

Central Role of Immunoglobulin (Ig) E in the Induction of Lung Eosinophil Infiltration and T Helper 2 Cell Cytokine Production: Inhibition by a Non-anaphylactogenic Anti-IgE Antibody

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Summary

Elevated levels of immunoglobulin (Ig) E are associated with bronchial asthma, a disease characterized by eosinophilic inflammation of the airways. Activation of antigen-specific T helper (Th) 2 cells in the lung with the subsequent release of interleukin (IL) 4 and IL-5 is believed to play an important role in the pathogenesis of this disease. In this study, we have used a non-anaphylactogenic anti-mouse-IgE antibody to investigate the relationship between IgE, airway eosinophil infiltration, and the production of Th2 cytokines. Immunization of mice with house dust mite antigen increased serum levels of IgE and IgG. Antigen challenge of immunized but not control mice induced an infiltration of eosinophils in the bronchoalveolar lavage associated with the production of IL-4 and IL-5 from lung purified Thy1.2⁺ cells activated through the CD3-T cell receptor complex. Administration of the anti-IgE monoclonal antibody (mAb) 6 h before antigen challenge neutralized serum IgE but not IgG and inhibited the recruitment of eosinophils into the lungs and the production of IL-4 and IL-5 but not interferon γ . Studies performed using an anti-CD23 mAb, CD23 deficient and mast cell deficient mice suggest that anti-IgE mAb suppresses eosinophil infiltration and Th2 cytokine production by inhibiting IgE-CD23-facilitated antigen presentation to T cells. Our results demonstrate that IgE-dependent mechanisms are important in the induction of a Th2 immune response and the subsequent infiltration of eosinophils into the airways. Neutralization of IgE by, for example, non-anaphylactogenic anti-IgE mAbs may provide a novel therapeutic approach to the treatment of allergic airway disease.

Bronchial asthma is characterized pathologically by an infiltration of eosinophils into the airway submucosa (1). Eosinophil activation results in the secretion of an array of highly charged cytotoxic cationic proteins such as major basic protein, and is believed to play a central role in the etiology of this disease by inducing damage to the airway epithelium (2), activation of sensory C-fibers (3), and the generation of proinflammatory mediators (4). The mechanisms by which these cells are recruited into the airways after antigen provocation, however, are less clear. Studies performed in experimental animals have suggested that CD4⁺ T cells of the Th2 phenotype play a critical role in the development of eosinophil accumulation, mediated by their ability to secrete IL-5 (5–7). Th2 cells also produce IL-4, which at least in murine systems is essential in instructing B cells to switch to IgE production (8). It has been demonstrated both in vitro (9–11) and in vivo (5, 12) that IL-4 is

also required for antigen naive CD4⁺ T cells to become committed to the Th2 phenotype. More recently, it has been demonstrated that a similar Th2 profile exists in the lungs of individuals with allergic airway disease (13, 14), further supporting the hypothesis that Th2-derived cytokines play an important role in the initiation and perhaps also in the maintenance of bronchial asthma.

In addition to eosinophilic inflammation of the airways, elevated levels of IgE in both the serum and bronchoalveolar lavage (BAL)¹ fluid are also associated with bronchial asthma (15–17). IgE-dependent mast cell activation is a central mechanism in antigen-induced bronchoconstriction mediated largely through the production of histamine and LTD4 (18). Although IgE has been implicated in the in-

¹Abbreviations used in this paper: BAL, bronchoalveolar lavage; DMA, dust mite antigen; M, mouse.

duction of extrinsic asthma, the relationship between IgE and the development of a Th2 immune response and eosinophilic inflammation of the airways is at present unknown. To investigate this further, we have used a non-anaphylactogenic rat anti-murine IgE antibody (termed 1-5) that does not induce activation of mast cells and basophils (19). In this study, we show that IgE-dependent mechanisms play an important role in Th2 cytokine production and in the development of eosinophilic inflammation of the airways and discuss the implication and therapeutic potential of IgE inhibition for the treatment of bronchial asthma.

Materials and Methods

Non-anaphylactogenic Anti-IgE 1-5 mAb

The rat IgG1 anti-mouse IgE 1-5 antibody is IgE isotype specific and reacts with free IgE but does not recognize IgE bound to mast cells (19). This antibody presumably recognizes an epitope within the FcεRI-binding region. As a result of this binding specificity, the antibody 1-5 does not induce histamine release from IgE-sensitized rat basophilic leukemia cells, and based on this property is termed non-anaphylactogenic.

Quantification of Eosinophil Recruitment to the Lung

BALB/C mice (20–25 g) were immunized intraperitoneally with 2 μg of house dust mite antigen (DMA) (Greer Laboratories, Lenoir, NC) in 0.2 ml of alum (Serva, Heidelberg, Germany) on day 0 and boosted with DMA/alum on day 14. Non-immunized control mice received two injections of alum alone. 7 d after the last immunization, animals were anesthetized with isoflurane and challenged by the intranasal route with 100 μg of DMA (in a volume of 50 μl). Mice were injected intraperitoneally with 200 μg of anti-IgE 1-5 mAb or matched isotype IgG control 6 h before the booster injection or airway challenge. 72 h after antigen inhalation, mice were anesthetized with urethane, the trachea cannulated, and BAL performed by five repeated lavages with 0.3 ml of saline injected into the lungs via the trachea. Total cell counts were performed, cytospin preparations were prepared, and a differential count of 200 cells was performed. In addition, 72 h after allergen challenge, blood was taken from the descending aorta, and serum levels of total IgE and IgG were measured by ELISA as described below.

Experiments were also performed in mast cell-deficient WBB6F₁/J-W/W^v and normal congenic mice as described above to determine the possible involvement of mast cell activation in the recruitment of eosinophils to the lung. Likewise, to investigate the possible contribution of IgE bound to the low-affinity IgE receptor (CD23), we administered a neutralizing anti-CD23 antibody (20, 21) (B3B4, 1 mg i.p.) 24 h before challenge and performed a BAL 72 h later. Finally, to determine whether the anti-IgE 1-5 mAb inhibits eosinophil infiltration via a CD23-dependent mechanism, we administered the anti-IgE 1-5 mAb 6 h before antigen provocation in CD23 wild-type or CD23-deficient mice (22).

Determination of Serum IgE and IgG1

Mouse IgG1 and IgE were determined by ELISA as described previously (23) with the following alterations. Briefly, microtiter plates were coated with rat anti-mouse (M) IgG1 LOMG1-2 (Zymed 04-6100) and rat anti-M IgE 4B3-39 (Ciba-Geigy Ltd.), respectively. After incubation with dilutions of serum samples,

bound mouse Ig was detected with biotinylated rat anti-M κ R.33-18-12 (kindly provided by Prof. G. Hämmerling, Krebsforschungszentrum, Heidelberg, Germany) and rat anti-M IgE 3-11 (Ciba-Geigy Ltd.), respectively. Antibodies 4B3-39 and 3-11 recognize different epitopes on the IgE molecule than the non-anaphylactogenic anti-IgE antibody 1-5 without inducing steric hindrance. Thus, the combination of anti-M IgE antibodies chosen for this assay resulted in a highly sensitive and isotype-specific detection of mouse IgE, which is not affected by an even large excess of anti-IgE 1-5 mAb in the serum sample.

In Vitro Experiments

Purification of Lung T Cells. To analyze the lung T cell cytokine profile, experiments were performed in BALB/c mice 72 h after antigen challenge as described previously (5). Mice were treated with anti-IgE 1-5 mAb or matched isotype control antibody 6 h before the booster injection or airway challenge. After the five repeated lavages for assessment of the inflammatory cell infiltrate, a further five lavages were performed with 1 ml of sterile PBS to remove remaining resident alveolar macrophages. The lungs were then perfused via the right ventricle with 5 ml of PBS containing 100 U/ml of heparin to remove any blood and intravascular leukocytes. The lungs were then removed and placed into DMEM containing 10% FCS, 2-ME (50 μM), L-glutamine (2 mM), sodium pyruvate (1 mM), Hepes (10 mM), and gentamycin (50 μg/ml). The lungs were then gently homogenized, the cell suspension was filtered through a 70-μm filter, and lymphocytes were enriched over a single step Ficoll gradient. B cells were depleted from the cell suspension using magnetic sheep anti-mouse Ig beads (Dynabeads; Dynal AS, Oslo, Norway). Cells were then labeled with Thy-1.2-FITC (PharMingen, San Diego, CA) and purified with a flow cytometer (Becton Dickinson, & Co., Mountain View, CA) as described previously (6). In all experiments, purity was >99.5% Thy1.2⁺.

Cell Culture. Lung Thy1.2⁺ cells were plated at a concentration of 2×10^5 in 96-well microtiter plates coated with an anti-CD3 antibody (2C11, 50 μg/ml). Cells were cultured for 72 h in the presence of human IL-2 (200 U/ml). Supernatants were harvested, and cytokine production was determined by ELISA as described elsewhere (12). The limits of detection were as follows: IL-4, 0.5 U/ml; IL-5, 100 U/ml; IFN-γ, 10 U/ml.

Results

Anti-IgE 1-5 mAb Neutralizes Serum IgE and Inhibits Eosinophil Infiltration. Antigen provocation of immunized mice resulted in a selective eosinophil infiltration in the airways (nonimmunized, 0.13 ± 0.09 eosinophils/ml $\times 10^5$; immunized, 7.5 ± 2.0 eosinophils/ml $\times 10^5$) associated with elevated levels of IgE (non-immunized, <200 ng/ml; immunized, 11.6 ± 3 μg/ml) and IgG (non-immunized, 665 ± 119 μg/ml; immunized, $3,418 \pm 919$ μg/ml). Treatment of mice with the anti-IgE 1-5 mAb (200 μg) 6 h before antigen challenge neutralized serum IgE by >80% (2.1 ± 0.5 μg/ml, $n = 6$, $P < 0.001$; Fig. 1 A) and inhibited the recruitment of eosinophils into the lungs (2.24 ± 0.86 cells/ml $\times 10^5$, $P < 0.02$; Fig. 1 B). In contrast, anti-IgE mAb 1-5 did not reduce serum IgG levels ($3,783 \pm 981$ μg/ml, $P > 0.05$).

Anti-IgE 1-5 mAb Inhibits the Production of Th2 Cytokines from Lung T Cells. Consistent with our previous results

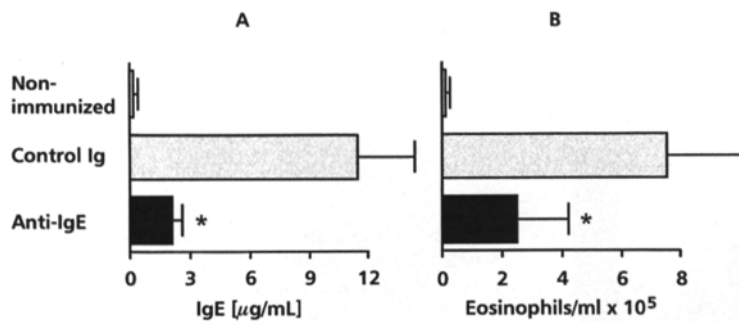


Figure 1. The effect of anti-IgE 1-5 mAb on levels of (A) serum IgE or (B) antigen-induced eosinophil infiltration in mice immunized with DMA. Levels of IgE and eosinophil number of nonimmunized mice (open bars) is shown for comparison. Anti-IgE 1-5 mAb (black bars) or isotype matched control Ig (grey bars) was administered 6 h before antigen challenge. Data are shown as the mean \pm SEM of five to seven animals in each group. Statistical significance was determined by Student's *t* test, and a value of $P < 0.05$ (*) was considered significant.

(5), airway antigen challenge of immunized mice results in the production of Th2 cytokines upon reactivation via cross-linking of the CD3-TCR complex. In contrast, activation of Thy1.2⁺ cells from the lungs of non-immunized mice produced IFN- γ and no IL-4 or IL-5. Administration of anti-IgE 1-5 mAb to immunized mice 6 h before allergen provocation inhibited the production of IL-4 by 95% (control Ig, 66 ± 6 U/ml; anti-IgE, 3 ± 1 U/ml) and IL-5 by 97% (control Ig, $2,500 \pm 145$ U/ml; anti-IgE, 267 ± 61 U/ml) but did not affect IFN- γ production (control Ig, 64 ± 8 U/ml; anti-IgE, 84 ± 3 U/ml) (Fig. 2).

Anti-IgE 1-5 mAb Mediated Suppression of Antigen-induced Eosinophil Infiltration, Th2 Cytokine Production, and Neutralization of Serum IgE Can Be Temporally Dissociated. To understand more fully the relationship between free serum IgE, eosinophil infiltration, and the production of Th2 cytokines, we administered the anti-IgE 1-5 mAb, either before the booster injection (i.e., 10 d before challenge) or 24 h before, 30 min before, or 24 h after challenge. At each time point of antibody administration, there was a comparable neutralization of free IgE in the serum. This was not due to an inhibition of the read-out assay by anti-IgE 1-5 mAb present in the serum sample, as we used an IgE ELISA that is not affected by the presence of the anti-IgE 1-5 mAb. The ability of anti-IgE 1-5 mAb to neutralize

IgE was temporally dissociated from the inhibition of eosinophil infiltration, in that anti-IgE 1-5 mAb only inhibited eosinophil infiltration when given within 24 h of the antigen provocation, and not before the booster injection or 24 h after the antigen challenge (Fig. 3). Likewise, administration of anti-IgE 1-5 mAb failed to inhibit the production of IL-4 and IL-5 when administered before the booster injection compared with animals treated with an isotype-matched control antibody (Fig. 4). These observations suggest that inhibition of antigen-induced eosinophil infiltration and Th2 cytokine production by anti-IgE 1-5 mAb cannot be simply explained by a neutralization of free serum IgE.

IgE-Dependent Triggering of the Fc ϵ RI on Mast Cells Cannot Account for the Effect of Anti-IgE 1-5 mAb on Eosinophil Recruitment. To explore the mechanism of action by which anti-IgE 1-5 mAb inhibits Th2 cytokine production and eosinophil recruitment, we next performed experiments in mast cell-deficient WBB6F₁/J-W/W^v and normal congenic mice. There was no significant difference in the degree of eosinophil infiltration in mast cell-deficient mice compared with congenic normal control mice ($n = 6$, $P = 0.41$; Fig. 5), suggesting that inhibition of IgE-dependent triggering of the Fc ϵ RI on mast cells cannot account for the inhibitory effect of anti-IgE 1-5 mAb on eosinophil in-

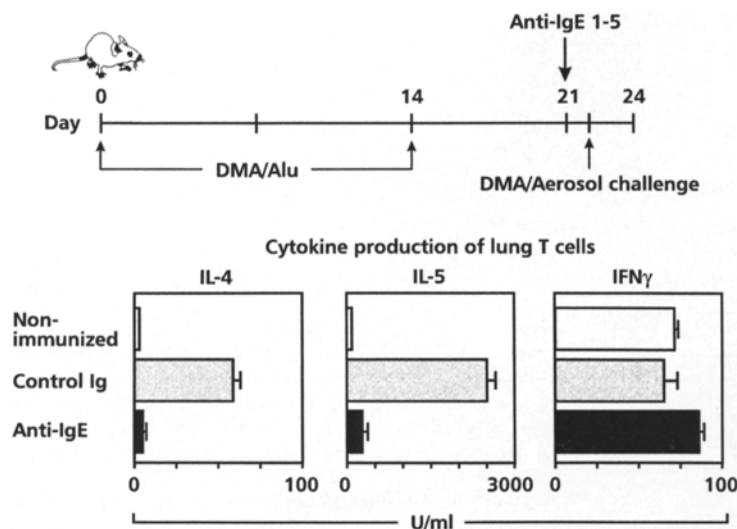


Figure 2. The effect of anti-IgE 1-5 mAb on production of cytokines from FACS[®]-sorted Thy1.2⁺ lung cells obtained from nonimmunized mice (open bars), immunized mice receiving control isotype Ig (grey bars), and immunized mice receiving anti-IgE (black bars). The anti-IgE 1-5 mAb (or isotype-matched control antibody) was administered 6 h before DMA challenge as illustrated. Data are shown as the mean \pm SEM of three individual cultures obtained from the pooled lungs of five to six mice in each group.

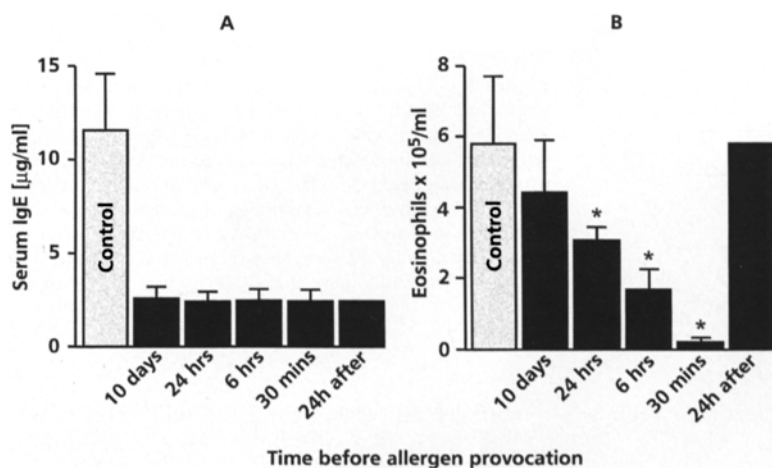


Figure 3. Temporal dissociation between the effect of anti-IgE 1-5 mAb on levels of (A) serum IgE and (B) antigen-induced eosinophil infiltration. The anti-IgE 1-5 mAb (black bars) was administered 10 d, 24 h, 6 h, or 30 min before antigen challenge or 24 h after antigen challenge. Data for the isotype-matched control Ig (grey bars) are shown for comparison. Data are shown as the mean \pm SEM of four to five animals in each group. Statistical significance was determined by Student's *t* test, and a value of $P < 0.05$ (*) was considered significant.

filtration and Th2 cytokine production. It is thus possible that anti-IgE mediates its effect through inhibition of IgE binding to CD23.

Anti-IgE mAb 1-5 Inhibits Eosinophil Infiltration via a CD23-dependent Mechanism. To determine the involvement of an IgE-CD23-dependent mechanism in antigen-induced eosinophil infiltration, we either administered an anti-CD23 mAb (B3B4) (20, 21) or used mice in which the gene for CD23 was deleted by homologous recombination (22). Administration of a mAb against CD23 24 h before antigen provocation inhibited the recruitment of eosinophils into the lungs by 60% (nonimmunized, 0.1 ± 0.1 eosinophils/ml $\times 10^5$, $n = 4$; immunized + control Ig, 7.5 ± 2.6 eosinophils/ml $\times 10^5$, $n = 4$; immunized + anti-CD23, 3.2 ± 0.5 eosinophils/ml $\times 10^5$, $n = 5$; $P < 0.05$; Fig. 6). Similarly, CD23-deficient mice had an impaired ability to recruit eosinophils into the lung (Fig. 7). However, whereas anti-IgE 1-5 mAb inhibited eosinophil recruitment in CD23 wild-type mice, there was no additional suppression of eosinophil infiltration by anti-IgE 1-5 mAb in CD23-deficient mice (control wild-type mice, 7.1 ± 1.0 eosinophils/

ml $\times 10^5$, $n = 6$; control wild-type + anti-IgE-treated mice, 1.19 ± 1.0 eosinophils/ml $\times 10^5$, $n = 4$, $P = 0.01$; CD23-deficient mice, 0.53 ± 0.20 eosinophils/ml $\times 10^5$, $n = 5$; CD23 deficient + anti-IgE 1-5-treated mice, 0.59 ± 0.34 eosinophils/ml $\times 10^5$, $n = 5$, $P = 0.9$; Fig. 7). These observations suggest that CD23-dependent mechanisms are involved in the recruitment of eosinophils into the lung and that anti-IgE 1-5 mAb affects the interaction of IgE with CD23 for the suppression of eosinophil recruitment.

Discussion

Elevated levels of serum IgE and the presence of eosinophils in the lung submucosa have long been associated with bronchial asthma (1, 16, 17, 24). However, it is unknown whether antigen-induced eosinophil accumulation in the lungs is dependent on IgE or whether they are independent manifestations of the allergic status. In this study, we formally demonstrate using an anti-IgE mAb that IgE is required for the recruitment of eosinophils into the lung after antigen provocation.

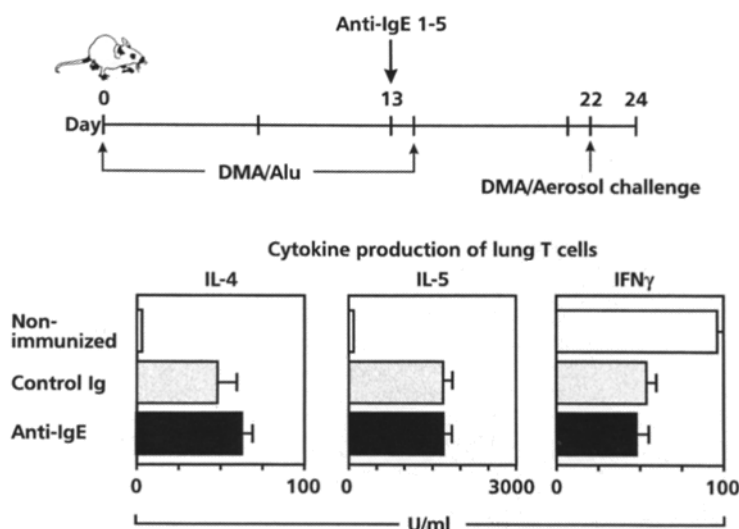


Figure 4. The effect of anti-IgE 1-5 mAb on production of cytokines from FACS[®]-sorted Thy1.2⁺ lung cells obtained from nonimmunized mice (open bars), immunized mice receiving control isotype Ig (grey bars), and immunized mice receiving anti-IgE (black bars). The anti-IgE 1-5 mAb or isotype-matched control Ig was administered during immunization as illustrated. Data are shown as the mean \pm SEM of three individual cultures obtained from the pooled lungs of five to six mice in each group.

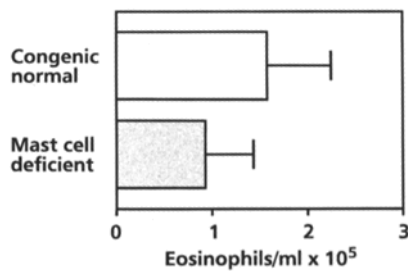


Figure 5. Antigen-induced eosinophil recruitment in mast cell-deficient mice (WBB6F₁/J-*W/W*^{*}) (grey bars) or congenic normal mice (open bars). Data are expressed as the mean ± SEM of six mice in each group. Statistical significance was determined by Student's *t* test, and a value of *P* < 0.05 (*) was considered significant.

The anti-IgE mAb used in this study has the unique property that it neutralizes free serum IgE, binds to membrane IgE⁺ B cells, and thus inhibits IgE production by these cells. However, this mAb fails to bind to IgE on FcεRI on mast cells and basophils (19; Heusser, C., manuscript in preparation). Based on these properties, the anti-IgE mAb does not induce mast cell/basophil activation and degranulation and, as such, is designated non-anaphylactogenic. Furthermore, this anti-IgE mAb inhibits IgE binding to a mast cell line and thus competes with FcεRI for IgE binding (19). Therefore, the epitope of this antibody is presumably located within the FcεRI binding region on IgE, which is mapped to the ε-CH3 domain.

Administration of anti-IgE 1-5 mAb neutralized serum IgE by >80%, without affecting serum levels of IgG. These observations are in agreement with our previous results demonstrating that administration of anti-IgE 1-5 mAb inhibited the production of antigen-specific IgE but not IgG responses (19). In the present series of experiments, it is shown that anti-IgE 1-5 mAb, but not the isotype-matched control antibody, inhibits the accumulation of eosinophils into the lungs, which leads us to conclude that IgE dependent mechanisms play an important role in the recruitment of eosinophils into the lungs.

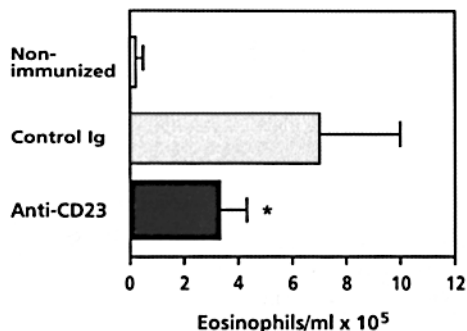


Figure 6. Inhibition of antigen-induced eosinophil recruitment by a CD23 mAb. Immunized mice were treated with anti-CD23 mAb (black bars) or isotype matched control Ig (grey bars), 24 h before antigen challenge. Data are expressed as the mean ± SEM of six mice in each group. Statistical significance was determined by Student's *t* test, and a value of *P* < 0.05 (*) was considered significant.

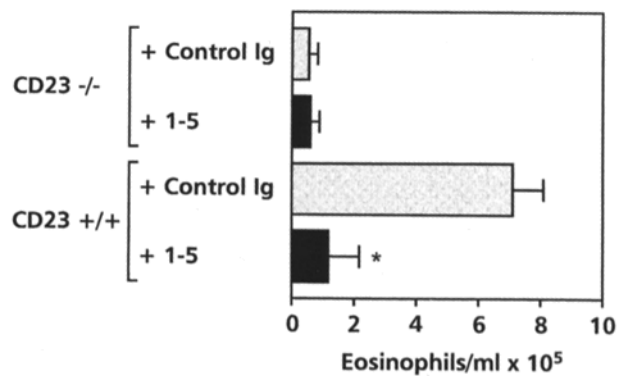


Figure 7. Antigen-induced eosinophil infiltration in CD23 wild-type or CD23-deficient mice. 6 h before antigen challenge, mice were treated with the anti-IgE (black bars) or matched isotype control Ig (grey bars). Data are expressed as the mean ± SEM of four to five mice in each group. Statistical significance was determined by Student's *t* test, and a value of *P* < 0.05 (*) was considered significant.

The precise mechanism(s) underlying the requirement of IgE in the recruitment of eosinophils to the lungs is at present unclear. However, previous studies have demonstrated that IgE-dependent mast cell activation *in vivo* leads to bronchoconstriction (25) and increased airway responsiveness (26). IgE-mediated mast cell triggering has also been suggested to play a role in other aspects of the allergic response by inducing leukocyte infiltration (27) and increasing collagen gene expression (28), which are in part dependent on TNF-α production (27, 28). Mast cell activation has also been shown to result in the secretion of IL-3, GM-CSF, IL-4, and IL-5 (29–31), which could account for the induction of eosinophil infiltration. However, under our experimental conditions, it is unlikely that mast cells are involved in the recruitment of eosinophils to the lung and that anti-IgE 1-5 mAb inhibits eosinophil accumulation via IgE bound to FcεRI on these cells, for two reasons. First, anti-IgE 1-5 mAb, while effectively preventing IgE binding to a mast cell line (32), would not be expected to readily displace high affinity prebound IgE, particularly in view of the relatively short time required to inhibit eosinophil infiltration. Second, in agreement with previous studies, we also report that mast cell-deficient mice develop a normal lung eosinophilic response to antigen provocation (33), suggesting that IgE-dependent mast cell degranulation is not a critical event in the recruitment of eosinophils into the lung and cannot explain the efficacy of the anti-IgE 1-5 mAb.

We and others have previously demonstrated that the recruitment of eosinophils into the airways is dependent on the activation of the Th2 subset of CD4⁺ T cells and the subsequent secretion of IL-5 (5, 6). Therefore, we next investigated whether anti-IgE 1-5 mAb can inhibit the production of Th2 cytokines. Our results demonstrate that administration of the anti-IgE 1-5 mAb inhibited the *ex vivo* production of IL-4 and IL-5 from lung T cells activated through the CD3-TCR complex. In contrast, there was no inhibition in the production of IFN-γ, demonstrating a

selective inhibition of T cell-derived Th2 cytokines. Thus IgE is not only required for the recruitment of eosinophils into the lung, but IgE-dependent mechanisms are also required for the activation of T cells and subsequent Th2 cytokine secretion. As eosinophil infiltration is dependent on IL-5 secreted by CD4⁺ T cells, we conclude that the inhibition of Th2 cytokine production by anti-IgE 1-5 mAb is the mechanism by which this antibody inhibits eosinophil infiltration of the lung.

Recent *in vitro* studies have demonstrated that antigen-IgE complexes, but not antigen-IgG complexes bound to CD23, allows B cells to facilitate antigen presentation to antigen-specific T cells, resulting in a greatly amplified T cell response (34, 35). The concept of IgE-dependent antigen affinity focusing has more recently been demonstrated *in vivo*, using anti-CD23 mAbs (36, 37) and CD23 deficient mice (38). We therefore investigated whether IgE bound to low affinity FcεRII is involved in the recruitment of eosinophils into the lungs after antigen provocation. Administration of anti-CD23 mAb inhibited antigen-induced lung eosinophil accumulation by >60%, raising the possibility that anti-IgE mAb inhibits eosinophil accumulation thus impairing IgE antigen focusing to antigen-specific CD4⁺ T cells in the lung, resulting in a failure of the Th2 cells to proliferate and secrete IL-5. This concept is further supported by the demonstration that eosinophil recruitment was attenuated in CD23-deficient mice. Moreover, anti-IgE 1-5 mAb failed to inhibit residual eosinophil infiltration in these mice. These observations may explain the recent demonstration that CD23-deficient mice fail to develop an increase in airway responsiveness after antigen challenge (39). However, whether CD23⁺ positive B cells (20) and/or dendritic cells (21) mediate antigen focusing to lung Th2 cells remains to be determined.

There was, however, a temporal dissociation between the ability of the anti-IgE 1-5 mAb to neutralize serum IgE and to inhibit eosinophil accumulation and the production of Th2 cytokines. Administration of anti-IgE 1-5 mAb during immunization effectively neutralized serum IgE but failed to suppress eosinophil infiltration and Th2 cytokine production, supporting the hypothesis that the mode of action of the anti-IgE 1-5 mAb is not simply related to neutralization of free serum IgE and may reflect the requirement for a critical concentration of anti-IgE 1-5 mAb for optimal inhibition of IgE-CD23 interactions.

Chimerized (40) or reshaped (41) anti-human IgE mAbs have also been reported as potential antiallergic compounds. Interestingly, one of these mAbs (40) was selected on the basis of its inability to induce histamine release from basophils and was shown to inhibit IgE-CD23 interactions, similar to the murine anti-IgE 1-5 mAb. In contrast, the reshaped anti-IgE mAb was selected to compete with IgE for binding to the FcεRI and mimic FcεRI binding activity and may not interfere with IgE-CD23. Based on the present data, our prediction would be that an anti-IgE mAb that interferes with IgE binding to both FcεRs (I and II) would be more effective in the treatment of allergic disorders.

The IgE-CD23-mediated antigen presentation, which apparently leads to an induction of Th2 cytokine production, could represent a positive feedback loop responsible for amplifying an allergic asthmatic response. Our results open up the possibility that inhibition of IL-4 production by anti-IgE mAb could prevent such an amplification of the immune response by preventing the switch of virgin IgM + B cells to IgE production and the subsequent "arming" with IgE of FcεRI and FcεRII on mast cells and APC, respectively. Moreover, as IL-4 is required not only to drive CD4⁺ T cells to a Th2 phenotype, but may also be required to sustain a Th2 immune response (42), treatment with anti-IgE mAb may also prevent this positive feedback mechanism. However, it still remains to be determined whether inhibition of eosinophil infiltration by anti-IgE 1-5 mAb is also associated with an attenuation of allergen-induced airway hyperreactivity.

In conclusion, IgE plays a critical role in the production of Th2 cytokines and in the development of eosinophil recruitment into the airways after antigen provocation. Although the precise mechanisms underlying this observation remain to be fully elucidated, there is evidence to suggest that this occurs via an inhibition of CD23/IgE-enhanced antigen processing and presentation to CD4⁺ T cells in lungs and subsequent activation and Th2 cytokine production. Our data suggest that inhibition of IgE represents a novel approach for the treatment of asthma, not only by preventing IgE binding to mast cells (resulting in inhibition of bronchoconstriction), but also by inhibiting the activation of Th2 cytokine-producing cells and the recruitment of eosinophils into the lungs. Finally, these results highlight the therapeutic potential of anti-IgE mAbs for the treatment of allergic disorders such as bronchial asthma.

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