction following one dose of infection and at one time point, it is unclear if the same effect can be generated in other conditions. Further study to explore the potential reasons for the differences, including more extensive examination of the influence of infection intensity, time and allergy models, could provide new insights into the mechanisms underlying the modulation of infection on allergy/asthma.

The most novel finding in this study is the possible involvement of DC in helminth infection-mediated inhibition of airway allergic reactions. Dendritic cell probably contributes to the reduction in allergen-driven Th2 cytokine production in the infected mice. There are at least three mechanisms that may account for the modulating effect of transferred SJDC on T-cell cytokine responses to allergen *in vivo*. First, SJDCs educated through parasitic infection can directly present allergen to T cells in recipient mice, as shown that DCs from *Chlamydia*-infected mice inhibit allergic response to OVA (36–38). Second, transferred SJDC can further influence the function of DCs in the recipient mice, leading to decreased Th2-like cell development. Third, SJDC can induce the development of other regulatory cells which subsequently inhibit allergen-specific Th2-like cells. Therefore, DC from infected mice may play an inhibitory role through direct and/or indirect mechanisms.

In line with the third potential mechanism, several epidemiological and experimental studies have shown an association between systemic and/or local increase in IL-10 production (12,16,18,19,22) and the generation of T regulatory cells (26,27,41), with the decrease of allergy in parasitic infections. In particular, Yang *et al.* (27) recently reported that injection of *Schistosoma* egg antigen or dead *SJ* eggs can induce CD4⁺ CD25⁺ T regulatory cells, which

was inhibitory for airway inflammation induced by OVA in a mouse model. They found that the immunization with these antigens or dead parasite eggs could increase the number and suppressive activity of T regulatory cells that produced IL-10. Although this finding was obtained from an antigen/dead egg injection study rather than live infection, it sheds some light on the mechanism by which *SJ* infection may modulate allergen-driven Th2 cytokine production. Consistently, we found that IL-10 production in the BALF of the SJDC recipient mice was dramatically higher than those without cell transfer or received NDC following OVA sensitization and challenge. The data suggest that enhancement of IL-10 production by SJDC transfer may be the basis for the inhibition of allergen-driven Th2 cytokine production and the inhibition of local allergic inflammation. The higher local IL-10 levels may be the result of higher production of this cytokine by antigen-presenting cells and T regulation cells. However, it should be noted that it still unclear whether the IL-10 levels in the infected mice following allergen exposure are also high. This is also a limitation for this study to conclude directly the role of IL-10 in helminth infection-mediated inhibition of allergy. Based on the data of DC transfer which showed enhanced IL-10 production in the SJDC recipients following allergen treatment, it appears reasonable to expect that this is the case. Nevertheless, further study to address specifically this question is warranted to obtain a solid conclusion.

Notably, the adoptive transfer of NDC also showed some influence on cytokine responses (Figures 7 and 8), although to a significantly lesser degree than SJDC. We think that this probably reflects a degree of nonspecific activation of DCs by the isolation and transfer process. It has been reported that the ex vivo handling process may have a nonspecific activation effect on DCs. However, it is also important to note that although the transfer of NDC had a significant inhibitory effect on Th2 cytokines, its effect on airway inflammation was mild (Figure 5) and had no significant effect on IL-10 production and allergen-specific IgE responses (Figure 6). In contrast, the transfer of SJDC had a significant inhibitory effect on Th2 cytokine, airway inflammation and IgE responses, with significantly increased IL-10 production. More important, although both NDC and SJDC had an inhibitory effect on Th2 cytokine production, the degree of inhibition by SJDC was significantly higher than that by NDC. As the transferred SJDC and NDC were in the same amount and had been isolated and transferred by the same processes, the results suggest that *SJ* infection indeed has an influence on DC function, which may contribute to the modulating effect of *SJ* infection on allergic reactions.

In conclusion, this study has demonstrated an inhibitory role of chronic *SJ* infection on the development of allergic airway inflammation, allergen-specific IgE production and Th2 cytokine responses. More importantly, we found that DC plays a critical role in this inhibitory effect. Further study on the molecular and cellular bases of the DC-mediated modulating effect will provide new insight into the mechanism of infection-mediated modulation of allergy/asthma and will be helpful for developing novel therapeutic and preventive approaches against these diseases.

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