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A Critical Role for Mouse CXC Chemokine(s) in Pulmonary Neutrophilia During Th Type 1-Dependent Airway Inflammation

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Ag-specific Th1 and Th2 cells have been demonstrated to play a critical role in the induction of allergic diseases. Here we have investigated the precise mechanisms of Th1-induced airway inflammation. Airway inflammation was induced in BALB/c mice by transfer of freshly induced OVA-specific Th1 or Th2 cells followed by OVA inhalation. In this model, both Th1 and Th2 cells induced airway inflammation. The former induced neutrophilia in airways, whereas the latter induced eosinophilia. Moreover, we found that Th1 cells induced more severe airway hyperresponsiveness (AHR) than Th2 cells. The eosinophilia induced by Th2 cell infusing was almost completely blocked by administration of anti-IL-5 mAb, but not anti-IL-4 mAb. In contrast, Th1-induced AHR and pulmonary neutrophilia were inhibited by the administration of anti-human IL-8R Ab, which blocks the function of mouse CXC chemokine(s). These findings reveal a critical role of mouse CXC chemokine(s) in Th1-dependent pulmonary neutrophilia and AHR. The Journal of Immunology, 2001, 167: 2349–2353.

Nonspecific airway hyperresponsiveness (AHR) and airway inflammation with eosinophils, lymphocytes, and neutrophils are characteristic features of pulmonary diseases such as asthma and sarcoidosis (1, 2). There is growing evidence to suggest that activated T cells are playing a major role in modulating the pathogenesis of asthma (3, 4). In particular, two types of Th cells, Th1 and Th2, have been demonstrated to play key efferent functions in allergic asthma (5). Th2 cells have been shown to play a crucial role in the development of airway inflammation with eosinophilic infiltration by producing IL-4, IL-5, and IL-13 (6–8). However, it has been reported that IFN-γ-secretting T cells were increased in bronchoalveolar lavage (BAL) fluid (BALF) of asthmatic patients (9, 10), and that Th1 cells can induce airway inflammation with neutrophilic infiltration (11–13). These results suggest that Th1 cells also play a crucial role in airway inflammation, but by a mechanism distinct from Th2 cells. Moreover, the precise mechanisms of Th1-induced airway inflammation remain to be elucidated.

To investigate the role of Th1 and Th2 cells during the effector phase of airway inflammation, BALB/c mice were infused with OVA-specific Th1 or Th2 cells and challenged with aerosolized OVA. Using this bronchial asthma model, it was confirmed that both Th1 and Th2 cells can induce airway inflammation by distinct mechanisms. The former induced neutrophilic infiltration in BALF, whereas the latter induced eosinophilic infiltration. Moreover, we found that mouse CXC chemokines (14) homologous to human IL-8 play a crucial role in Th1-dependent AHR and neutrophilia in airways.

Materials and Methods

Mice

BALB/c mice were purchased from Charles River Breeding Laboratories (Kanagawa, Japan). OVA233–359-specific I-A d-restricted TCR-transgenic mice (DO11.10) on a BALB/c background were kindly donated by K. Murphy (Washington University School of Medicine, St. Louis, MO) (15). All mice were female and were used at 5–6 wk of age.

Generation of Th1 and Th2 cells from DO11.10 TCR-transgenic mice

CD4+ CD45RB+ naive Th cells were isolated from DO11.10 TCR-transgenic mouse spleen cells using cell sorting (FACS Vantage; BD Biosciences, San Jose, CA). OVA-specific Th1 and Th2 cells were induced from purified naive Th cells as described previously (16). Functional differentiation of these cells was confirmed by measuring intracellular cytokine-producing ability by flow cytometry as described previously (16). The cytokine activities in BALF were determined by ELISA (Endogen, Cambridge, MA) according to the manufacturer’s directions. The minimum detectable concentrations for IFN-γ, IL-2, IL-4, and IL-5 were 18, 34, 15, and 20 pg/ml, respectively.

Induction of allergic asthma in Th1- or Th2 cell-transferred mice by OVA inhalation

Th1 or Th2 cells (1 × 106) in 0.2 ml PBS were injected into the tail vein of normal recipient BALB/c mice. One day after the cell transfer, mice were exposed daily to OVA (100 mg/ml in 0.9% saline for 30 min) for 3 days. The aerosol was generated by a nebulizer (DeVilbiss 646 nebulizer; DeVilbiss, Somerset, PA) driven by compressed air at 18 L/min. Seventy-two hours after the first OVA inhalation, mice were subjected to pulmonary function testing and sacrificed for BAL analysis.
AHR was measured by methacholine (Mch)-induced airflow obstruction as previously reported (17). Briefly, mice were placed into whole-body plethysmographs (Buxco Electronics, Troy, NY) interfaced with computers using differential pressure transducers. Measurements were performed of respiratory rate, tidal volume, and enhanced pause. Airway resistance is expressed as: $P_{	ext{Enh}} = [(T_{	ext{Exp}} - 1) \times 2P_{	ext{EF}}/P_{	ext{IF}}]$, where $P_{	ext{Enh}}$ = enhanced pause, $T_{\text{Exp}}$ = expiratory time (seconds), $T_r$ = relaxation time (seconds), $P_{\text{EF}}$ = peak expiratory flow (milliliters), and $P_{\text{IF}}$ = peak inspiratory flow (milliliters per second). Increasing doses of Mch were administered by nebulization (for 1 min), and enhanced pauses were calculated over the subsequent 3 min.

Characterization of cells in BALF

After measuring airway reactivity, the trachea was cannulated with a polyethylene tube through which the lungs were gently lavaged three times with 0.8 ml PBS containing 0.1% BSA. Cells were stained with hematoxylin peroxidase, and differentials were performed based on morphology and staining characteristics. Supernatants of BALF were kept frozen at $-20^\circ$C until use. Cytokine levels were measured in samples of BALF from each animal by ELISA as described above.

Administration of Abs

Th2 cell-transferred mice received 0.5 mg neutralizing anti-IL-4 mAb (11B11; BD Pharmingen, San Diego, CA) or anti-IL-5 mAb (TRFK-5; BD Pharmingen) i.p. 30 min before and 24 h after OVA inhalation. Anti-mouse IL-5R homologue Ab (18) or control Ab, produced by K. Matsushima (University of Tokyo, Tokyo, Japan) were administered by both intranasal (10 $\mu$g) and i.v. (50 $\mu$g) injection 30 min before OVA inhalation in Th1 cell-transferred mice.

Results and Discussion

Th1 and Th2 cells were generated from naive Th cells isolated from DO11.10-derived naive Th cells as described in Materials and Methods. Ten days after initiation of culture, the differentiation of Th1 cells (A) and Th2 cells (B) was confirmed by intracellular staining of cytoplasmic IFN-$\gamma$ and IL-4 in anti-CD3-stimulated cells. The cytokine production profile of in vivo-transferred Th1 and Th2 cells was also confirmed by measuring IL-4 (C) and IFN-$\gamma$ (D) levels in BALF of mice after inhalation of aerosolized saline or OVA. Cytokine levels were determined by ELISA. The bars represent the mean $\pm$ SE of three mice.

FIGURE 2. Characterization of cellularity in BALF of Th1 cell- or Th2 cell-transferred mice exposed to aerosolized saline or OVA. Mice transferred with Th1 or Th2 cells were challenged with aerosolized saline or OVA. After harvesting BALF of the mice, the number of cells (total cells, macrophages, neutrophils, eosinophils, and lymphocytes) were counted using cytospin preparation from BALF. The bars represent mean $\pm$ SE of three mice. $*, p < 0.05$ **, $p < 0.01$ ***, $p < 0.005$ as compared with saline-challenged mice. □, Th1-transferred mice challenged with saline; □, Th1-transferred mice challenged with OVA; □, Th2-transferred mice challenged with saline; and □, Th2-transferred mice challenged with OVA.
In contrast to Th2, the role of Th1 cells in airway inflammation remains unclear. Seventy-two hours after the first aerosol exposure, mice were examined for AHR with increasing concentrations of Mch in whole-body plethysmographs. As illustrated in Fig. 5, both mice that received Th1 cells and mice that received Th2 cells developed AHR to Mch after OVA inhalation. Mice that received unstimulated CD4\(^{+}\)T cells from DO11.10 and inhaled OVA had no lung inflammation (data not shown). These findings demonstrate for the first time that Th1 cells can induce significant AHR after Ag exposure in addition to airway neutrophilia. In our model, Th1 cells induced higher levels of AHR as compared with Th2 cells. There are discrepancies between our results presented here and previous reports by Cohn et al. (13, 23), in which they reported that Th1 cells did not induce AHR. This discrepancy might be because of different experimental methods for the induction of Th1 and Th2 cells, the number of transferred cells, or a schedule for OVA exposure. Moreover, we used BALB/c mice as recipients, whereas Hansen et al. used SCID mice. Airway responsiveness to Mch in BALB/c mice is 2-fold greater than that in SCID mice. It is likely that activation status of the cells transferred to BALB/c and to SCID mice are different. Therefore, the mechanism of induction of AHR after Ag stimulation in our experiments and theirs might be different. Another potentially important difference in this study and the one performed by Cohn is the method used for the determination of AHR. We measured change in enhanced pause after Mch inhalation; on the contrary, Cohn et al. had measured pulmonary resistance in response to i.v. acetylcholine. The i.v. challenge of the mice with bronchoconstrictive agents might not solely reflect physiologic stimulation in airway smooth muscles. The measurement of AHR in unrestrained, conscious animals is reported to be a valid indicator of this condition (17). We have confirmed the reproducibility of Th1-induced AHR in >20 experiments using an unrestrained AHR measurement system.

It has been generally assumed that Th1 cells can suppress Th2-dependent allergic responses. This idea has raised the possibility of manipulating the Th1-Th2 balance in vivo as a potential treatment of asthma. Indeed, after successful immunotherapy, allergic patients show an increase in IFN-\(\gamma\) levels and a decrease in IL-4 levels (24–26). Furthermore, infection with Mycobacterium tuberculosis induces immunodeviation toward Th1 type, which is associated with a reduced incidence of asthma (27). Thus, in vivo manipulation of Th1-Th2 balance toward Th1-dominant immunity appeared to have therapeutic benefit on Th2-dependent allergic reactions. However, our data suggest that the activation of Th1 immunity to inhaled Ag can cause severe airway inflammation accompanied with neutrophilic infiltration and AHR. The features

**Figure 3.** Distinct airway inflammation in Th1 cell- and Th2 cell-transferred mice after exposure to aerosolized OVA. BALB/c mice were transferred with Th1 (A and C) or Th2 cells (B and D). Twenty-four hours after cell transfer, the mice were challenged with aerosolized saline (A and B) or OVA (C and D), and the cellularity of BALF was examined after staining with H&E or hematoxylin plus peroxidase. Mouse eosinophils were stained brown by peroxidase. E–G, Effect of anti-IL-5 mAb or anti-IL-4 mAb on Th2-induced cellular infiltration into the airways. Th2 cell-transferred mice were injected i.p. with 0.5 mg control mAb (E), anti-IL-5 mAb (F), or anti-IL-4 mAb (G) twice 30 min before and 24 h after the first OVA inhalation. Typical photographs were taken under the microscope.

**Figure 4.** The chemokine production profile of in vivo-transferred Th1 and Th2 cells after OVA inhalation. BALF were recovered before and 6 and 24 h after third OVA inhalation in Th1-transferred mice (○) and Th2-transferred mice (●) eotaxin (A), KC (B), and MIP-2 (C) levels were determined by ELISA (R&D Systems, Minneapolis, MN). The bars represent the mean ± SE of four to five mice; *; p < 0.05; **, p < 0.01, as compared with Th2-transferred mice.
of this inflammation are consistent with the inflammation observed in human severe asthma, chronic bronchitis, and sarcoidosis (1, 28). Our observations are supported by adoptive transfer studies demonstrating that Th1 cells do not prevent Th2-induced airway inflammation but, rather, cause severe airway inflammation (29–31). We also confirmed that in vitro-activated Th1 cells could not prevent Th2-induced airway inflammation (data not shown). Thus, in vivo activation of Th1 responses was able to ameliorate Th2-dependent allergy, whereas infusion of in vitro-activated Th1 cells failed to counterbalance Th2-mediated inflammation and, rather, caused more severe inflammations.

Finally, we investigated the mechanisms underlying Th1-induced airway inflammation and AHR. Th1 cells play a critical role for regulating cell-mediated immunity through the production of high levels of IFN-γ (32, 33). Therefore, we investigated the role of IFN-γ in Th1-induced airway inflammation. However, no significant inhibitory effect of anti-IFN-γ mAb on neutrophilia and AHR was observed (data not shown). Next, we investigated the role of chemokine(s). CXC chemokine(s) have been demonstrated to play an important role in recruitment of neutrophils to inflamed tissues (34–36). Although mice do not have an IL-8R (CXCR1) nor do they have IL-8, several mouse CXC chemokines (CXCR2 ligands) including MIP-2, KC, LPS-induced CXC chemokine, and lungkine are homologs of human IL-8 (14). Abs against CXCR2, which is a mouse homolog of the human IL-8R, have been used successfully to define the role of mouse CXC chemokines in immune diseases (14, 18). Therefore, we investigated the effects of anti-human IL-8R Ab, which cross-reacts with mouse CXCR2 (18), on Th1-induced airway inflammation. As shown in Fig. 6, both Th1-induced neutrophilia (Fig. 6A) and AHR (Fig. 6B) were completely inhibited by the administration of anti-human IL-8R Ab. The important role of mouse CXC chemokine(s) in Th1-induced neutrophilia was further supported by the fact that larger amounts of KC and MIP-2 were detected in the BALF after OVA inhalation in Th1-transferred mice than in the BALF in Th2-transferred mice (Fig. 4). Furthermore, it has been reported that the level of the human CXC chemokine IL-8 is increased in patients with inflammatory airway disease, such as acute asthma, chronic obstructive pulmonary disease, and cystic fibrosis, characterized by high neutrophil numbers (37, 38). Mouse homolog of human IL-8 (CXC chemokines), produced by bronchial epithelial cells, venous endothelial cells, alveolar macrophages, and neutrophils, have also been implicated as important mediators of lung immunity in airway inflammation models (36, 39). To test the ability to produce neutrophil chemotactant upon Ag stimulation in T cells, T cells were cultured with APCs and OVA peptide. Neither Th1 cells nor Th2 cells produced detectable amounts of KC and MIP-2 upon Ag stimulation (data not shown). As reported by Knott et al. (40) and also by us, although CD4+ T cells themselves were not the source for these chemokines, they were essential for chemokine production. It remains unclear what types of cells and cytokines are required for production of CXC chemokine(s) that induce Th1-dependent recruitment of neutrophils into airways.

Several investigators have reported the critical role of CXC chemokines and CXCR2 in immune diseases with neutrophilia. Sonoda et al. (18) showed that MIP-2 and its related molecules regulate neutrophil migration into the vagina in a sexual cycle-dependent manner. In their model, neutrophil migration was blocked by the administration of anti-human IL-8R Ab, and its effect was partially reversed by injection of anti-MIP-2 Ab. During the preparation of this paper, Knott et al. (40) reported that neutrophil infiltration into BALF at 6 h after OVA inhalation in DO11.10 mice is regulated by KC and MIP-2. They showed that a combination of anti-κCD and anti-MIP-2 Ab reduced neutrophil influx, but neither Ab alone prevented...
neutrophil numbers in BALF. We also have evidence that anti-MIP-2 alone cannot prevent Th1-induced neutrophilia (data not shown). Taken together, these results suggest that Th1 cell-induced neutrophil migration into BALF and AHR appear to be mediated by several CXCR2 ligands including KC and MIP-2.

In conclusion, the present work demonstrates that, in addition to Th2 cells, Th1 cells can induce significant AHR. Although Th2-induced eosinophil recruitment into airways was inhibited by anti-IL-5 Ab, Th1-induced neutrophil recruitment and AHR were inhibited by anti-human IL-8R Ab, which cross-reacted with mouse CXCR2. Thus, Th1 cells and Th2 cells induce airway inflammation and AHR by totally distinct mechanisms. These findings demonstrate for the first time that Th1 cells play a critical role in pulmonary neutrophilia coupled with the production of CXC chemokine(s).

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References


