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Cutting Edge: IL-4-Independent Induction of Airway Hyperresponsiveness by Th2, But Not Th1, Cells[1]

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We investigated the role of Th1 or Th2 cells in airway hyperresponsiveness (AHR), because both IFN-γ and IL-4 and IL-5-producing CD4 T cells have been identified in the airways of asthmatics. After transfer of in vitro-generated TCR transgenic Th1 or Th2 cells and exposure to inhaled Ag, Th2 cells induced AHR and airway eosinophilia, whereas Th1 cells induced neutrophilic inflammation without AHR. Next, to determine the precise effector function of IL-4 in Th2 cell-induced AHR, we transferred IL-4$^{-/-}$ Th2 cells into wild-type and IL-4$^{-/-}$ recipient mice. After exposure to inhaled Ag, both groups of mice exhibited AHR with markedly reduced airway eosinophilia. Thus, IL-4 production by Th2 cells is not essential for the induction of AHR, but is critical for the migration of eosinophils from lung tissue into the airways. ***The Journal of Immunology,*** **1998, 161: 3813–3816.**

Airway hyperresponsiveness (AHR)[3] is one of the defining features of asthma and is believed to result from chronic inflammation of the bronchial mucosa. Airway inflammation in asthma is characterized by the presence of activated T cells, mast cells, and eosinophils, and recent studies of patients with severe asthma show an increase in airway neutrophils (1–3). Numerous studies of bronchoalveolar lavage (BAL) and biopsies from asthmatic airways have shown an increase in CD4 positive T cells producing Th2-like cytokines, IL-4, IL-5, and IL-13 (4, 5), and have suggested the importance of these cytokines in AHR. In contrast, others have shown an increase in IFN-γ secreting Th1-like cells (6, 7). In sarcoidosis, a disease characterized by the presence of activated Th1 cells infiltrating the lung, AHR is often present (8, 9). Data from animal models of allergic inflammation implicate a role for Th2 cells in AHR; however, AHR has also been reported in animal models of Th1-driven inflammation (10, 11). Thus, the role of Th1 or Th2 cells in the induction of AHR has not been clearly defined.

Priming naive CD4 T cells results in the generation of Th1 or Th2 effector cells. The cytokine environment in which a naive CD4 T cell is stimulated can determine its differentiative fate, such that T cells stimulated in the presence of IL-4 become Th2 cells, and those stimulated in the presence of IL-12 become Th1 cells. IL-4 is required for the generation of Th2 cells (12). Thus, studies delineating the role of IL-4 in both airway inflammation and AHR have been difficult, because in the absence of IL-4 there is defective Th2 cell priming as well as a subsequent loss of Th2 effector response.

To investigate the direct effect of Th1 and Th2 cells on AHR, we generated Th1 and Th2 cells in vitro from TCR transgenic CD4 T cells. We have previously shown that these populations of OVA-specific Th1 or Th2 effector cells, once transferred into recipient mice, can be activated in the lung after exposure to inhaled OVA, and their patterns of cytokine production persist in vivo (13). We showed that Th2 cells induced many of the histopathologic features of asthma, including airway eosinophilia and mucus hypersecretion, whereas Th1 cells induced airway neutrophilia and had no effect on mucus production. In this report we show that AHR is induced by Th2 cells, but not Th1 cells. To definitively assess the effector role of IL-4 in AHR, we generated IL-4$^{-/-}$ Th2 cells in vitro, transferred them into recipient mice, and exposed them to inhaled OVA. These data show that Th2 cells induce AHR in the absence of IL-4.

Materials and Methods

Generation of Th1 or Th2 cells and cell transfer

Th1 or Th2 cells were generated from BALB/c DO11.10 or DO11 IL-4$^{-/-}$ mice, which are transgenic for the TCR recognizing OVA peptide 323–339 (pOVA$^{323–339}$) (Ref. 14; kindly provided by K. Murphy, Washington University, St. Louis, MO). DO11.10 CD4 T cells were isolated from splenocytes by negative selection, and syngeneic T-depleted splenocytes were used as APCs and treated with mitomycin-C as previously described (15). All cultures were set up in flasks containing equal numbers of CD4 T cells and APCs at a concentration of 0.5×10^6 cells/ml. To generate Th1 cells, cultures contained pOVA$^{323–339}$ (5 μg/ml), IL-12 (5 ng/ml) (Genetics Institute, Cambridge, MA), and anti-IL-4 (11B11) at inhibitory concentration. To generate Th2 cells, cultures contained pOVA$^{323–339}$ (5 μg/ml),

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[3] Abbreviations used in this paper: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; pOVA$^{323–339}$, OVA peptide 323–339; ACh, acetylcholine.

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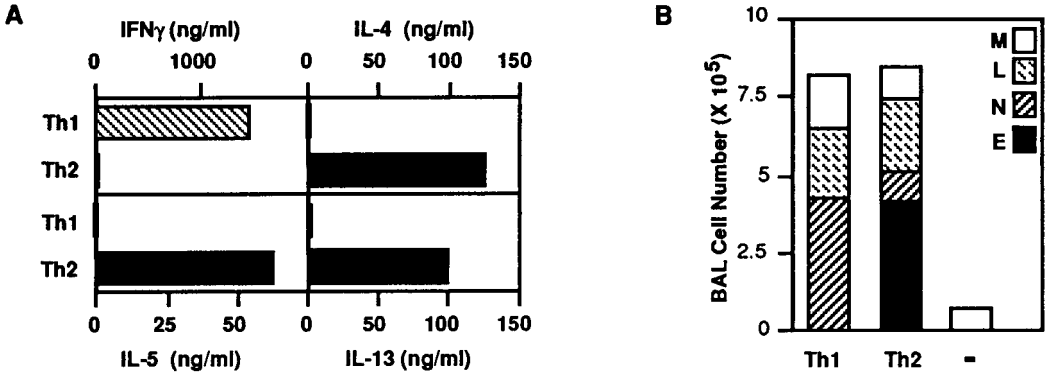

FIGURE 1. *A*, Cytokine production by Th1 or Th2 cells. At the time of transfer into recipient mice, in vitro-generated Th1 or Th2 cells were cultured with APCs and $pOVA^{323–339}$. Supernatants were collected after 48 h, and cytokine ELISAs were performed. *B*, BAL cell recovery in mice after transfer of cells, Th1, Th2, or none (−), and exposure to inhaled OVA. Differential counts were performed on cytospins of cells recovered from BAL from individual mice. M, macrophages; L, lymphocytes; N, neutrophils; E, eosinophils. Mean cell counts are shown ($n = 5$ mice per group). One experiment is shown and is representative of three experiments.

IL-4 (200 U/ml) (Collaborative Research, Waltham, MA), and anti-IFN-γ (XMG1.2) at inhibitory concentration. Cultured Th1 or Th2-like cells were harvested after 4 days and washed with PBS, and 5×10^6 cells were injected i.v. into syngeneic BALB/c or BALB/c IL-$4^{-/-}$ recipients (The Jackson Laboratories, Bar Harbor, ME). At the time of transfer, FACS (Becton Dickinson, Mountain View, CA) analysis was performed on Th1 and Th2 cell preparations. The transferred cell populations were determined to be >95% CD4 and TCR transgene positive using anti-CD4 (Quantum Red-L3T4, Sigma, St. Louis, MO), and DO11.10 TCR-specific, anti-clonotypic Ab, KJ1–26, and fluorescein isothiocyanate-avidin D (Vector Laboratories, Burlingame, CA). Beginning 1 day after transfer of cells, mice were challenged for 20 min daily for 7 days with inhaled 1% OVA in PBS, using an ultrasonic nebulizer (UltraAir NE-U07, OMRON, Vernon Hills, IL) as previously described (13). Twenty-four hours after the last exposure, mice were subjected to pulmonary function testing or sacrificed for analysis of airway inflammation.

Bronchoalveolar lavage

BAL was performed by cannulation of the trachea and lavage with 1 ml of PBS. Cytospin preparations of BAL cells were stained with Dif-Quik (Baxter Healthcare, Miami, FL), and differentials were performed on 200 cells based on morphology and staining characteristics.

Cytokine assays

At the time of transfer, an aliquot of Th1- or Th2-like cells or naive CD4 T cells were retained for restimulation. A total of 5×10^5 CD4 T cells/ml and 5×10^5/ml freshly isolated BALB/c APCs were cultured with $pOVA^{323–339}$ (5 μg/ml), and supernatants were collected at 48 h. ELISAs were performed as previously described (13). The lower limit of sensitivity for each of the ELISAs was 0.6 ng/ml (IFN-γ), 15 pg/ml (IL-4), 0.025 ng/ml (IL-5), 0.030 ng/ml (IL-13).

Lung physiology

Airway responsiveness to five doses of i.v. acetylcholine (ACh) (0.1–10 mg/kg) was determined. Mice were anesthetized (50 mg/kg pentobarbital, 1.8 g/kg urethane), intubated with a 20-gauge stainless steel catheter through which they were ventilated (Harvard Apparatus, Holliston, MA) with 100% O_2 at a tidal volume of 9 μl/g at 150 breaths/min after paralysis with 0.5 mg/kg pancromonium bromide. A 27-gauge needle connected to a catheter was inserted into the tail veil for drug delivery before placement in a volume displacement body plethysmograph (Penn Century, Philadelphia, PA). Continuous measurements of airway pressure and thoracic flow were obtained using a computerized data acquisition system (Buxco Electronics, Sharon, CT), and pulmonary resistance was computed (16). Mean baseline and peak pulmonary resistance (±SE) after each dose was used for statistical analysis. ACh dose responses were analyzed by repeated measures analysis of variance.

Results and Discussion

Th2 but not Th1 cells induce AHR to ACh

To investigate how different T cell subsets activated in the lung effect AHR, Th1 and Th2 cells were generated from CD4 T cells isolated from TCR transgenic DO11.10 mice and transferred into syngeneic recipient mice as previously described (13). Consistent with our previous findings, CD4 T cells stimulated to differentiate into Th1 cells produced high levels of IFN-γ and low to undetectable IL-4 and IL-13, whereas the cells stimulated to differentiate into Th2 cells secreted high levels of IL-4, IL-5, and IL-13 and minimal IFN-γ (Fig. 1*A*). After transfer of cells, mice were exposed to inhaled OVA or PBS. Mice that received Th1 or Th2 cells and inhaled OVA exhibited airway inflammation with similar number of cells isolated by BAL, but the characteristics of the infiltrating leukocytes were different. Neutrophilia was observed in mice that received Th1 cells, whereas mice that received Th2 cells and inhaled OVA had airway eosinophilia (Fig. 1*B*). Mice that received Th1 or Th2 cells and inhaled PBS had no lung inflammation (data not shown). Mice that received no cells and inhaled OVA showed no inflammation, and BAL cells recovered showed differentials that were similar to those of naive mice with >97% macrophages.

The airway inflammation induced by Th1 or Th2 cells after exposure to inhaled OVA was accompanied by an increase in baseline pulmonary resistance (units = cm H_2O/ml) compared with mice that received aerosolized OVA alone (−/OVA, 1.7 ± 0.18; Th1/OVA, 2.27 ± 0.40; Th2/OVA, 2.13 ± 0.33; $p < 0.001$ Th1/OVA and Th2/OVA vs −/OVA). Despite the difference in baseline resistance, only mice that received Th2 cells showed a dose-dependent increase in airway resistance upon challenge with ACh when compared with mice that received either inhaled OVA and Th1 cells or no cells (Fig. 2). Therefore, once activated in the airways, Th2 cells, but not Th1 cells, induce AHR.

Th1 cells induced a neutrophilic inflammatory response when activated by inhaled Ag, yet there was no evidence of AHR. Neutrophilic inflammation has been found in asthmatic patients, including recent studies showing increased neutrophils in the BAL of patients with acute, severe asthma (1). AHR is a typical feature of patients with chronic bronchitis who characteristically have airway neutrophilia (17), and sarcoidosis, a disease in which activated Th1 cells are found in the lung (8). Our studies suggest that neither activated Th1 cells nor airway neutrophilia are sufficient to induce AHR in these diseases.

Th2 cells induce AHR in the absence of IL-4

IL-4 is a marker of Th2 cell activation in asthma. It has been identified in BAL and biopsies of asthmatic patients (5, 18). However, it has been difficult to dissect the effector function of IL-4 in inflammation and AHR from its critical role in the generation of

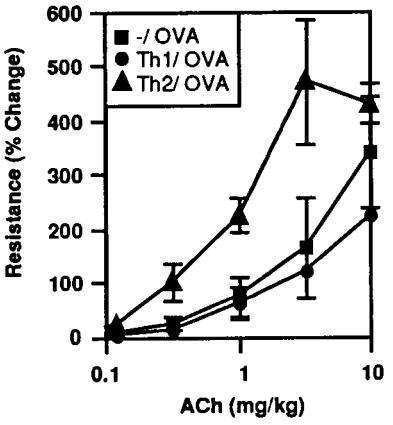

FIGURE 2. Airway reactivity to ACh after transfer of cells and exposure to inhaled OVA. The change in pulmonary resistance from baseline was determined in response to increasing doses of i.v. Ach in mice after transfer of cells, Th1, Th2, or none (−), and exposure to inhaled OVA (n = 3–5 mice per group); $p < 0.0008$ Th2/OVA vsTh1/OVA; $p < 0.04$; Th2/OVA vs −/OVA in three experiments.

Th2 cells. Several lines of evidence indicate that when T cells are primed in vivo in IL-4-deficient, IL-4R-deficient, or STAT6-deficient mice, or during blockade with anti-IL-4 Ab, there is defective Th2 cell generation (12, 19–25). Instead, Th1-predominant cell populations result and are recruited to the lung after Ag challenge, and the effects on airway inflammation and AHR often reflect the presence of Th1 cells and IFN-γ (26–28) as much as the absence of IL-4. Thus, when AHR was absent using these experimental systems, it was likely due to a Th1-predominant response in the airways. Furthermore, in studies in which IL-4 effects were blocked after priming of Th2 cells but before Ag challenge, there is conflicting data with respect to AHR (22, 24) and airway eosinophilia (22, 29, 30).

Because Th2 cells induced AHR when recruited and activated in the respiratory tract, we looked specifically at the effector functions of Th2 cells in the absence of IL-4. We generated Th2 cells from DO11.10 and DO11.10 IL-$4^{-/-}$ mice. Since, in the absence of IL-4, Th2 cells are not generated, DO11.10 IL-$4^{-/-}$ CD4 cells were stimulated with $pOVA^{323–339}$ and APCs in the presence of IL-4. At the time of transfer, Th2 cells from IL-$4^{-/-}$ mice produced no IL-4, but secreted high levels of IL-5, IL-10, and IL-13, and these cytokines were comparable to the levels secreted by wild-type DO11.10 Th2 cells (Fig. 3*A*). Thus, equivalent populations of Th2 cells were generated, with the exception of the production of IL-4, and were transferred into wild-type BALB/c mice.

Mice that received wild-type Th2 cells or IL-$4^{-/-}$ Th2 cells and inhaled OVA showed comparable levels of inflammation in the respiratory tract as indicated by similar numbers of cells recovered from BAL (Fig. 3*B*). In the absence of IL-4, Th2 cell activation resulted in a marked reduction in BAL eosinophils and a concomitant increase in neutrophils. FACS staining on BAL with anti-CD4 and the anti-clonotypic Ab, KJ1.26, showed that TCR transgenic CD4 T cells made up similar percentages of the total cells, 9% (±0.7) in mice that received wild-type Th2 and 11% (±2.2) in mice that received IL-$4^{-/-}$ Th2 cells and inhaled OVA. Mucus production was induced equally in mice that received wild-type Th2 or IL-$4^{-/-}$ Th2 cells and inhaled OVA as previously shown (13). These studies indicate that along with IL-5, which is known to be essential for eosinophil recruitment to areas of inflammation, IL-4 is critical for the development of BAL eosinophilia.

Both mice that received wild-type or IL-$4^{-/-}$ Th2 cells and inhaled OVA showed increased baseline pulmonary resistance compared with mice that received no cells and aerosolized OVA (data not shown). Responsiveness to i.v. ACh was increased in mice that received either wild-type Th2 cells or IL-$4^{-/-}$ Th2 cells when compared with mice that received no cells and inhaled OVA (Fig. 4). This finding was reproducible in both wild-type and IL-$4^{-/-}$ recipient mice, thus eliminating the possibility that endogenous cells in wild-type BALB/c recipient mice could influence AHR. These data show that AHR is induced by Th2 cells in the complete absence of IL-4, and Th2 cell production of IL-4 is important for the development of BAL eosinophilia.

The striking reduction in BAL eosinophilia and increase in neutrophils observed in mice that received IL-$4^{-/-}$ Th2 cells was previously shown by Hogan et al. (31) when they immunized and challenged IL-$4^{-/-}$ mice with OVA. However, in immunized IL-$4^{-/-}$ mice it was unclear if the absence of eosinophils in the BAL was related to the presence of activated Th1 cells or to the lack of IL-4 (31). We also found, as did Hogan, that when IL-$4^{-/-}$ Th2 cells were activated in the respiratory tract, there was lung tissue eosinophilia despite the reduction in BAL eosinophils (data not shown). Thus, although AHR does not correlate with BAL eosinophilia, the presence of tissue eosinophils may be responsible for AHR in mice that received IL-$4^{-/-}$ Th2 cells.

Our previous studies with normal, nontransgenic OVA specific IL-$4^{-/-}$ Th2 cells from BALB/c mice showed that in the absence of IL-4, recruitment of inflammatory cells to the lung did not occur and could be stimulated by exogenous administration of TNF-α

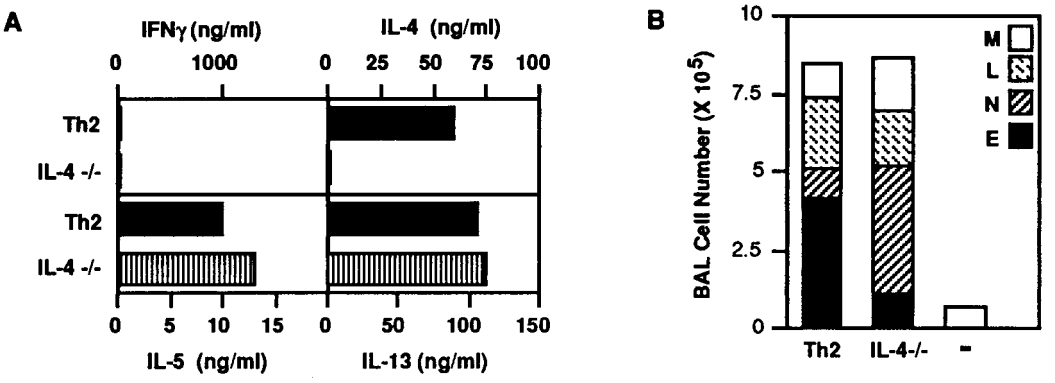

FIGURE 3. *A*, Cytokine production by Th2 or IL-$4^{-/-}$ Th2 cells. At the time of transfer into recipient mice, in vitro generated Th2 or IL-$4^{-/-}$ Th2 cells were cultured with APCs and $pOVA^{323–339}$. Supernatants were collected after 48 h, and cytokine ELISAs were performed. *B*, BAL cell recovery in mice after transfer of cells, Th2, IL-$4^{-/-}$ Th2 (IL-$4^{-/-}$), or none (−), and exposure to inhaled OVA. Differential counts were performed on cytospins of cells recovered from BAL from individual mice. M, macrophages; L, lymphocytes; N, neutrophils; E, eosinophils. Mean cell counts are shown (n = 5 mice per group). One experiment is shown and is representative of three experiments.

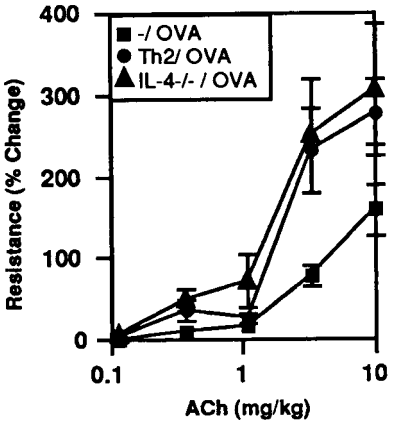

FIGURE 4. Airway reactivity to ACh induced by Th2 cells in the absence of IL-4. The change in pulmonary resistance from baseline was determined in response to increasing doses of i.v. ACh in BALB/c recipient mice after transfer of cells, Th2, IL-$4^{-/-}$ Th2 (IL-$4^{-/-}$), or none (−), and exposure to inhaled OVA (n = 5–6 mice per group); $p < 0.01$ Th2/OVA vs −/OVA; $p < 0.04$ IL-$4^{-/-}$ Th2/OVA vs −/OVA. One experiment is shown and is representative of two experiments.

(13). In the current studies, however, IL-$4^{-/-}$ TCR transgenic cells from DO11.10 mice were recruited to the lung equally well as shown by similar numbers of TCR transgenic cells in the BAL. We believe that recruitment in the TCR transgenic system is due to a 10- to 20-fold increase in cytokines produced by the transgenic Th2 cells. We are currently investigating the cytokines involved in Th2 cell recruitment to the lung in the TCR transgenic and nontransgenic systems.

It remains unclear how Th2 cells control AHR. These data together with our previous studies show that Th2 cells directly induce multiple features of asthma; AHR, airway eosinophilia, and mucus hypersecretion. IL-4 has both local and systemic effects that relate to asthma and atopy, yet AHR can be induced in its absence. Eosinophilic inflammation following Th2 cell activation remains a possible mechanism of AHR. It is also possible that IL-13, which shares many of the biologic effects of IL-4, can stimulate AHR in its absence, or that IL-13 mediates AHR distinct from functions of IL-4.

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