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Introduction

Allergens are known to induce the formation of IgE antibodies which, upon binding to high-(FcεRI) and low (FcεRII)-affinity IgE-receptor-bearing cells, mediate allergen-induced effector functions. By this interaction, mast cells and basophils are activated and thereby release an array of mediators that provoke various physiological changes

associated with allergies. Several approaches have been envisaged to inhibit IgE antibody formation and/or actions. Neutralizing anti-IgE antibodies are interesting principles for IgE inhibition [1–3]: they can be selected for isotype specificity and have a long half-life. However, conventional anti-IgE antibodies will induce activation of IgE-sensitized cells (e.g. mast cells, basophils) in a way similar to allergen. In order to bypass such anaphylactic reactions, the anti-IgE

Demonstration of the Therapeutic Potential of Non-Anaphylactogenic Anti-IgE Antibodies in Murine Models of Skin Reaction, Lung Function and Inflammation

Abstract

Background: Allergies and allergic asthma are believed to be mediated by allergen-specific IgE antibodies. We have investigated the therapeutic potential of inhibiting endogenous IgE by a non-anaphylactogenic anti-mouse IgE antibody 1–5 with respect to its effects on antigen-induced skin reaction, lung function changes and lung inflammation in mice. **Methods:** Mice were immunized with benzylpenicillinoyl-KLH or ovalbumin, and antigen-mediated skin reaction, bronchoconstriction, bronchopulmonary hyperresponsiveness (BHR) and lung eosinophilic inflammation determined in anti-IgE 1–5-treated versus untreated animals. **Results:** Application of anti-IgE 1–5 inhibited (by 90%) the serum IgE and, 3–4 days after onset of treatment, blocked the antigen-induced skin reaction. Furthermore, the antibody also inhibited (by 90%) the antigen-induced infiltration of eosinophils into the lung. This latter effect seems to be mediated by blocking the IgE-CD23 interaction and indicates that lung eosinophilic inflammation also depends on IgE. Moreover, when applied to rats passively sensitized with mouse IgE, antibody 1–5 inhibited the antigen-induced bronchoconstriction. A similar effect could be seen in actively immunized mice, where antibody 1–5 was able to inhibit (by 70%) the ovalbumin-induced bronchoconstriction as well as BHR. **Conclusions:** In summary, non-anaphylactogenic anti-IgE antibodies can markedly inhibit IgE levels and IgE-mediated allergic reactions. Since bronchoconstriction, BHR and lung eosinophilic inflammation can be suppressed, such antibodies may be attractive principles for the treatment of allergic asthma.

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antibody must have a particular epitope specificity [1–4], namely it must be directed against a site/area of the IgE molecule which is located within or near the structures recognized by the FcεRI and II, which are both located within the C_H3 domain. Such an antibody would not recognize receptor-bound IgE. Corresponding antibodies to human IgE have already been generated [2, 4] and shown to be unable to trigger histamine release from sensitized human basophils [2, 3, 5], as they compete with the FcεRI interaction for IgE and thus are of non-anaphylactogenic nature [3]. The existence of naturally occurring anti-IgE antibodies has been known for many years [6]. It has been speculated that naturally occurring anti-human IgE of the anaphylactic and non-anaphylactic type may amplify and counteract endogenous IgE responses. We have generated a non-anaphylactogenic anti-mouse IgE antibody [1, 3] and have investigated its potential for the inhibition of antigen-induced skin reactions, bronchoconstriction, bronchopulmonary hyperresponsiveness (BHR) and lung eosinophilic inflammation.

Materials and Methods

Immunization and Treatment of Mice. Mice (Balb/c and BP2) were immunized with benzylpenicillinoyl (BPO)-KLH intraperitoneally or with ovalbumin in Al(OH)₃ subcutaneously and boosted 2–3 weeks thereafter as described [7, 8]. Mice were treated with indicated amounts of the rat IgG1 non-anaphylactogenic anti-mouse IgE antibody 1–5 [3] or normal rat IgG by intraperitoneal or intravenous application.

Detection of Serum IgE. IgE levels were determined as described [7], using anti-IgE antibody 4B3-39 for coating and 3-11 for detection, allowing to determine IgE levels in the presence of antibody 1–5.

In vivo Read-Out Analyses. Skin reactions were determined after intradermal injection of BPO-BSA followed by intravenous administration of Evans blue solution, as described [9]. Lung eosinophil infiltration was determined after antigen challenge by inhalation as described [8], and pulmonary bronchoconstriction and BHR were determined after intravenous ovalbumin application in anesthetized mice, as described [10].

Results

Characterization of the Non-Anaphylactogenic Anti-IgE Antibody 1–5

We have generated a non-anaphylactogenic anti-IgE antibody to mouse IgE, termed '1–5', which specifically binds IgE (cross-reactivity to other isotypes <0.01%) and recognizes IgE-expressing hybridoma B cells as tested by FACS analyses. However, in contrast to conventional anti-IgE antibodies, this antibody does not recognize IgE-sensitized PB-3 [11] mouse basophilic cells. Furthermore, antibody

1–5 efficiently inhibited (by 80–90%) ¹²⁵I-labeled mouse IgE binding to these cells (IC₅₀ value of 0.2 µg/ml). The functional properties of FcεRI-bearing basophils/mast cells was tested using IgE-sensitized rat basophilic leukemia (RBL) cells from which histamine release could be induced by conventional anti-IgE antibodies [12, 13]. As tested over a wide range of antibody concentrations, antibody 1–5 was unable to induce histamine release from IgE-sensitized RBL cells. Thus, antibody 1–5 was termed 'non-anaphylactogenic'.

Inhibition of IgE Levels in Mice by Anti-IgE Antibody 1–5

Balb/c mice previously immunized with BPO-KLH were boosted and treated with 200 µg antibody 1–5 on the day before the boost and on days 0, 1 and 4 after the boost. The BPO-specific antibody levels in control mice reached 20–50 µg/ml. Total and antigen-specific IgE levels were inhibited by 85% in treated groups, but not in groups treated with nonspecific rat IgG. On the other hand, the levels of IgG anti-BPO antibody were not affected by the treatment. In situations with lower serum IgE levels (1–10 µg/ml), one application of antibody 1–5 (200 µg) resulted in 95% reduction in serum IgE. In order to investigate whether the effect of antibody 1–5 was merely the result of IgE neutralization or whether it was also due to an inhibition of new IgE production from IgE-producing cells, the number of IgE-anti-BPO-antibody-producing splenic cells was also determined. Whereas the number of IgE-anti-BPO-antibody-producing cells (control 20 per 10⁶ cells) was inhibited by 50%, the number of IgG-anti-BPO-antibody-producing cells (control 275 per 10⁶ cells) was not affected. These data indicate that antibody 1–5 inhibited IgE antibodies in vivo in an isotype-specific way by inducing clearance of free IgE antibodies as well as by reducing the number of IgE-producing cells.

Inhibition of Antigen-Induced Skin Reactions by Anti-IgE Antibody 1–5

In a first set of experiments, it was shown that intradermal injection of 0.1 µg of conventional anti-IgE antibody into antigen-immunized mice induces a strong skin reaction, whereas even a 100-fold excess of antibody 1–5 had no effect. Furthermore, rats passively sensitized with IgE anti-BPO showed a strong skin reaction to BPO-BSA. This skin reaction, which was measured 30 h after IgE application, could be fully prevented if rats were previously treated intravenously with 1 mg antibody 1–5, but not if treated after IgE application. In order to investigate the effect of antibody 1–5 on the antigen-induced skin reaction of an endogenous im-

mune response, BPO-KLH-immunized mice were treated intraperitoneally with 500 and 250 μ g of antibody 1–5 on days 0 and 3, respectively. Partial inhibition of the skin reaction could be observed on day 3, whereas the reaction on day 4 was completely inhibited. This slow onset of action is in accordance with the understanding that skin reactions are mediated by high-affinity binding of IgE to mast cells and therefore would not readily be displaced. Thus, cell-bound IgE will presumably decrease with time due to capture of released IgE during the on/off interaction with the Fc ϵ RI.

Inhibition of Antigen-Induced Lung Eosinophil Infiltration by Antibody 1–5

An important feature of lung inflammation after antigen challenge in sensitized animals is the infiltration of eosinophils. Thus, the effect of anti-IgE antibodies on antigen-induced infiltration of eosinophils was also investigated. Mice were immunized as described above and challenged once with ovalbumin by inhalation. This treatment induced a pronounced infiltration of eosinophils into the airways (>60-fold over background). Application of 200 μ g antibody 1–5 given before antigen challenge resulted in a 60–90% suppression of this eosinophil infiltration. Interestingly, optimal inhibition was achieved when mice were treated shortly before challenge, whereas the longer the pretreatment period was, the lower the inhibition observed (treatment periods before challenge and suppressive effects were: 30 min, 97%; 6 h, 72%; 24 h, 48%; 10 days, 24%).

As the effect was rapid in onset, the mechanism is unlikely to be mediated via high-affinity IgE-Fc ϵ RI interaction. In order to examine the involvement of CD23 in this effect, studies were performed with anti-CD23 antibodies and CD23-deficient mice. Treatment with anti-CD23 antibodies induced inhibition of lung eosinophil infiltration by 58%. On the other hand, eosinophil infiltration in CD23-deficient mice was only 7% of the one obtained in control mice, and this residual response was not inhibited by anti-IgE antibodies. These results suggest that anti-IgE antibodies inhibit lung eosinophil infiltration by blocking IgE-CD23 interaction.

Inhibition of IgE-Mediated Bronchoconstriction by Anti-IgE Antibody 1–5

In order to investigate the role of IgE in antigen-induced bronchoconstriction, F344 rats were passively given 200 μ g mouse IgE against BPO. Intravenous challenge of rats 4 days later with BPO-BSA resulted in a significant airway narrowing (90% increase in lung resistance). Pretreatment of rats with anti-IgE administered 14 h before passive immunization significantly reduced the antigen-induced in-

crease in lung resistance (49 and 100% suppression with 0.5 and 2.0 mg antibody application, respectively). Furthermore, administration of anti-IgE (2 mg per animal) 24 h after passive immunization with IgE again inhibited the BPO-BSA-induced bronchoconstriction by 96%. Thus, antigen challenge of rats, passively sensitized with mouse IgE, causes a significant bronchoconstriction which could be prevented by treatment with a non-anaphylactogenic anti-IgE antibody.

Inhibition of Antigen-Induced Bronchoconstriction and Pulmonary Hyperreactivity by Antibody 1–5

As antigen-induced bronchoconstriction seems to be IgE dependent, we investigated the effect of anti-IgE antibody 1–5 on anaphylactic bronchoconstriction in actively immunized BP2 mice. Intravenous challenge of immunized mice with ovalbumin resulted in an increase in pulmonary resistance (by 138%). Intravenous treatment of mice with 330 μ g antibody 1–5 given 1 or 3 days before challenge inhibited the bronchoconstriction by 68 and 79%, respectively.

In order to test the effect of anti-IgE antibody on BHR, mice were similarly immunized and 4 times challenged with antigen by the inhalation route. Such mice expressed enhanced sensitivity to serotonin (PD₅₀ of 20 μ g/kg versus 39 μ g/kg for nonchallenged mice). Treatment of mice with 1 mg of 1–5 antibody given 3 times (namely before each immunization and before challenge) resulted in 70% inhibition of BHR, whereas treatment with an irrelevant antibody had no significant effect. These results indicate that *in vivo* inhibition of IgE by a suitable neutralizing anti-IgE antibody results in the inhibition of antigen-induced bronchoconstriction and BHR.

Discussion

It has been well established that IgE antibodies mediate allergic reactions. However, it remains to be clearly shown to what degree this antibody class is responsible for allergen-mediated bronchoconstriction, BHR and lung eosinophilic inflammation. Furthermore, some discrepancy exists as to the obligatory role of IgE in antigen-induced skin reaction. Whereas studies with Fc ϵ R α -chain-deficient mice have demonstrated skin reactions to be dependent on IgE-mediated Fc ϵ RI triggering [14], studies with IgE-deficient mice showed that other mechanism (presumably via IgG antibodies) could also mediate anaphylactic reactions [15]. It was therefore of interest to investigate whether neutralization of IgE would result in the inhibition of antigen-induced skin reactions and anaphylactic bronchoconstriction.

Studies with anti-IgE antibodies in mice have shown that IgE responses can be efficiently suppressed over a long period [16, 17]. Furthermore, anti-IgE antibodies were shown to inhibit the allergen-induced contraction of human airways in vitro [18, 5]. We have investigated the potential of IgE neutralization by anti-IgE antibodies in the inhibition of antigen-induced skin reactions and pulmonary responses in mice. In order to bypass anaphylactic reactions, we have developed a rat anti-mouse IgE antibody (termed 1–5) [1, 3] that does not trigger receptor-bound IgE similar to corresponding anti-human IgE antibodies [2–4]. This antibody does not recognize receptor-bound IgE, as it presumably binds to the same area on IgE as FcεR I and II and thus competes with these receptors for IgE binding. Indeed, antibody 1–5 does not induce histamine release from IgE-sensitized RBL cells and thus is of non-anaphylactogenic nature.

One might question whether inhibition of IgE could ever be achieved to a high enough degree, as cross-linking of as few as 0.1% of IgE receptors on basophils is sufficient to trigger partial mediator release [19]. It was therefore of interest to observe that the treatment of actively immunized mice with antibody 1–5 resulted, within 4 days, in the complete inhibition of antigen-induced skin reactions. Several explanations may be considered. It is clear that neutralization of IgE is independent of its antigen specificity, whereas triggering by antigen only applies to antigen-specific IgE, which represents only a fraction of total IgE. Thus, depending on the proportion of specific to total IgE, antigen-specific cell triggering could be prevented with a relatively limited IgE inhibition. Furthermore, recent studies (see article by D. MacGlashan et al. in this issue, pp. 45–48) demonstrate that reduction of serum IgE levels by *in vivo* anti-IgE antibody treatment resulted in a 30-fold reduction in the FcεRI density on human basophils and mast cells thus reducing the chance of triggering these cells by specific IgE and corresponding allergens. Moreover, in an *in vivo* setting, antigen-specific IgG antibodies may counteract IgE-mediated triggering. On the other hand, our experiments with passive IgE administration in rats demonstrate that anti-IgE given 24 h afterwards can prevent bronchoconstriction (within 3 days) independent of the above-mentioned considerations. Moreover, certain IgE effects may be mediated by low-affinity FcεRII (CD23) interaction (see below) which are efficiently inhibited by anti-IgE [3] and which, furthermore, may require a higher degree of receptor occupancy for cell triggering. Such and other factors could explain why incomplete IgE suppression (e.g. by 90%) could result in inhibition of *in vivo* IgE-mediated reactions.

Recent studies imply that (allergic) asthma is genetically linked to serum IgE levels [20] and demonstrate a close association between serum IgE and BHR [21]. In this study, we showed that IgE alone is able to mediate antigen-induced anaphylactic bronchoconstriction, as demonstrated by passive IgE application in rats. As this response could be inhibited by anti-IgE 1–5, it was therefore of interest to investigate the therapeutic potential in actively immunized mice. As demonstrated, antigen-induced anaphylactic bronchoconstriction in actively immunized mice could be inhibited by over 70%. Interestingly, in both cases, preliminary experiments indicate that inhibition of bronchoconstriction could also be achieved if anti-IgE was administered after (rather than before) either injection of IgE or induction of an IgE response. However, in these situations, higher concentrations of anti-IgE seem to be required. Importantly, in a mouse model of repetitive airway challenge resulting in BHR, the antibody 1–5 was able to inhibit this clinically important parameter by 70% if mice were treated during immunization and challenge. Thus, anti-IgE is able to improve lung function parameters.

Another important feature of allergic asthma is lung eosinophilic inflammation. We have recently reported that an anti-IgE antibody is able to efficiently inhibit lung eosinophil infiltration [8]. Studies with anti-CD23 antibodies and CD23-deficient mice indicated that this effect was mediated via the blockade of IgE-CD23 interaction, presumably preventing IgE-mediated antigen presentation to T cells. Indeed, after antigen provocation, anti-IgE treatment resulted in a strong and Th2-cytokine-selective inhibition of IL-4 and IL-5 production from lung T cells [8]. These results demonstrate that anti-IgE inhibits lung eosinophilic inflammation by a CD23-mediated mechanism.

Based on the understanding that non-anaphylactogenic anti-IgE antibodies are able to inhibit *in vivo* IgE-mediated phenomena such as antigen-induced skin reactions, bronchoconstriction, BHR and eosinophilic inflammation, such antibodies represent an attractive intervention principle for the treatment of allergies and allergic asthma. In this line, chimeric [22] and reshaped humanized [4, 23] non-anaphylactogenic anti-human IgE antibodies have been generated for *i.v.* and *s.c.* applications. These antibodies proved to be well tolerated and to inhibit serum IgE in a dose-dependent way [22] and showed efficacy in clinical trials in seasonal rhinitis during natural allergen exposure and in asthmatic patients after allergen provocation.

The neutralization of IgE with non-anaphylactogenic anti-IgE antibodies represents an intervention at an early and central stage of the disease process and, due to its IgE-specific nature, can be viewed as disease specific. It is possible

that severe rhinitis and/or steroid-resistant allergic asthmatic patients may profit from such treatment. Further clinical studies should prove the medical benefit and would identify the patient population that will best profit from therapy with non-anaphylactogenic anti-IgE antibodies.

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