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*J Immunol* 1998; 161:919-926; http://www.jimmunol.org/content/161/2/919

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Resistance to Antigen-Induced Airway Hyperresponsiveness Requires Endogenous Production of IL-12

Andrea Keane-Myers,* Maria Wysocka,† Giorgio Trinchieri,† and Marsha Wills-Karp2*

We have demonstrated previously that susceptibility of murine strains to the development of allergic airway responses is associated with a type 2 cytokine pattern. In the present study, we examine the in vivo role of IL-12 in the immune response to allergen exposure in susceptible (A/J) and resistant (C3H/HeJ, C3H) strains of mice. OVA sensitization and challenge induced significant increases in airway reactivity in A/J mice as compared with their PBS-challenged controls, while no increases in airway reactivity were observed in OVA-challenged C3H mice. OVA exposure of A/J mice resulted in marked increases in the Th2 cytokines, IL-4 and IL-10, in the bronchoalveolar lavage fluid, whereas increases in IFN-γ were observed in C3H mice. Strikingly, anti-IL-12 mAb (1 mg/mouse) treatment resulted in threefold increases in airway reactivity in OVA-challenged resistant C3H mice, concomitant with significant increases in bronchoalveolar lavage levels of Th2 cytokines and decreases in IFN-γ. IL-12 depletion of C3H mice also suppressed OVA-specific serum IgG2a levels and increased both serum OVA-specific IgG1 and IgE levels. Blockade of endogenous IL-12 levels in susceptible A/J mice resulted in further augmentation of type 2 immune responses. These results demonstrate that endogenous production of IL-12 is essential for resistance to Ag-induced airway hyperresponsiveness, and furthermore, that dysregulation of IL-12 production may lead to the development of deleterious type 2 immune responses to inhaled allergens. * The Journal of Immunology, 1998, 161: 919–926.

The incidence, morbidity, and mortality from asthma have been increasing worldwide over the last decade. The cardinal features of this disease are airway hyperresponsiveness, eosinophilic inflammation, and elevated serum levels of IgE. Allergic asthma is thought to arise when predisposed individuals mount inappropriate specific immune responses to inhaled allergens. The pathology associated with asthma is thought to be mediated by CD4+ T lymphocytes producing the type 2 cytokines, IL-4 and IL-5, as both mRNA and protein levels of these cytokines are elevated in bronchial biopsies (1), bronchoalveolar lavage (BAL)3 cells (2, 3), and blood (2) of allergic patients as compared with normal individuals. Since these cytokines promote the accumulation and activation of eosinophils (4, 5) as well as IgE synthesis by B cells (6), this cytokine pattern has been thought to be important in the pathogenesis of asthma. Although considerable circumstantial evidence exists for this hypothesis in human asthma, the use of animal models of allergic inflammation has allowed more in depth examination of the importance of Th2 cytokines in the pathogenesis of allergic inflammation. In a murine model, we have provided evidence that the development of airway hyperresponsiveness and pulmonary eosinophilia following allergen provocation is in fact CD4+ T cell dependent (7). Furthermore, we have noted that inbred murine strains exhibit differential susceptibility to mounting allergic responses (8, 9). Development of allergen-induced airway hyperresponsiveness in susceptible strains (i.e., A/J) is associated with increases in lung levels of type 2 cytokines. In contrast, resistance, which is characteristic of C3H/HeJ mice, is associated with a polarized type 1 cytokine response to Ag provocation. The pivotal role of the Th2 cytokines, IL-4 and IL-5, in both the recruitment of eosinophils into the murine lung after Ag challenge and the subsequent airway hyperresponsiveness, has been demonstrated in several murine studies in which the genes for these two cytokines have been disrupted or the proteins have been neutralized in vivo (10–13). For example, neither IL-4−/− nor IL-5-deficient mice develop eosinophilic inflammation or airway hyperresponsiveness in response to Ag sensitization and challenge (10, 11). Although substantial evidence supports a role for Th2 cytokines in the pathogenesis of asthma, the factors that lead to the development of the pathogenic type 2 cytokine pattern in response to inhaled Ags are not well understood.

Several studies (14–16) have highlighted the predominant role of cytokines during the initiation of antigenic stimulation in directing the differentiation of CD4+ T cell precursors. In vitro, using naive CD4+ T cells from TCR-αβ transgenic mice, IL-4 and IL-12 were clearly shown to drive the development of CD4+ T cell precursors toward, respectively, the Th2 and the Th1 functional phenotype (15, 16). Consistent with the necessity of IL-4– for induction of Th2 cell differentiation is the finding that IL-4−/− mice are defective in Th2 cytokine production (17). Conversely, IL-12 p40−/− mice are defective in IFN-γ production and almost completely lack the ability to generate a Th1 response (18). In a very well-characterized murine model of susceptibility to leishmanial infection, skewed production of these two critical immunoregulatory cytokines is thought to be important in susceptibility and resistance of murine strains to infections with Leishmania major (19). Specifically, resistance of C57BL/6J mice to leishmanial infection is thought to be due to enhanced production of IL-12 and/or reduced IL-4 production (20), whereas susceptibility of BALB/c mice appears to be associated with enhanced IL-4 production.

1 Abbreviations used in this paper: BAL, bronchoalveolar lavage.

Received for publication November 11, 1997. Accepted for publication March 10, 1998.

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and/or reduced IL-12 production or responsiveness (21, 22). Recent in vitro studies in T cell clones derived from murine strains exhibiting skewed cytokine patterns suggest that polarization to a Th2 cytokine response may actually occur as a result of loss of responsiveness of T cells to IL-12 (23). Although the exact mechanisms of this loss of responsiveness to IL-12 are not known, these studies highlight the importance of IL-12 signaling in prevention of harmful type 2 responses.

In the present study, we examine the role of endogenous IL-12 in the differential susceptibility of murine strains to allergen-induced airway hyperreactivity. Our results demonstrate that IL-12 depletion induces airway hyperresponsiveness, pulmonary eosinophilia, and Th2 cytokine production following Ag exposure in formerly resistant C3H mice, while exacerbating these responses in susceptible A/J mice. These studies suggest that IL-12 plays a critical role in the regulation of airway responses to allergen provocation, and that susceptibility to the development of allergic disorders may be due to dysregulation of this critical immunoregulatory cytokine.

Materials and Methods

Animals

Six-week-old male A/J and C3H/HeJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were housed in a laminar flow hood in a virus-free animal facility for the duration of the experiments. The studies reported in this work conformed to the principles for laboratory animal research outlined by the Animal Welfare Act and the Department of Health, Education, and Welfare (National Institute of Health) guidelines for the experimental use of animals (n = 6–8 mice/experimental group).

Specific reagents

Anti-IL-12 mAb was prepared from hybridoma C17.8 (rat IgG2a), as previously described (24), and an isotype-matched control Ab (IgG2a) was purchased from PharMingen (San Diego, CA).

Ag challenge and anti-IL-12 mAb administration

A/J and C3H mice were sensitized by an i.p. injection of 10 μg OVA in PBS (200 μl) or PBS alone for control animals. Two weeks later, mice were challenged with 50 μl of 1.5% solution of OVA in PBS or PBS alone for controls by aspiration, as previously described (25). To determine the effects of in vivo IL-12 depletion on the development of Ag-induced airway hyperresponsiveness, Ag-sensitized (OVA-sensitized and OVA-challenged) and control (PBS-sensitized and PBS-challenged) mice were treated by i.p. injection of either 1 mg/mouse of rat anti-mouse IL-12 (IgG1) or an isotype-matched control Ab 48 h before aspiration allergen challenge. Measurement of allergic responses was made 96 h following aspiration challenge.

Airway responsiveness measurements

Airway responsiveness to i.v. acetylcholine challenge was measured as previously described, with minor modifications (7, 13). Briefly, mice were anesthetized with sodium pentobarbital (17.5 mg/ml), intubated with a 20-gauge tracheal cannula, and ventilated at a rate of 120 breaths per minute with a constant tidal volume of air (0.2 ml). Airway pressure was measured with a pressure transducer via a port of the tracheal cannula. Muscle paralysis was provided by i.v. administration of decamethonium bromide (25 mg/kg). After establishment of a stable airway pressure recording, acetylcholine was injected i.v. (50 μg/kg) and the changes in airway pressure were recorded. Airway responsiveness was defined by the time-integrated change in peak airway pressure (airway-pressure-time index, APTI; cm H₂O/g).

Assessment of airway inflammation

After airway responsiveness measurements, lungs were lavaged thoroughly with 1 ml HBSS plus 10% FBS without calcium or magnesium. The lavage fluid was centrifuged (300 × g for 10 min), and the supernatant was removed for cytokine analysis and immediately frozen at −80°C. The cells were resuspended in 1 ml HBSS plus 10% FBS and counted with a hemocytometer using trypan blue dye exclusion as a measure of viability. Cytospin slides were made and BAL cell differential percentages were determined on slide preparations stained with Diff-Quik (Baxter, McGaw, IL). At least 500 cells were differentiated by light microscopy on conventional morphologic criteria.

Quantitation of cytokine protein levels in BAL fluid

IL-4, IFN-γ, and IL-10 protein levels were measured in a 1-ml volume of unconcentrated BAL fluid by sandwich ELISA, as previously described (13). ELISAs were conducted using matching Ab pairs obtained from Pharmingen, according to the manufacturer’s instructions. The following Ab pairs were used for ELISA detection of IL-4, IL-10, and IFN-γ, respectively: BVD4-1D11 and BVD6-24G2; JES5-2A5 and SXC-1; and R46A2 and XMG1.2. OD readings of samples were converted to picograms per milliliter using values obtained from standard curves generated with varying concentrations of recombinant IL-4, IL-10, and IFN-γ (2000 pg/ml to 10 pg/ml). The limit of detection was 10 pg/ml for each assay.

OVA-specific IgG1 and IgG2a ELISA assays

Sera were obtained from blood taken during exsanguination of the animals after airway measurements. IgG subclass-specific ELISAs were used to quantitate OVA-specific IgG1 and IgG2a Ab levels in serum. Briefly, 96-well Costar (Corning, NY) ELISA plates were coated with 50 μl of OVA (100 μg/ml) in HBSS overnight at room temperature. Sixteen hours later, wells were blocked by the addition of PBS/10% FBS (200 μl/well) for 2 h at room temperature. Following blocking, the plates were washed with PBS/Tween-20, sera were added (100 μl/well of a 1/100 dilution in PBS/1% FBS), and plates were incubated overnight at 4°C. Plates were then washed with PBS-Tween and incubated with biotin-conjugated anti-mouse IgG1 (1:2000; γ1-chain specific) or anti-mouse IgG2a (1:2000; γ2a-chain specific; Pharmingen, San Diego, CA; 100 μl/well) for 1 h at room temperature. Following a final washing procedure, plates were blotted dry and developed with 100 μl/well of an avidin peroxidase solution (ABTS; Kirkegaard and Perry, Gaithersburg, MD). Plates were read at 405 nm.

Quantitation of IgE

Sera were obtained from blood taken during exsanguination of the animals after airway measurements, and 100 μl (1/50 dilution in 1% FBS in PBS) was added per well. An IgE-specific ELISA was used to quantify total IgE Ab levels in serum using matching Ab pairs (R35-72 and R32-92) obtained from Pharmingen, according to the manufacturer’s instructions. OD readings (405 nM) of the samples were converted to picograms per milliliter using values obtained from standard curves generated with varying concentrations of IgE (2000 pg/ml to 5 pg/ml), and the final concentration of IgE in the serum was obtained by multiplying by the dilution factor.

Data analysis

Data are summarized as mean ± SEM. Significant treatment effects were determined using analysis of variance (Statview; Abacus Concepts, Berkeley, CA). If differences among groups were significant (p < 0.05), Fisher’s least significant difference test was used to distinguish between pairs of groups.

Results

In vivo depletion of IL-12 exacerbates Ag-induced airway hyperresponsiveness in both susceptible and resistant strains of mice

To determine the effect of IL-12 depletion on the development of allergic airway responses in resistant and susceptible strains of mice, PBS- or OVA-challenged A/J and C3H mice were given anti-IL-12 or an isotype-matched mAb by i.p. injection 48 h before Ag challenge. As previously reported, airway reactivity to acetylcholine challenge in OVA-challenged A/J mice was increased significantly compared with PBS-challenged control mice (Fig. 1A). In contrast, no increase in responsiveness to acetylcholine was observed in Ag-challenged C3H animals (Fig. 1B). Strikingly, IL-12 blockade before Ag challenge rendered C3H mice susceptible to Ag-induced airway hyperresponsiveness (Fig. 1B), and exacerbated the response seen in A/J mice (Fig. 1A). Surprisingly, IL-12 depletion of PBS-challenged mice of both strains significantly increased their responsiveness to acetylcholine challenge as compared with their controls (Fig. 1, A and B). Isotype control Ab administration had no significant effect on airway reactivity in either PBS- or OVA-challenged mice of either strain.
The effect of anti-IL-12 Ab administration on OVA-induced pulmonary eosinophilic inflammation was assessed by enumerating eosinophils in the lavage fluids. The number of eosinophils found in the BAL of non-Ag-challenged animals is typically very low (Fig. 2, A and B). After Ag challenge, the number of eosinophils was increased significantly in both A/J and C3H mice, although the total numbers of eosinophils differed dramatically in these two mouse strains, with A/J mice having more than 10-fold more eosinophils than C3H mice. This Ag-induced pulmonary eosinophilia was further enhanced after depletion of IL-12 in both strains of mice. There was no discernible effect of anti-IL-12 treatment on eosinophil numbers in PBS-challenged mice of either strain.

Anti-IL-12 mAb treatment induces type 2 cytokine production in the lung

To determine the effects of IL-12 depletion on in vivo type 1 and type 2 cytokine production, protein levels of IL-4 and IFN-γ were measured in the BAL fluids by ELISA. OVA challenge of presensitized A/J mice significantly increased BAL levels of the type 2 cytokine, IL-4 (Fig. 3A). In contrast, OVA challenge did not increase the levels of IL-4 in C3H mice over that seen in control animals (Fig. 3B). Strikingly, when OVA-exposed C3H animals were depleted of IL-12, a significant increase in BAL levels of IL-4 levels was observed. Anti-IL-12 treatment did not further increase IL-4 levels in OVA-challenged A/J mice, but did increase the levels of IL-4 in A/J mice challenged with PBS. A similar enhancement in IL-4 levels was not observed in PBS-challenged C3H mice.

As IL-12 is essential for the optimal production of IFN-γ by T cells and NK cells (26), we determined whether depletion of endogenous IL-12 would affect IFN-γ production in the lungs of resistant and susceptible mice. As we have noted previously, OVA challenge of A/J mice did not result in significant increases in BAL levels of IFN-γ when compared with their PBS controls (Fig. 4A). In contrast, OVA challenge of resistant C3H mice resulted in a
significant elevation in IFN-γ levels in the BAL of C3H mice (Fig. 4B). As expected, treatment with anti-IL-12 significantly reduced the concentration of IFN-γ in OVA- and PBS-challenged mice regardless of strain, although the results were more striking in the C3H animals. Surprisingly, the isotype-matched control mAb increased the concentration of IFN-γ in both the A/J and C3H PBS controls, suggesting again that this control was eliciting a biologic effect.

It has been suggested that IL-10 and IL-12 are capable of reciprocal antagonistic regulation (27); therefore, we examined the levels of IL-10 in the BAL from both A/J and C3H mice. Ag challenge significantly increased IL-10 levels in the lungs of A/J mice (Fig. 5), whereas there was no significant increase in C3H mice. However, depletion of IL-12 resulted in enhanced Ag-induced IL-10 production in both A/J and C3H mice (Fig. 5), suggesting that the endogenous production of IL-10 was down-regulated by IL-12 in these animals. Interestingly, the isotype control mAb decreased the Ag-induced increase in IL-10 in the A/J mice, but had no apparent effect on the already low levels of IL-10 in the C3H group.

These cytokine data suggest that Ag challenge results in a predominant Th2 cytokine pattern in the lungs of susceptible A/J mice, while a Th1 pattern is elicited in the lungs of the resistant strain. As IL-12 depletion in both strains enhances the production of Th2 cytokines and suppresses IFN-γ levels, these studies suggest that IL-12 may negatively regulate the production of Th2 cytokines in vivo during exposure to inhaled Ags.

Effects of IL-12 depletion on OVA-specific IgG1 and IgG2a Ab levels

To examine the in vivo effects of IL-12 depletion on the humoral immune response to pulmonary Ag exposure, we examined serum levels of OVA-specific IgG1 and IgG2a Abs in PBS- and OVA-challenged mice that received either the anti-IL-12 mAb or an isotype control Ab. This method has been used previously to assess the relative influence of type 1 vs type 2 cytokines in vivo, since...
the production of the mouse γ1 subclass is augmented by IL-4 and inhibited by IFN-γ, whereas that of γ2a is augmented by IFN-γ and inhibited by IL-4 (28, 29). As expected, the concentration of OVA-specific Abs in the PBS control groups was not above background (data not shown). However, the concentration of OVA-specific IgG subclasses was increased significantly after challenge with OVA. As shown in Figure 6, A/J mice produce dramatically higher levels of OVA-specific IgG1 Abs than C3H mice, while the levels of OVA-specific IgG2a Abs in the serum of the two strains are similar. Administration of anti-IL-12 mAb before Ag challenge significantly elevated serum levels of IgG1 in C3H mice, while it did not result in further enhancement of serum levels of this Ab isotype in A/J mice. Conversely, IL-12 depletion resulted in significant suppression of serum IgG2a levels in both strains of mice. These results support the findings from the cytokine measurements in that C3H exhibit a type 1 cytokine response, whereas A/J exhibit a predominant type 2 response. Following IL-12 depletion, C3H mice shift to a type 2 response. IL-12 treatment also significantly decreased the concentration of IgG2a found in C3H mice, confirming that anti-IL-12 treatment significantly decreased IFN-γ levels in both strains of mice. These data reinforce the concept that a switch had occurred in C3H animals from a strong type 1 response to a type 2 response, concomitant with increased susceptibility to airway hyperresponsiveness.
for six to eight mice per group. *Denotes values significantly different from those of the OVA control groups \((p < 0.05)\). Data were analyzed as described in Materials and Methods.

Discussion

In this study, we describe a model in which murine strains exhibit differential susceptibility to the development of allergen-induced airway hyperresponsiveness. In this model, OVA challenge induced significant increases in airway responsiveness to acetylcholine in A/J mice, while no changes in airway reactivity were observed in C3H mice. Airway hyperresponsiveness in the A/J strain is accompanied by significant increases in eosinophils in the BAL fluids and marked elevations in serum OVA-specific IgG1 and total IgE levels. However, significant levels of OVA-specific IgG2a were also observed in these mice, suggesting that some endogenous IFN-\(\gamma\) was also produced in this strain. This conclusion is also supported by the fact that IL-12 blockade in these animals exacerbated the development of allergic airway responses. Furthermore, the development of allergic responses in this strain was concomitant with significant elevations in the type 2 cytokines, IL-4 and IL-10, in the lavage fluid. Conversely, in the resistant C3H/HeJ strain, OVA-specific IgG2a levels were found with no significant elevations in serum IgE levels or IL-4 levels. However, significant increases in eosinophils were noted, although the magnitude of the eosinophil response was markedly lower than in A/J mice. In contrast to the cytokine pattern observed in A/J mice, significant increases in IFN-\(\gamma\) levels were found in OVA-challenged C3H animals, with no increases in IL-4 or IL-10 levels. Although IL-5 levels were not measured in this study, due to the small quantity of BAL fluid recoverable from individual mice, we have previously found that IL-5 levels are elevated in A/J mice, with only low levels being detected in C3H mice. These observations suggest that both murine strains are clearly sensitized to the Ag and develop local pulmonary responses to the OVA challenge. Although the immune response to allergen exposure in these strains appears to be complex, susceptibility to allergen-induced airway hyperresponsiveness is associated with elevations in lung IL-4 production and subsequent IgE synthesis, whereas resistance is associated with the lack of these two type 2 immune responses. Interestingly in this model, hyperresponsiveness does not appear to correlate with BAL eosinophilia.

As IL-12 has been shown to be the primary determinant of Th1 cell differentiation (16, 18, 26) and has been shown to prevent development of type 2 immune responses in several murine models of immune activation and infection (20, 22, 31, 32), we sought to determine the role of endogenous IL-12 production in the differential susceptibility of A/J and C3H/HeJ mice to allergen-induced airway hyperresponsiveness. In this study, we demonstrate that in vivo IL-12 depletion renders normally resistant C3H mice susceptible to OVA-induced airway hyperresponsiveness (AHR), IgE production, and type 2 cytokine production. Specifically, IL-12 depletion resulted in significant increases in airway responsiveness to acetylcholine in C3H mice. Eosinophil numbers in the lavage fluid of IL-12-depleted C3H animals were more than seven times greater compared with those observed in OVA-sensitized C3H mice receiving the isotype-matched control Abs (Fig. 2). In addition, a switch to a predominant IgG1 and IgE Ab profile was observed as compared with their natural IgG2a pattern. These changes were associated with a skewed type 2 cytokine pattern in the lungs of OVA-challenged IL-12-depleted C3H mice compared with their non-IL-12-depleted controls. The association of the shift to a type 2 response in the C3H animals with increases in airway hyperresponsiveness further supports the role of type 2 responses in the pathogenesis of airway hyperresponsiveness. Interestingly, in susceptible A/J mice, a type 2 response is observed in the absence of depleting IL-12 Abs and is further exacerbated following IL-12 blockade. These findings support our previous finding that administration of IL-12 to susceptible A/J mice suppressed the development of allergen-induced airway responsiveness concomitant with suppression of Th2 cytokines (31), a finding that has been confirmed in other murine models of allergic inflammation.

Augmentation of IgE production in anti-IL-12-treated OVA-exposed mice

Since IgE is thought to be important in the pathogenesis of early and late phase allergic responses (30), we measured the total serum IgE levels in Ag-exposed, anti-IL-12-treated and control animals. Ag challenge significantly increased the concentration of IgE in the serum of A/J mice over that seen in PBS-challenged controls (Fig. 7). In contrast, no Ag-induced increase was observed in the C3H animals (Fig. 7). Administration of anti-IL-12 before challenge significantly elevated the levels of serum IgE in C3H mice, but did not further enhance the concentration of IgE observed in the A/J mice. These results also support the importance of endogenous IL-12 levels in determining the nature of the immune response mounted in response to exposure to inhaled Ags.

![FIGURE 7. Levels of total IgE in OVA- and PBS-challenged, anti-IL-12-treated A/J (A) and C3H (B) mice. Data are the means ± SEM of IgE levels for six to eight mice per group. *Denotes values significantly different from those of the OVA control groups (p < 0.05). Data were analyzed as described in Materials and Methods.](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Downloadedfrom)

![A. A/J mice](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Downloadedfrom)

![B. C3H mice](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Downloadedfrom)
The ability of IL-12 to effectively suppress type 2 cytokine production has also been shown previously in T cells from allergic donors (34) and in parasite-infected animals (32). IL-12 depletion surprisingly increased airway reactivity in control animals of both strains, suggesting that endogenous cytokines influence airway tone even in the absence of overt inflammation. This increase in airway reactivity was not associated with increases in the cellularity of the BAL fluid, but was, however, associated with increases in BAL IL-4 levels in PBS-challenged A/J mice. These results suggest that the balance of IL-4 and IL-12 production in the lung may regulate processes important in maintenance of airway tone. In addition, it suggests that IL-4 even in the absence of inflammation may induce changes in airway function. However, studies in IL-4 transgenic animals, which were not hyperresponsive compared with their wild-type littermates, do not support this conclusion (35).

Many of the effects of IL-12 in vitro and in vivo are thought to be mediated via the induction of IFN-γ production by T cells or NK cells (26); however, IFN-γ-independent effects of IL-12 have also been reported. In this regard, we (31) have shown previously that the inhibitory effects of IL-12 administration on Ag-induced airway hyperresponsiveness in mice were IFN-γ dependent. This hypothesis is supported by the finding that IL-12 depletion, which results in development of hyperresponsiveness in formerly resistant C3H animals, is associated with reduction in IFN-γ levels. In addition, other studies have shown the effectiveness of both aerosol (36) and gene delivery of IFN-γ (37) in inhibiting Ag-induced airway hyperresponsiveness.

In the present study, removal of endogenous IL-12 after active immunization resulted in a switch from a predominant IgG2a Ab profile to one of IgG1 and IgE in C3H mice. This observation suggests that the lack of IgE production normally in these mice is due to the ability of endogenously produced IL-12 to suppress B cell production of IgE. These findings are consistent with both in vitro (38) and in vivo (39) studies demonstrating that IL-12 administration suppresses Ag-driven IgE production. However, several studies indicate that IL-12 is effective at suppressing IgE production only when given at the time of initial immunization (33). The reason for the effectiveness of IL-12 removal at increasing IgE levels after the initial Ag sensitization in the present study is unknown. However, a recent study by Chvatichko et al. (40) suggested that OVA challenge of mice results in production of IgE and IgG1 in the germinal center in the lung itself. Thus, IL-12 blockade at the time of lung Ag challenge in C3H mice may remove an inhibitory influence on local Ab production and allow local IgE production to occur.

The mechanism by which IL-12 suppresses Ag-driven IgE production is unclear. As IL-12 does not directly affect B cell class switching (38), its effect on IgE synthesis may be due to its ability to regulate the relative quantities of IFN-γ and IL-4 generated by Ag-specific lymphocytes. As these two cytokines reciprocally regulate the IgE response, enhanced IFN-γ production would favor suppression of Ag-specific IgE production as well as enhance the proportion of IL-4-secreting T cells (41). This hypothesis is supported by the fact that IFN-γ levels in C3H mice are high, whereas IL-4 levels are low in the absence of anti-IL-12 mAb treatment. However, following anti-IL-12 mAb administration, the reverse is true. However, King et al. (38) have shown that IL-12 can suppress IgE synthesis in vitro independently of IFN-γ.

The fact that IL-12 blockade in resistant C3H mice renders them susceptible to the development of allergen-induced airway hyperresponsiveness suggests that they produce high endogenous levels of IL-12 following Ag challenge of the lung. Conversely, the potentiation of allergic airway responses in A/J mice induced by IL-12 depletion would suggest that IL-12 production in the lungs of A/J mice may be reduced. Taken together with our previous finding that exogenous administration of IL-12 to A/J mice ablates their allergic responses, we may conclude that dysregulation of IL-12 production in these mice may lead to their inherent susceptibility to the development of allergen-induced airway hyperresponsiveness and type 2 cytokine pattern. Possible mechanisms for the Th2-polarized cytokine pattern in A/J mice might include alterations in genes controlling the expression of either p40 or p35 or in its receptor subunits. Conversely, alterations in genes regulating production of cytokines and/or mediators that modulate IL-12 production, such as the type 2 cytokines (i.e., IL-4, IL-13, or IL-10), TGF-β (26), PGE₂ (42), or certain complement receptors (43), may occur in A/J mice. In this regard, the enhanced production of IL-10 observed in the lavage of A/J mice may be responsible for apparently suppressed IL-12 production and subsequent polarization to a type 2 immune response. Recent studies have suggested that polarized type 2 immune response may be the result of a loss of responsiveness to IL-12 (23, 44). Studies in BALB/c mice that develop type 2 immune responses to leishmanial infection suggest that IL-4 produced early during the immune response may be responsible for the unresponsiveness of T cells to IL-12, allowing their differentiation toward a Th2 phenotype (44). As A/J mice exhibit high levels of IL-4 in response to Ag exposure, alterations in genes controlling IL-4 production may in fact be the primary defect.

The notion that dysregulated IL-12 levels may be important in allergic airway responses is supported by a recent study in human asthmatics. In this study, bronchial biopsies from asthmatic patients demonstrate fewer cells staining positive for IL-12 p40 as compared with normal controls (45). Furthermore, after successful steroid treatment, significant increases in the number of cells expressing p40 were observed. Conversely, those patients that were resistant to steroid treatment did not show an increase in the numbers of IL-12-expressing cells.

In summary, we have described a murine model of differential susceptibility to allergen-induced airway hyperresponsiveness in which certain murine strains are either naturally resistant or susceptible to the development of allergic asthma. Susceptibility to airway hyperresponsiveness and allergic inflammation is associated with a polarized type 2 cytokine profile, whereas resistance is associated with the production of the type 1 cytokine IFN-γ. We demonstrate that natural resistance to Ag-induced airway hyperresponsiveness in murine strains is dependent on endogenous production of IL-12, whereas susceptibility most likely results from altered IL-12 production or responsiveness. As our results suggest that IL-12 production and/or signaling are critical to prevention of deleterious type 2 immune responses in the lung following inhalation of ubiquitous allergens, the model described herein should prove useful in elucidating the mechanisms governing IL-12 dysregulation in allergic individuals.

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