Chlamydia Infection Induces ICOS Ligand-Expressing and IL-10-Producing Dendritic Cells That Can Inhibit Airway Inflammation and Mucus Overproduction Elicited by Allergen Challenge in BALB/c Mice

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Chlamydia Infection Induces ICOS Ligand-Expressing and IL-10-Producing Dendritic Cells That Can Inhibit Airway Inflammation and Mucus Overproduction Elicited by Allergen Challenge in BALB/c Mice

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Our previous study has shown that the adoptive transfer of dendritic cells (DCs) freshly isolated from Chlamydia-infected mice (iIDCs), unlike those from control naive mice (iNDCs), can inhibit systemic and cutaneous eosinophilia induced by OVA exposure. In the present study, we examined the mechanism by which iIDC inhibits allergen-specific Th2 cell differentiation in vitro and in vivo. The study revealed that iIDCs exhibited higher surface expression of CD8α and the ICOS ligand (ICOS-L), as well as higher IL-10 and IL-12 production than iNDCs. In vitro DC:CD4+ T cell coculture experiments showed that iIDCs could inhibit allergen-specific Th2 cell differentiation and that the inhibitory effect could be abolished by the blockage of IL-10 or IL-12 activity. More interestingly, the coblockade of IL-10 and the ICOS-L showed synergistic effect in enhancing allergen-driven Th2 cytokine production. Furthermore, adoptive transfer of iIDCs, but not iNDCs, to OVA sensitized mice significantly inhibited airway eosinophilia and mucus overproduction following intranasal challenge with OVA. Overall, the data demonstrate a critical role played by ICOS-L-expressing and IL-10-producing DCs from Chlamydia-infected mice in the infection-mediated inhibition of allergic responses. The Journal of Immunology, 2006, 176: 5232–5239.

Allergic asthma is a chronic inflammatory disorder characterized by reversible airflow obstruction, airway eosinophilic inflammation, and mucus overproduction (1, 2). Allergen-specific Th2 cells play a major role in both initiation and progression of allergic asthma by orchestrating the inflammatory responses (3). In the past few decades, the prevalence of allergic diseases has dramatically increased, especially in developed countries. An explanation for this phenomenon is the so-called “hygiene hypothesis,” which proposes that the reduced or altered infectious burdens due to improved hygiene diminishes the inhibitory effect of certain natural infections on allergic diseases (4). This hypothesis has gained considerable support from epidemiological studies that showed an inverse relationship between the rate of infection and the prevalence of allergic diseases (5, 6). Experimental evidence from animal model studies has also demonstrated the inhibitory effect of various infections on the allergic reactions (7–9).

The biological basis for the inverse relationship between infection and allergy is still debatable. A relatively more generally accepted explanation is that the induction of Th1 responses by some infections may cross-regulate Th2 responses induced by an allergen (10, 11). More recently, some reports suggest that the induction of Th2 cell unresponsiveness and the induction of regulatory T cells by infectious agents may contribute to the inhibition of allergic responses (12–16).

Dendritic cells (DCs) are the most efficient APCs in priming naive T cells and directing T cell differentiation by providing polarizing signals to T cells. Based on the functional preference in directing T cell response, DCs can be categorized into at least three subtypes, i.e., DC1, DC2, and regulatory DC (designated DCreg). The DC subsets may represent different cellular lineages and/or stages of maturation of these cells that may vary in phenotype, cytokine production, and function (17–19). Our previous study (20, 21) has shown that Chlamydia infection can inhibit allergic reaction in a mouse model and that DCs play an important role in the infection-mediated inhibition of allergy. In particular, we have shown that the adoptive transfer of DCs isolated from Chlamydia-infected mice (iIDCs), but not DCs isolated from naive mice (iNDCs), suppressed allergic responses in recipient mice upon OVA sensitization and cutaneous challenge. Interestingly, although we have found a higher IL-12 production by iIDC and a Th1-promoting effect of these cells in an in vitro DC:CD4+ T coculture system, the in vivo effect of the iIDC appeared to be more evident in reducing allergen-specific Th2 cytokine production rather than increasing allergen-specific Th1 cytokine (IFN-γ) production (21). This finding suggests that the adoptive transfer of the iIDC is more likely to result in a less responsiveness of the allergen-specific Th2 cells in vivo. To test the possibility that chlamydial infection may induce tolerogenic/regulatory DCs that may inhibit the development of allergen-specific Th2 cell responses, in the present study we analyzed the phenotype and the cytokine profile of the DCs from Chlamydia-infected mice and their role in modulating allergen-driven CD4+ T cell responses in vitro. In addition, we examined the effect of adoptive transfer of iIDCs on...
airway allergic reaction instead of cutaneous challenge with allergen as shown in the previous report (21). We found that iDCs expressed higher levels of CD8α and the ICOS ligand (ICOS-L) on the cell surface and produced higher amounts of IL-10 and IL-12 as compared with iNDCs. The blockage of IL-10 or IL-12 activity in the iDC:CD4+ T cell coculture could reverse the inhibitory effect of iDCs on Th2 cytokine production by allergen-specific CD4+ T cells. Moreover, the coblockade of IL-10 and ICOS-L in the culture showed a synergistic effect on the increase of Th2 cytokine production by allergen-specific CD4 T cells. The data suggest that, in addition to inducing IL-12-producing DC1-like cells that may promote allergen-specific Th1 cell development, chlamydial infection may induce IL-10-producing and ICOS-L-expressing DCs, which cause reduced responsiveness of allergen-specific Th2 cells.

Materials and Methods

Animals

Female BALB/c mice (7–10 wk old) were bred at the University of Manitoba (Winnipeg, Manitoba, Canada) breeding facility. All animal care and procedures were in accordance with guidelines issued by the Canadian Council on Animal Care. Reagents

Complete RPMI 1640 medium was prepared as previously described (21). Fluorescence-conjugated anti-CD11c, anti-CD8α, anti-CD80, anti-CD86, and anti-CD4 Abs and matched isotype controls were purchased from BD Pharmingen. A PE-conjugated and purified anti-ICOS-L Ab (clone HK5.3) was purchased from eBioscience. Pair Abs for ELISA analysis of IFN-γ, IL-4, IL-5, IL-10, and IL-12 were purchased from BD Pharmingen. Paired Abs for IL-13 ELISA were purchased from R&D Systems. A purified, neutralizing anti-IL-10 mAb (JESS-2A5) and an anti-IL-12 (clone 15.6) mAb for in vitro analysis were purchased from BD Pharmingen. Recombinant mouse IL-10 and IL-12 were purchased from BD Pharmingen and PeproTech, respectively. Injection of mice and isolation of DCs

Mice were inoculated intranasally with 1×103 inclusion-forming units (IFU) of Chlamydia trachomatis mouse pneumonitis (MoPn) to generate respiratory tract infection as previously described (21). Fourteen days postinfection, spleens were aseptically collected and DCs were isolated using a MACS CD11c column (Miltenyi Biotec) according to manufacturer’s instructions. The purity of the isolated CD11c+ DC was >95% based on flow cytometric analysis. The isolated CD11c+ cells from Chlamydia-infected mice are designated as iIDCs (21). The CD11c+ cells isolated from age- and sex-matched naive C57BL/6 mice are designated as iNDCs (21). Surface phenotyping of DCs was performed by double staining 1×106 cells with FITC-conjugated anti-CD11c and other marker-specific, PE-conjugated Abs for 30 min on ice. After 30 min of incubation, the cells were fixed, washed, and analyzed on EPICS ALTRA flow cytometer (Beckman Coulter). To test the spontaneous production of cytokines by the freshly isolated DCs, the cells were cultured with complete medium using 96-well culture plates at 5×103 cells/well for 48–72 h. IL-12 and IL-10 in culture supernatants were measured by ELISA as described (21).

Purification of CD4+ T cells and the setup of DC-T cell coculture

Naive CD4+ T cells were isolated from the spleen of DO11.10 OVA peptide-specific TCR-αβ transgenic mice (BALB/c background) by using a MACS positive selection column. The naive CD4+ T cells (5×105 cells/well) were cocultured with iDCs or iNDCs at 1:10 (DC:T cell) ratios in the presence of OVA (100 μg/ml). Cell culture supernatants were collected at 48–72 h for cytokine analysis. As indicated in particular experiments, purified anti-IL-10 (clone JESS-2A5) and anti-IL-12 (clone C15.6) mAbs (BD Pharmingen) were added to the coculture wells at 5 μg/ml or rIL-10 (0.2–20 ng/ml) or rIL-12 (0.2–20 ng/ml) was added to either block endogenous cytokine activity or to supplement exogenous cytokines. Anti-ICOS-L mAb (clone HK5.3, purchased from eBioscience) was added in indicated wells at 5 μg/ml.

Sensitization and challenge of mice with allergen and adaptive transfer of DCs

Mice were sensitized i.p. with 4 μg OVA in 2 mg of Al(OH)3 or OVA in 2 mg of Al(OH)3 adjuvant (alum). Fourteen days after sensitization, the mice received iIDC or iNDC by adoptive transfer through i.v. injection. For adoptive transfer, splenic CD11c+ cells isolated by a MACS CD11c column were first washed in protein-free PBS and then injected into the tail vein of the syngeneic recipient mice (5×106 DC/mouse). Two hours following adoptive transfer, mice were challenged intranasally with 50 μg OVA (40 μl). Control mice were sensitized and challenged in the same manner but without DC transfer. Mice were sacrificed at 7 days after OVA challenge, and different tissue samples were collected for further analysis.

Bronchoalveolar lavage (BAL) and leukocyte differentials

The mouse trachea was cannulated, and the lungs were washed twice with 1 ml of PBS. The BAL fluids were centrifuged immediately. The supernatants were collected for the measurement of cytokines, and the cells were resuspended for cell counting and BAL smears. Cytokines (IL-4, IL-5, IL-13, and IFN-γ) were measured by ELISA. For leukocyte differential the BAL smear slides were air-dried, fixed, and stained with a Hema-3 stain set (Fisher Scientific). The numbers of monocytes, lymphocytes, neutrophils, and eosinophils per 200 cells were counted based on cellular morphology and staining characteristics. Blood smears from mice were prepared and stained by the same reagent for blood leukocyte differentials.

Histopathological and immunohistochemical analysis

Lungs were collected, fixed with 10% formalin, embedded, sectioned, and stained by H&E. Slides were examined for pathological changes under light microscopy. Mucus and mucus-containing goblet cells within the bronchial epithelium were analyzed by a periodic acid-Schiff staining kit (Sigma-Aldrich). The histological mucus index was measured by Image-Pro Plus software (Media Cybernetics) as described (22). For analysis of VCAM-1 and eotaxin expression, lung tissues were snap frozen in liquid nitrogen when the mice were killed and stored at −80°C until sectioning. Frozen sections (10 μm) were placed on slides and fixed with 99.6% acetone. For VCAM-1 staining, slides were incubated with rat anti-mouse VCAM-1 (clone 429; BD Pharmingen) or isotype-matched control Abs followed by biotin-conjugated anti-rat IgG (BD Pharmingen) developed with Texas Red (Vector Laboratories). For eotaxin staining, slides were incubated with purified goat anti-mouse eotaxin Ab that was purchased from R&D followed by PE-conjugated anti-goat IgG secondary Ab purchased from Jackson Immunoresearch. Eukaryotic and epithelial cells were stained with anti-pan-cytokeratin (B311.1) (Calbiochem) followed by a FITC-conjugated, anti-mouse IgG secondary Ab (Sigma). The blocking reagent was purchased from DakoCytomation. IL-5R expression on bone marrow cells was determined as previously described (21).

RT-PCR analysis

Total RNA was extracted from DC preparations using Trizol reagent (Invitrogen Life Technologies) as described (21). The first-strand cDNA was generated from 1.2 μg of total RNA using Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) and oligo(T) primer. Real-time PCR was performed using LightCycler PCR system (Roche). Murine-specific primer pairs used were: GAPDH, 5′-AAGCAGCCCTCCTGTGAG-3′ (sense) and 5′-TCACGATCATACGACG-3′ (antisense); and IL-10, 5′-GTGGTCCAGCTATTCGCA-3′ (sense) and 5′-ACCTCTACGCCCTCAAAAG-3′ (antisense); and IL-13, 5′-GGCCACGAGAATA-3′ (sense) and 5′-AATCTGGAAGAACTGGAGA-3′ (antisense). A Master SYBR Green I (Roche) detection system was used, and the reactions generated a melting temperature dissociation curve enabling quantitation of the PCR product. All genes were normalized to GAPDH. The presence of mRNA specific for ICOS-L was determined by semiquantitative RT-PCR. The PCR primer sets were: ICOS-L, 5′-CTACTCACGTTACGACGGC-3′ (sense) and 5′-GATGGTGTTAGCTAGTGC-3′ (antisense); and β-actin, 5′-GGTGGCGCCCTTATGACACCA-3′ (sense) and 5′-TCTTCTTGTTCGACGATC-3′ (antisense).

Statistical analysis

Unpaired Student’s t test was used to determine statistic significance between the groups. A p value of <0.05 was considered significant.
Results

DCs isolated from MoPn-infected mice express higher levels of CD8α and ICOS-L and produce higher levels of IL-10 and IL-12

To determine the potential phenotypic changes in DCs following chlamydial infection, we isolated DC from the spleens of infected mice (iIDC) and naive mice (iNDC) using a DC isolation system (MACS CD11c column). The isolated DCs were double stained with anti-CD11c and mAbs specific for other surface markers related to DC function and classification. As shown in Fig. 1A, a higher percentage of iIDCs expressed CD8α compared with iNDCs (41.9% vs 24.7%). The expression of MHC class II (I-A) (95.2 vs 87.0%) and costimulatory CD80 (61.6 vs 47.3%) molecules on iIDCs also appeared to be higher compared with iNDC. The mean channel fluorescence (X-mean) of MHC class II molecule (I-A) in iIDCs was significantly higher than that in iNDCs (23.9 vs 13.6). Interestingly, iIDCs expressed significantly higher levels of ICOS-L than did iNDCs as determined by flow cytometry and RT-PCR (Fig. 1, B–D). The data demonstrate that DCs isolated from MoPn-infected and naive mice differ in the expression of the cell surface markers that are considered to be related to DC function.

To study the cytokine profiles of the iIDC and the iNDC, the expression of mRNA for cytokines by these freshly isolated cells and the cytokine protein levels in the ex vivo DC cultures were determined by real-time RT-PCR and ELISA, respectively. Real-time RT-PCR analysis showed that freshly isolated iIDCs expressed significantly higher levels of mRNA for IL-10 as well as for IL-12 as compared with iNDC (Fig. 2A). Similar differences were observed when the cytokine proteins in the supernatants of ex vivo iIDC and iNDC cultures were measured (Fig. 2B). For the other tested cytokine messages (IFNα, IFNβ, and TGFβ), no significant difference between iIDCs and iNDCs was found by RT-PCR analysis (data not shown). The results indicate that iIDC and iNDC are different in cytokine profile, especially in IL-10 and IL-12 production.

IL-10 and IL-12 production and ICOS-L expression contribute to the inhibitory effect of iIDC on Th2-like OVA-specific CD4+ cell development

To examine whether the cytokine (IL-10 and IL-12) production and ICOS-L expression by iIDC play a role in modulating CD4+ T cell responses, we tested the effect of the neutralizing anti-IL-10/anti-IL-12 mAbs and anti-ICOS-L Ab on allergen-specific CD4+ T cell differentiation in the DC:CD4+ T cell coculture system (Fig. 3). The CD4+ T cells were isolated from naive transgenic DO11.10 mice that express a TCR specific for the OVA peptide. As shown in Fig. 3A and similar to our previous report (21), iIDC significantly reduced OVA-specific Th2 CD4 T cell differentiation as demonstrated by significantly reduced IL-4 and IL-5 production and increased IFNγ production in the coculture. However, when anti-IL-10 mAb was added to block endogenous IL-10 in the cocultures of iIDCs and naive CD4+ T cells, the production of both Th2 (IL-4 and IL-5) and Th1 (IFNγ) cytokines by the allergen-specific CD4 T cells was dramatically increased (Fig. 3A). In contrast, the blockade of endogenous IL-12 alone increased Th2 cytokine but not IFNγ production (Fig. 3A). Moreover, the addition of exogenous rIL-10 to the iIDC:CD4+ T cell coculture reduced both IL-4 and IFNγ production in a concentration-dependent manner (Fig. 3B). Not surprisingly, the addition of rIL-12 inhibited IL-4 production but increased IFNγ production (Fig. 3B). More interestingly, although the blockade of ICOS-L alone failed to alter cytokine production by OVA-specific CD4+ T cells, a synergistic effect of increasing both Th2 and Th1 cytokine production was found when both IL-10 and IL-12 were blocked in the iIDC:CD4+ T cell coculture (Fig. 3C). These results suggest that IL-10 production and ICOS-L expression by iIDCs play an important role in the inhibition of allergen-specific Th2 cell differentiation.

Adaptive transfer of iIDC inhibits systemic and airway eosinophilia induced by allergen exposure

We have reported that adaptive transfer of iIDC could inhibit the allergic reaction induced by allergen sensitization and cutaneous challenge in C57BL/6 mice (21). In this study we further tested the effect of iIDC adaptive transfer on airway allergic reaction in...
BALB/c mice. As shown in Fig. 4 and Table I, the adoptive transfer of iIDC had a significant inhibitory effect on the allergic responses in OVA sensitized/challenged BALB/c mice. The iIDC recipient mice showed significantly reduced eosinophilia in circulating peripheral blood (Table I) and expressed far fewer IL-5Rα-chains on bone marrow cells, suggesting the decrease of eosinophil precursor cells (Fig. 4A). The adoptive transfer of iIDCs also dramatically inhibited local eosinophil infiltration in the lung. The control mice without iIDC adoptive transfer showed a large amount of eosinophils in BAL (Fig. 4B) and severe eosinophil infiltration in the bronchial submucosa and alveolar and perivascular sheaths following OVA challenge (Fig. 5, A and B). In the mice that received iIDCs, the proportions of eosinophils in BAL (Fig. 4B) and pulmonary tissues (Fig. 5, A and B) were remarkably reduced. Because airway mucus overproduction is another hallmark of asthmatic reaction, we also tested the effect of iIDC adoptive transfer on allergen-induced mucus production. The results showed that the mucus-containing goblet cells, mucus secretion, and bronchial epithelial hyperplasia induced by OVA sensitization/challenge were markedly decreased in iIDC-transferred mice (Fig. 5C). The histological mucus index, a quantitative way to measure airway mucus production (22), was decreased from 26.57 ± 1.32% in control mice to 13.18 ± 3.74% in iIDC-transferred mice (p < 0.05). In contrast, the adoptive transfer of iNDCs showed much less effect on airway inflammation and mucus production (Figs. 4B and 5C). The results clearly indicate that iIDC transfer is able to inhibit allergic airway eosinophilia and mucus overproduction.

Adoptive transfer of iIDC inhibits VCAM-1 and eotaxin expression in the lung following allergen challenge

VCAM-1 expression and eotaxin production in the airways play an important role in eosinophil infiltration to the lung in asthmatic reactions. To elucidate the mechanism by which iIDC reduced airway allergic inflammation, we examined the effect of iIDC transfer on eotaxin and VCAM-1 expression in the lung by immunohistochemical staining. High density VCAM-1 and eotaxin expression was observed in mice without DC transfer (Fig. 5, D and E). In sharp contrast, undetectable VCAM-1 (Fig. 5D) and eotaxin expression (Fig. 5E) was observed in mice with iIDC transfer. However, mice that received iNDCs, unlike those that received iIDCs, showed similar levels of VCAM-1 and eotaxin expression as those without DC transfer (Fig. 5, D and E). These results suggest that the inhibitory effect of iIDC transfer on airway eosinophilia may be mediated by the inhibition of VCAM-1 and eotaxin expression.

Adoptive transfer of iIDC inhibits Th2 cytokine production in the lung following allergen airway challenge

Mounting local Th2 cytokine responses is one of the major mechanisms by which allergens induce allergic reactions. To elucidate the effect of iIDC on airway cytokine patterns, we examined the cytokine profiles in the BAL fluids of the mice that received iIDCs following local OVA challenge. The results showed that the levels of Th2 cytokines, including IL-4, IL-5, and IL-13, produced in the lung of the mice that received iIDCs were significantly lower than those the mice without iIDC transfer (Fig. 6). The level of IFNγ, the Th1 cytokine, in the BAL fluids was undetectable in all of these groups. The results demonstrate that iIDC transfer can modulate the cytokine pattern in airways following allergen exposure.

Discussion

We reported previously that adoptive transfer of iIDCs can inhibit allergic responses induced by OVA sensitization and cutaneous challenge (21). In the present study we examined the effect of iIDC
transfer in allergic airway inflammation and studied the mechanism by which iIDCs modulate allergen-specific CD4+ T cell response. We found that iIDCs expressed significantly higher levels of CD8α and ICOS-L and produced higher levels of IL-10 and IL-12 than iNDCs. The blockade of IL-10, IL-12, and ICOS-L (with coblockade of IL-10) abolished the inhibitory effect of iIDCs on Th2 cytokine production by allergen-specific CD4+ T cells. Furthermore, we showed that the adoptive transfer of iIDCs to OVA-sensitized mice is capable of suppressing allergic airway inflammation following intranasal OVA challenge. The data further extend our knowledge on the mechanisms by which infection inhibits allergy, especially in regard to the role of DC in this process.

The most significant finding in this study is the higher IL-10 production and ICOS-L expression by iIDCs. IL-10, which is produced by DCs, has been shown to be related to the induction of immunological unresponsiveness and the suppression of immune reactions. The present study showed that IL-10 plays an important role in iIDC-mediated inhibition of allergen-driven Th2 cytokine production. There are at least three potential mechanisms that might account for the inhibitory effect of IL-10 produced by iIDCs on allergic reaction and Th2 cell development. First, IL-10 may have a direct regulatory function on allergen-specific T cell differentiation and allergic reaction (23). For example, the presence of IL-10 may result in the nonresponsiveness of allergen-specific Th2 cell progenitors during DC:CD4+ T cell interaction, thus reducing Th2 cytokine production. In addition, IL-10 can reduce eosinophil survival and eotaxin production, resulting in reduced eosinophil recruitment to the sites of allergic inflammation (24, 25). Second, IL-10-producing DCs can induce regulatory T cells such as Tr1 cells (7, 14, 26). Akbari et al. (27) have found that IL-10-producing DCs can...
stimulate the development of Ag-specific T regulatory cells that also produce high levels of IL-10, resulting in the inhibition of allergic responses. Moreover, they have shown that the development of regulatory T cells was dependent on the presence of both IL-10 and the ICOS-ICOS-L interaction, which were provided by DCs (28). Interestingly, in the present study we found that iIDCs produced higher levels of IL-10 and also expressed higher levels of ICOS-L than iNDCs (Fig. 1). More importantly, we found that although the blockade of ICOS-L alone failed to show significant effect on Th2 cytokine production, the coblockade of IL-10 and ICOS-L exhibited dramatic synergy in increasing Th2 cytokine production by allergen-specific CD4+ T cells (Fig. 3C). Finally, IL-10, which is produced by iIDCs, may influence the development and the function of endogenous DCs in recipient mice, subsequently inhibiting the development of allergen-specific Th2 cells. Obviously, further study is necessary to clarify whether some or all of these mechanisms play a role in the iIDC-mediated inhibition of allergy and allergen-specific Th2 cell development. It should also be mentioned that although the results of our previous experiments suggest that the expressions of IL-10 and ICOS-L by iIDCs play an important role in modulating Th2 cytokine production, there is no data suggesting that the inhibition of Th2 cells is dependent on these molecules. Further studies using DCs from mice that are deficient on these molecules will directly examine the potential link of the expression of these molecules by DCs and the inhibition of allergic responses.

The fact that certain pathogens/microbial products can induce IL-10-producing DCs that show an immunoregulatory function has been reported in several previous studies (29–34). For example, CD11c+ cells isolated from mice exposed to Mycobacterium vaccae produce high levels of IL-10 and have a regulatory function (31). A Bordetella pertussis-derived molecule, filamentous hemagglutinin, has shown to be able to induce the production of IL-10 by DCs, which promote the differentiation of regulatory T cells (32). In a Candida albicans study, it was found that the invasive hyphal form of the fungus, rather than commensal yeast, triggered DCs to produce high levels of IL-10 (33). Moreover, TLR binding and the cross-linking of the mannose receptor on the DC surface have been shown to induce the production of IL-10 by DCs (30, 35). The function of IL-10 production induced by pathogen exposure may have an opponent effect on host-pathogen interaction. On one hand, IL-10 may lead to dysfunctional immune protection, thus providing an opportunity for immune evasion by the microbes (32, 34). On the other hand, IL-10 production may be beneficial to the host, given the fact that IL-10 is essential in regulating immune responses and thus preventing an excessive inflammatory response that may be detrimental (27, 30, 36). The function of IL-10 production induced by pathogen exposure may have an opponent effect on host-pathogen interaction. On one hand, IL-10 may lead to dysfunctional immune protection, thus providing an opportunity for immune evasion by the microbes (32, 34). On the other hand, IL-10 production may be beneficial to the host, given the fact that IL-10 is essential in regulating immune responses and thus preventing an excessive inflammatory response that may be detrimental (27, 30, 36). The present study suggests that, in addition to contributing to pathogen-host interaction, bacterial infection can induce IL-10-producing DCs that can modulate immune responses in the host to unrelated Ags such as allergens. Notably, the inhibitory role of chlamydial infection on asthma is not supported by clinical studies, and some epidemiological studies have even suggested that chlamydial infection/colonization of the respiratory tract in humans appears to be associated with asthma, especially in adults (37, 38). The reason for this discrepancy is unclear. However, the chlamydial species that was used in this study (C. trachomatis) is different from the chlamydial species (Chlamydia pneumoniae) that was found to be associated with asthma. It is also possible that the experimental system in the present study more likely models the functions of dendritic cells rather than the activity of a specific bacterial colonizer of the respiratory tract.

DCs in mouse represent a very heterogeneous cell population. Cell surface markers appear to be related to, although not exclusively defining, DC subpopulations (39–45). The potential DC subpopulation within iIDCs that is responsible for IL-10 and IL-12 production and ICOS-L expression following chlamydial infection
is unknown. It has been documented that CD8+/H9251+/H11001 DCs can make either IL-12 or IL-10 in response to different combinations of microbial stimulus and CD40L (46). It is unclear whether the increased CD8+/H9251+/H11001 DC subpopulation observed in iIDCs is responsible for the increased IL-10, the increased IL-12, or both. Similarly, it is unclear which DC subpopulation represents the increased ICOS-L expression on iIDCs. However, it is possible that the IL-10-producing DCs comprise the subpopulation of DCs that express ICOS-L. This speculation is based on the observation that the coincubation of IL-10 and ICOS-L of iIDCs showed strong synergy in enhancing Th2 cytokine production by allergen-specific CD4 T cells (B311.1) followed by PE-conjugated and FITC-conjugated secondary Abs as described in Materials and Methods. The orange color represents c mothlin on bronchial epithelial cells.

Further define the DC subtype(s) induced by chlamydial infection that inhibits allergic reaction.

Interestingly, the present study showed similar and additive effects of IL-10 and IL-12 on the inhibition of Th2 cytokine production by allergen-specific CD4 T cells in the iIDC:CD4+ T cell coculture (Fig. 3A). Because IL-12 can promote Th1 responses that may be inhibitory for Th2 responses, iIDCs can inhibit allergen-driven Th2 cytokine production through the enhancement of allergen-specific Th1 responses. Our current hypothesis is that chlamydial infection can induce at least two subtypes of DC, one producing IL-10 and expressing ICOS-L, thus leading to Th2 cell unresponsiveness or the generation of Th2-inhibiting regulatory T cells, and the other producing IL-12, which enhances Th1 cell development and results in the inhibition of Th2 cells. The inhibitory effect of the different DC subsets on allergic reaction could be
additive and even synergistic, although the dominance of each mechanism may vary depending on the allergen model, which differs in regard to in vivo location, the time course, the type of exposed allergen, etc. Further studies that test this hypothesis, especially those that explore the nature of the interaction among the various subtypes of DCs, will deepen our understanding of the mechanisms underlying infection-mediated inhibition of allergy.

Disclosures

The authors have no financial conflict of interest.

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