Cutting Edge: Limiting MHC Class II Expression to Dendritic Cells Alters the Ability to Develop Th2-Dependent Allergic Airway Inflammation

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In allergic airway inflammation, dendritic cells (DCs) are required for Th2 generation, recruitment, and activation in the respiratory tract. DCs have been shown to be necessary and sufficient for the induction of Th1 immune responses. In Th2 immunity and allergic airway inflammation, the ability of a DC to function as the sole APC has not been tested. We show that CD11c/Aβb mice with MHC class II expression restricted to CD11c-expressing DCs develop airway neutrophilia rather than allergic airway inflammation. Although CD11c/Aβb mice are capable of Th2 recruitment and activation in the lung, Th2 priming in CD11c/Aβb mice results in IFN-γ production. Effective Th2 generation and allergic airway inflammation was achieved in CD11c/Aβb mice after treatment with anti-IFN-γ. These studies show that DCs alone cannot drive the development of Th2 cells but require an additional MHC class II signal to stimulate effective Th2 immunity. The Journal of Immunology, 2009, 183: 1523–1527.

Asthma is characterized by the activation of CD4+ T cells in the respiratory tract resulting in airway eosinophilia, mucus hypersecretion, increased chitinase activity, and episodic airway obstruction (1, 2). The generation of Th2 cells and their recruitment into and activation in the lung require expression of MHC class II (MHC II) (3, 4). Dendritic cells (DCs) provide signals that direct the priming of naive CD4 T cells, including presentation of Ag in the context of MHC II, expression of costimulatory molecules, and secretion of cytokines. Indeed, Ag presentation by DCs has been shown to be sufficient for the development of Th1-dependent immune responses (5). However, the sufficiency of Ag presentation by DCs in the generation of Th2-dependent responses such as allergic airway inflammation is unknown.

In vivo depletion of DCs inhibits the priming of Th2 cells in response to Ag and the adjuvant alum (6). Similarly, DC depletion during secondary aerosolized Ag challenge blocks eosinophilic infiltration, mucus production, and bronchial hyperreactivity (4). Thus, DCs are necessary for the development of allergic airway inflammation. Yet, studies have shown that other cells, including B cells, mast cells, eosinophils, and basophils contribute to Th2 development and allergic airway inflammation, perhaps through the production of cytokines or expression of costimulatory molecules (7–12). The in vivo requirement for Ag presentation by these accessory cell populations during Th2 generation, recruitment, and reactivation in the lung remains unclear.

To determine whether MHC II-dependent Ag presentation by DCs is sufficient to induce Th2 cell differentiation and allergic airway inflammation, we chose to take advantage of CD11c/Aβb mice with MHC II expression restricted to CD11c-expressing DCs.

Materials and Methods

Mice

CD11c/Aβb, Aβb–/–, Aβb–/–, and OT-II(Thy1.1+) mice were bred in our facility. C57BL/6 and TCRc–/– mice were purchased from The Jackson Laboratory. Mice 6–10 wk of age were used in all experiments. These studies were reviewed and approved by the Yale University Animal Care and Use Committee (New Haven, CT).

Reconstitution and adoptive transfer

CD11c/Aβb, Aβb–/–, and TCRc–/– mice were reconstituted with 107 CD4+ T cells isolated from syngeneic C57BL/6 mice and between 1 and 4 × 106 CFSE-labeled naive CD4 OT-II TCR transgenic (Tg) cells isolated by negative selection (13). The following day, mice were immunized i.p. with 50 μg of OVA (fraction V; Sigma-Aldrich) in 2 mg of alum. To induce allergic airway inflammation, mice were immunized i.p. on days 1 and 6 and challenged with inhaled 1% OVA in PBS using an ultrasonic nebulizer for 20 min daily for 3 days. Five hundred micrograms of (XMG1.2) or control rat IgG1 (Innovative Research) was administered i.p. on immunization days 1 and 6. For adoptive transfer of Th2 cells, Th2 cells were generated from OT-II mice (13). Cultured Th2 cells (2 × 105) were injected i.v. and the following day mice were challenged for 20 min daily for 7 days with inhaled...
1% OVA in PBS. One day after the last exposure, mice were sacrificed for analysis of airway inflammation.

Analysis of airway inflammation

Bronchoalveolar lavage (BAL) and lung inflammatory cells were isolated (13). Cytospin preparations of BAL cells were stained with Diff-Quik (Baxter Healthcare) and differentials were performed on 200 cells based on morphology and staining characteristics. FACS analysis for CD4 and Thy1.1 was performed to identify the transferred OT-II TCR Tg cells. DCs were identified by FACS gating on low autofluorescent CD11c+ cells (14). Lungs were inflated and fixed with formalin and stained with H&E or period acid-Schiff. A histological museum was calculated using period acid-Schiff-stained lung sections (15).

Data analysis

Data are reported as mean ± SE. Statistical significance was determined by an unpaired Student’s t test.

Results and Discussion

To determine whether DCs are sufficient to drive the development of allergic airway inflammation, we used CD11c/Aβ+ mice that have equivalent numbers and normal localization of DCs in the spleen and lymph nodes (LNs) (5). In CD11c/Aβ+ mice, CD11b+ and CD8α+ conventional DCs (cDCs) in LNs and spleen have similar expression of I-Aβ compared with that of DCs from wild-type mice (5, 16), whereas lung DCs from naïve CD11c/Aβ+ mice express modestly lower I-Aβ (Fig. 1; mean fluorescence intensity: 1106 ± 28 vs 553 ± 5, p < 0.0001). CD11c-low plasmacytoid DCs as well as B cells and macrophages lack expression of I-Aβ (5).

CD11c/Aβ+ mice lack MHC II expression on the cortical thymic epithelium and have no MHC-restricted CD4+ T cells, which are essential for the development of allergic airway inflammation (1, 5). We therefore reconstituted the mice with polyclonal CD4 T cells and OT-II CD4 (OVA TCR Tg) cells 1 day before i.p. immunization with OVA and alum. TCRα−/− mice were used as controls because they have wild-type expression of I-Aβ but, like CD11c/Aβ+, would depend on transferred cells for an immune response to Ag.

Following OVA/alum immunization and inhaled OVA challenge, airway and lung eosinophilia were clearly present in Aβ−/− and TCRα−/− mice. The inflammatory response was MHC II dependent, as Aβ−/− mice had no airway infiltrates. Pulmonary eosinophilia in Aβ−/− and TCRα−/− mice was associated with mucus metaplasia and high chitinase activity in BAL fluid, characteristic of pulmonary Th2 responses (Fig. 2). CD11c/Aβ+ mice also developed airway inflammation; however, the infiltrating cells were predominantly neutrophils (Fig. 2D). Lungs from OVA-immunized and –challenged CD11c/Aβ+ mice showed inflammation, but mucus metaplasia was minimal and chitinase activity was low. The reduced pulmonary inflammation in both CD11c/Aβ+ and TCRα−/− mice compared with Aβ−/− mice likely reflects a lack of endogenous CD4 T cells and limited reconstitution by the transferred CD4 T cells. These studies show that Ag presentation restricted to DCs can induce airway inflammation; however, the inflammation is neutrophil predominant.

The development of allergic airway inflammation in this model involves two steps. Intraperitoneal immunization with OVA/alum induces Th2 differentiation in the mesenteric LN and spleen, and inhaled OVA stimulates primed Th2 cells to be recruited to and activated in the respiratory tract. Both of these steps have been shown to require CD11c-expressing DCs (4, 6). We therefore asked whether CD11c/Aβ+ mice could effectively recruit to and activate effector Th2 cells in the respiratory tract and develop allergic airway inflammation. We generated OT-II Th2 cells in vitro with wild-type splenic APCs and transferred 2 × 10^6 effector Th2 cells into CD11c/Aβ+, TCRα−/−, Aβ−/+, or Aβ−/− mice. Mice were then exposed to inhaled OVA. As expected, Aβ−/− mice did not exhibit lung inflammation. However, CD11c/Aβ+, TCRα−/−, and Aβ−/− all had comparable numbers of total inflammatory cells and OT-II CD4 T cells in the lung (not shown) and developed dramatic pulmonary eosinophilia and mucus metaplasia (Fig. 3). Thus, lung cDCs can effectively recruit Th2 cells to the respiratory tract, leading to robust allergic airway inflammation and other Th2 effector responses characteristic of asthma.

Because there was no evidence of a lung-specific immune defect in CD11c/Aβ+ mice, we next investigated whether the lack of allergic airway inflammation reflected altered T cell priming in the mesenteric LN and spleen after i.p. immunization with OVA in alum. Mice were reconstituted with polyclonal CD4 T cells and CFSE-labeled OT-II cells followed by immunization with OVA in alum. Three days later, OT-II T cell proliferation was comparable in mesenteric LNs (Fig. 4, A and B) and spleen (not shown) from CD11c/Aβ+ and TCRα−/− mice. We found that 60 ± 5% of OT-II cells in mesenteric LNs of CD11c/Aβ+ mice and 58 ± 10% of OT-II cells in mesenteric LNs of TCRα−/− mice had undergone between one and four cycles of proliferation. On day 13, CD11c/Aβ+ and TCRα−/− spleens had similar numbers of OT-II cells and cDCs and comparable expression of I-Aβ (supplemental Fig. S1). Spleen cells were restimulated...
with pOVA\textsuperscript{323–339} and supernatants were assessed for cytokines. As expected, splenic cells from TCR\textsuperscript{H9251/H11002} mice produced IL-13, IL-4, and low IFN-\gamma (Fig. 4C). Surprisingly, CD11c/A\textsubscript{H9252}b splenic cells produced high levels of IFN-\gamma and minimal IL-4 and IL-13. Splenic cells from A\textsubscript{H9252}b mice produced very low levels of IFN-\gamma and no detectable IL-4 or IL-13. Thus in CD11c/A\textsubscript{H9252}b mice a Th2 stimulus such as OVA in alum leads to DC activation of CD4 T cells and CD4 T cell proliferation, yet production of predominantly IFN-\gamma. Hence, when these cells are recalled to the lung by inhaled OVA, CD11c/A\textsubscript{H9252}b mice do not develop allergic airway inflammation.

The fact that MHC II expression on cDCs is not sufficient to effectively prime Th2 cells but can activate naive CD4 T cells to proliferate suggests that another cell expressing

![FIGURE 2](image-url) Allergic airway inflammation is absent in CD11c/A\textsubscript{H9252}b mice. CD11c/A\textsubscript{H9252}b, TCR\textsuperscript{H9251/H11002}, A\textsubscript{H9252}b, and A\textsubscript{H9252}b mice were immunized with OVA/alum followed by inhaled OVA challenge. A, Total BAL cells. B, BAL eosinophils (Eos). C, Lung eosinophils (Eos). D, BAL polymorphonuclear neutrophils (PMNs). E, Histologic mucus index. F, Chitinase bioactivity in fluorescence units. G–I, Lung histopathology (H&E stain; original magnification: 100) of TCR\textsuperscript{H9251/H11002} (G), CD11c/A\textsubscript{H9252}b (H), and A\textsubscript{H9252}b (I) mice. Mean values ± SEM are shown (n = 5 to 6 mice per group). One experiment is shown that is representative of three experiments. *, p < 0.03; †, p < 0.008; ‡, p < 0.0008; all compared with TCR\textsuperscript{H9251/H11002} mice.

![FIGURE 3](image-url) Adoptive transfer of Th2 cells leads to allergic airway inflammation in CD11c/A\textsubscript{H9252}b mice. OT-II Th2 cells (2 \times 10\textsuperscript{6}) were transferred into CD11c/A\textsubscript{H9252}b, TCR\textsuperscript{H9251/H11002}, and A\textsubscript{H9252}b mice after reconstitution with naive CD4 T cells from C57BL/6 mice. Mice were immunized with OVA in alum and sacrificed 56 h later. Mesenteric LNs and spleen were isolated and assessed by flow cytometry gating on CD4 and Thy1.1-expressing OT-II cells. A, Histograms showing CFSE fluorescence (FL1) in mesenteric LN OT-II cells in individual mice. B, Mean fluorescence intensity (MFI) in FL1 channel (n = 3 mice per group). C, Spleen cells were isolated on day 13, restimulated with the OVA peptide pOVA\textsuperscript{323–339}, and supernatants were tested for cytokines. One experiment is shown that is representative of three experiments. *, p < 0.03 compared with TCR\textsuperscript{−/−} mice; †, p < 0.0009 compared with A\textsubscript{H9252}b mice.

![FIGURE 4](image-url) CD11c/A\textsubscript{H9252}b and TCR\textsuperscript{−/−} mice have comparable CD4 cell proliferation, but CD11c/A\textsubscript{H9252}b mice produce IFN-\gamma. CFSE-labeled CD4 OT-II cells (2.5 \times 10\textsuperscript{6}) were transferred into CD11c/A\textsubscript{H9252}b, TCR\textsuperscript{−/−}, and A\textsubscript{H9252}b mice after reconstitution with naive CD4 T cells from C57BL/6 mice. Mice were immunized with OVA in alum and sacrificed 56 h later. Mesenteric LNs and spleen were isolated and assessed by flow cytometry gating on CD4\textsuperscript{+} and Thy1.1-expressing OT-II cells. A, Histograms showing CFSE fluorescence (FL1) in mesenteric LN OT-II cells in individual mice. B, Mean fluorescence intensity (MFI) in FL1 channel (n = 3 mice per group). C, Spleen cells were isolated on day 13, restimulated with the OVA peptide pOVA\textsuperscript{323–339}, and supernatants were tested for cytokines. One experiment is shown that is representative of three experiments. *, p < 0.03 compared with TCR\textsuperscript{−/−} mice; †, p < 0.0009 compared with A\textsubscript{H9252}b mice.
MHC II must provide signals that promote Th2 development. Given the previous work demonstrating a critical role for CD11c+ DCs in Th2 induction by OVA in alum (6), these data suggest that cDCs act in concert with other MHC II-expressing cell populations to initiate Th2 immune responses.

A Th1 response to OVA/alum was also the outcome in mice deficient in IL-4, lacking Th2 signaling pathways, or mice that were treated with IFN-γ or IL-12 (17–22). To define a mechanism controlling the Th1-predominant immune response to OVA in alum in CD11c−/− mice, we tested whether altering the cytokine milieu during CD4 T cell priming would restore a Th2 response. We treated mice with anti-IFN-γ or a control Ab followed by immunization with OVA/alum and challenged with inhaled OVA. CD11c−/− mice treated with anti-IFN-γ developed pulmonary eosinophilia, increased mucus (not shown), and chitinase activity, whereas CD11c+/− mice treated with control Ab did not (Fig. 5, A and B). IFN-γ-producing, OVA-responsive OT-II cells were increased and the ratio of IL-4 to IFN-γ was low in the lungs of CD11c−/− mice treated with control Ab, whereas CD11c+/− mice treated with anti-IFN-γ had a marked reduction in IFN-γ-producing, OVA-responsive OT-II cells and an increase in the ratio of IL-4 to IFN-γ to a level comparable to that of TCRα−/− mice (Fig. 5C). These studies show that IFN-γ can regulate the Th1-predominant immune response to OVA/alum in CD11c−/− mice. Blockade of IFN-γ permits the generation of a Th2 response and the development of allergic airway inflammation in CD11c+/− mice. This suggests that MHC II-expressing cDC do not provide a cytokine milieu supportive of Th2 development.

These data show that effective Th2 immunity requires class II MHC expression on cells other than cDCs. Because prior studies indicate that cDCs are required to generate Th2 immune responses to OVA/alum (6), it is likely that an alternative APC interacts with the naive T cell in conjunction with cDC to activate Th2 immunity. cDCs were also shown to be insufficient to induce Th2 immunity in the gut mucosal surface, suggesting that alternative APCs may be necessary universally in Th2 generation (23). MHC II expression on this accessory APC may insure specific targeting, but its major role may be to provide a cytokine milieu supportive of Th2 development, because the signal for T cell proliferation can be provided by cDCs. Another explanation is that an alternative APC is sufficient on its own to stimulate an immune response. Potential candidates that express MHC II and have the capacity to stimulate Th2 development include basophils, eosinophils, macrophages, and B cells (8, 10, 23–25). Traditional theory suggests that cDCs can provide all of the necessary signals for CD4 T effector cell priming. These data show that effective Th2 immunity requires class II MHC expression on cells in addition to cDCs.

Disclosures
The authors have no financial conflict of interest.

References


Figure S1. I-A<sub>β</sub><sup>b</sup> expression in spleen of immunized CD11c/A<sub>β</sub><sup>b</sup> mice. CD11c/A<sub>β</sub><sup>b</sup>, A<sub>β</sub><sup>b</sup>+/− and A<sub>β</sub><sup>b</sup>−/− mice were immunized twice with OVA in alum i.p. and sacrificed on day 13. Flow cytometry on spleen cells gated on CD11c<sup>hi</sup> DCs shows equivalent expression of I-A<sub>β</sub><sup>b</sup> in CD11c/A<sub>β</sub><sup>b</sup> and A<sub>β</sub><sup>b</sup>+/− mice compared to A<sub>β</sub><sup>b</sup>−/− mice. A<sub>β</sub><sup>b</sup>+/− gray line, CD11c/A<sub>β</sub><sup>b</sup> black line, A<sub>β</sub><sup>b</sup>−/− shaded curve.