

Role for IgE in Airway Secretions: IgE Immune Complexes Are More Potent Inducers Than Antigen Alone of Airway Inflammation in a Murine Model

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IgE is present in airway secretions from human patients with allergic rhinitis and bronchial asthma. However, the contribution of IgE present locally to the overall airway inflammation is not well understood. We hypothesize that Ag-specific IgE can capture airborne Ags and form immune complexes. These immune complexes may function as potent inducers of immune responses in the lung, contributing to the perpetuation of airway inflammation. BALB/c mice were first sensitized with OVA in alum systemically and then challenged with nebulized OVA. Bronchoalveolar lavage (BAL) fluid from these mice contained significant amounts of IgE, of which >50% was Ag specific. The IgE levels in airway secretions remained elevated for more than 15 days after the termination of Ag exposure. Significant amounts of IgE-OVA immune complexes were detected in BAL fluid from the OVA-challenged mice. For comparison of IgE immune complexes vs Ag alone, we treated OVA-immunized mice with intranasal administration of trinitrophenyl-OVA or trinitrophenyl-OVA-anti-DNP IgE. Those treated with the immune complexes showed significantly higher levels of IL-4 and more pronounced eosinophilia in BAL fluid than did those receiving the Ag alone. The IgE immune complexes did not augment the inflammatory response in high affinity IgE receptor (FcεRI)-deficient mice. We conclude that IgE present in the airways can capture the Ag and that the immune complexes thus formed may augment allergic airway response in an FcεRI-dependent manner. Thus, IgE present in airway secretions may facilitate Ag-mediated allergic airway inflammation. *The Journal of Immunology*, 2000, 164: 2667–2673.

Immunoglobulin E has long been regarded as a major molecular component of atopic diseases, including asthma (1). Clinical studies have found a close association between asthma and serum IgE levels as well as IgE-dependent skin test reactivity to allergens (2). In addition, allergen-specific IgE has been detected in nasal mucosa from allergic patients (3), and IgE synthesis and heavy chain class switching to IgE occur in B cells from the nasal mucosa of allergic individuals (4). Furthermore, IgE is consistently present in airway secretions from human patients with allergic rhinitis and bronchial asthma (5) and is detected in nasal washes from allergic patients receiving nasal challenge with specific allergens (6). Allergen-specific IgE is detected in bronchoalveolar lavage (BAL)³ fluids in asthmatic patients obtained with or without bronchial provocation with the allergen (7). It is believed that mast cells present in airway tissue are sensitized by the allergen-specific IgE and, on exposure to a specific allergen, are activated to produce potent mediators that are responsible for some of the symptoms in allergic airway diseases. The functional significance of IgE found in airway secretions is, however, not

well understood. In this study, we hypothesize that Ag-specific IgE can capture airborne Ags and form immune complexes. Further, these immune complexes may function as more potent inducers of immune responses in the lung and thus contribute to the perpetuation of airway inflammatory responses. We have used a mouse model of asthma to test this hypothesis.

Animal models of human asthma have drawn considerable attention in recent years. A mouse model has been particularly popular, in which mice are primarily sensitized with an Ag systemically and then challenged with the same Ag through the airways. The animals develop a Th2 response in the lung with prominent eosinophilia, production of Th2 cytokines, and development of airway hyperresponsiveness (8–10). This model has been valuable, especially in conjunction with the use of genetically engineered mice lacking specific gene products, in the elucidation of molecular and cellular mechanisms responsible for allergic airway inflammation and airway hyperresponsiveness. It is evident that T cells play an important role (11, 12). The roles of various cytokines, including IL-4 (13, 14), IL-5 (15, 16), IL-6 (17), IL-13 (18–20), and IL-16 (21) in promoting, and IL-12 (22, 23) in suppressing inflammation have been demonstrated. Consistent with the presence of a Th2-like response, elevation of serum IgE is also a prominent feature of this mouse model.

The airway inflammation developed in this model can be conveniently studied through analysis of BAL fluid obtained from the mice. Typically, allergic airway inflammation is accompanied by a prominent increase in the numbers of eosinophils and elevation of levels of Th2 cytokines, such as IL-4 and IL-5, in the BAL fluid. However, there is no report documenting the detection of IgE in the fluid in this mouse model. In this study, we found the presence of Ag-specific IgE in both free and Ag-complexed forms in BAL fluid from mice with allergic airway inflammation. We also identified a potential role for the IgE present in the airway secretions by demonstrating that in vitro-formed IgE immune complexes, when

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Received for publication June 7, 1999. Accepted for publication December 14, 1999.

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¹ This work was supported by National Institutes of Health Grants AI-39620 and AI-42244 to F.-T.L. and Smokeless Tobacco Research Grant 0654 to S.-S.C. This is publication 257 of La Jolla Institute for Allergy and Immunology.

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³ Abbreviations used in this paper: BAL, bronchoalveolar lavage; OPD, o-phenylenediamine dihydrochloride; TNP, trinitrophenyl; FcεRI, high affinity IgE receptor.

administered through the airways, are more potent than Ag alone in inducing airway inflammatory responses in mice previously sensitized with the Ag.

Materials and Methods

Mice

Female BALB/cBYJ mice (The Jackson Laboratory, Bar Harbor, ME) 6–8 wk of age were used in all experiments. High affinity IgE receptor (Fc ϵ RI)-deficient (Fc ϵ RI $^{-/-}$) mice and Tg1101 mice with a defective murine Fc ϵ RI- α chain gene but carrying a human Fc ϵ RI- α gene were obtained from R.W. Johnson Pharmaceutical Research Institute, San Diego, CA. In the latter mice, mast cells express functional Fc ϵ RI receptor that recognizes both murine and human IgE (24).

Reagents

Mouse monoclonal IgE specific for dinitrophenol (25) and that specific for a ragweed Ag (26) were obtained previously. Goat anti-mouse IgE and rabbit anti-mouse IgE were prepared as described previously (25), and were affinity purified by using mouse anti-ragweed Ag monoclonal IgE (26). Trinitrophenylated OVA (TNP-OVA) was prepared as described (27). The IgE immune complexes were prepared before use by mixing 20 μ g anti-DNP IgE with either 2 or 4 μ g TNP-OVA followed by incubation at room temperature for 30 min.

Immunization and challenge protocols

Both control and OVA mice were immunized with 10 μ g of OVA (chicken egg albumin from Sigma, St. Louis, MO) in 2 mg aluminum hydroxide gel (alum) i.p. After 14 days, the mice were given aerosolized OVA for 30 min each day from days 1 to 6 on consecutive days. Control mice were exposed to aerosolized PBS, pH 7.2. This was accomplished by placing the mice in a Plexiglass chamber connected to a nebulizer that generates an aerosol mist. In other experiments, OVA-immunized mice were first anesthetized using metofane (Pitman-Moore, Washington Crossing, NJ) and then challenged by administering intranasally 25–50 μ l OVA, TNP-OVA, or mixtures of TNP-OVA and IgE (the concentrations used for individual experiments are shown in *Results*).

Collection of BAL fluid

The BAL fluid was collected after various time intervals following airway Ag challenge. The mice were anesthetized by using pentobarbital (Sigma) at 100 μ g/g body weight, the trachea was cannulated, and a BAL was performed with 1 ml pyrogen-free PBS. The lavage fluid collected was centrifuged at 400 $\times$ g, and cells were separated from the fluid. The fluid was again centrifuged at 1000 $\times$ g to remove cellular debris and was then stored at -70°C until it was evaluated for its cytokine and Ig levels as well as the presence of IgE-OVA immune complexes.

Enumeration of cells

Total viable cell numbers were determined by trypan blue exclusion using a hemocytometer. Differential cell counts were determined by cytocentrifuging cells onto slides and staining them with either Wright-Giemsa stain (Sigma) or Leukostat staining kit (Fisher Scientific, Pittsburgh, PA).

Quantitation of IL-4

IL-4 in the BAL fluid was quantitated by a sandwich ELISA. The buffers, diluents, and washes used between steps in this assay were according to the PharMingen Cytokine ELISA Protocol (PharMingen, San Diego, CA). Briefly, 96-well plates (Maxisorp, Nunc, Roskilde, Denmark) were coated overnight with the capture Ab 11B11 (National Cancer Institute, Biological Response Modifier Program) at 4°C and then blocked for 1 h with 1% BSA in PBS. Recombinant IL-4 standard (a gift from Dr. J. Ohara, Denver, CO.) and BAL fluid samples were added, and the plates were incubated for 2 h at room temperature. The bound cytokine was detected with a biotinylated secondary Ab (PharMingen), with an incubation period of 2 h at room temperature, followed by HRP-avidin (Becton Dickinson, Mountain View, CA), at a dilution of 1:3,000, with an incubation period of 30 min at room temperature. The plates were developed by addition of the substrate, o-phenylenediamine dihydrochloride (OPD), followed by incubation in the dark on a Variac shaker for 30 min before reading them in an ELISA plate reader (Spectramax 250, Molecular Devices, Sunnyvale, CA) at 490 nm. The calculations were done with the computer program provided with the plate reader.

Quantitation of IgE

Total IgE in the BAL fluid was quantitated with a sandwich ELISA protocol. Briefly, the plates were coated with affinity-purified rabbit anti-IgE overnight at 4°C and then blocked with 1% BSA in PBS for 1 h at 37°C . The BAL fluid samples and appropriate dilutions of a standard IgE preparation were placed in the wells, and the plates were incubated for 3 h at 4°C . Sample blank wells did not receive BAL fluid but were otherwise treated similarly. The bound IgE was detected with polyclonal goat anti-IgE Abs (incubation for 1 h at 37°C), followed by HRP-conjugated rabbit anti-goat Abs (incubation for 1 h at 37°C). The plates were developed by addition of OPD and read in an ELISA plate reader at 490 nm.

In an initial experiment, due to nonavailability of anti-OVA specific IgE Ab, the actual concentration (nanograms per milliliter) of OVA-specific IgE in BAL fluid was determined indirectly by batch absorption of 100 μ l BAL fluid with either BSA-Sepharose (control) or OVA-Sepharose (test). The unabsorbed IgE level was determined by the total IgE ELISA as described above. The percent OVA-specific IgE present in BAL fluid was then calculated as a reciprocal value of (IgE not absorbed by OVA-Sepharose/IgE not absorbed by BSA-Sepharose) $\times$ 100.

This pooled BAL fluid contained 30 ng/ml OVA-specific IgE and was subsequently used as the standard in ELISA to determine OVA-specific IgE levels in BAL fluids from other experiments. For this, 96-well plates were coated with 10 μ g/ml OVA overnight at 4°C and the plates were then blocked as for total IgE. The standard and experimental BAL fluids were placed at several dilutions and incubated for 2 h at room temperature. Then, plates were washed nine times in PBS, and 100 μ l biotinylated anti-IgE (PharMingen) at 1 μ g/ml were placed in each well. After 2 h of incubation at room temperature, the plates were washed as before and incubated with avidin-HRP conjugate for 30 min. After washing, the plates received the OPD substrate and were read 30 min later as described above for total IgE.

Quantitation of IgE immune complexes

IgE-OVA complexes in the BAL fluid were detected by a sandwich ELISA. The plates were coated with rabbit anti-OVA Abs overnight at 4°C and then blocked as described above. Samples were placed in the wells and the plates were incubated for 3 h at 4°C . The bound IgE was detected by goat anti-mouse IgE Abs followed by HRP-coupled rabbit anti-goat Abs (Zymed, San Francisco, CA) and substrate as described above. Experimental values were read off a standard curve prepared with serial dilutions of in vitro prepared anti-DNP IgE-TNP-OVA complexes (see *Reagents*).

Statistics

Data were analyzed using Statview software. An unpaired Student two-tailed *t* test was used to compare samples from different treatments as shown under individual experiments in *Results*.

Results

Ag-specific IgE is present in BAL fluid from mice with allergic airway inflammation

As shown in Fig. 1, IgE was detectable in BAL fluid from mice exposed to just one dose of aerosolized OVA, when the fluid was obtained 24 h after the Ag exposure. Significantly higher levels were detected after six Ag exposures, at both 3 and 24 h after the last exposure. Control mice that were sensitized to OVA but were exposed to aerosolized PBS did not show detectable levels of IgE. The levels of IgE in general correlated with eosinophilia. To determine whether the IgE present in the BAL fluid was Ag specific, samples of the BAL fluid taken 24 h after the last aerosol were adsorbed with OVA-Sepharose 4B, or BSA-Sepharose 4B as a control, and the concentrations of IgE in the supernatants were determined by ELISA. By this method, it was determined that $>50\%$ of the IgE secreted into the airways was Ag specific (Table I).

IgE persists in airway secretions after aerosolized Ag exposure

To determine whether IgE persists in airway secretions after cessation of Ag exposure, we treated OVA-sensitized mice with six doses of aerosolized OVA and then monitored the amounts of IgE in BAL fluid after various intervals following the last Ag challenge. Data are presented for two experiments with different and overlapping time intervals (Fig. 2). Both the total and ova-specific

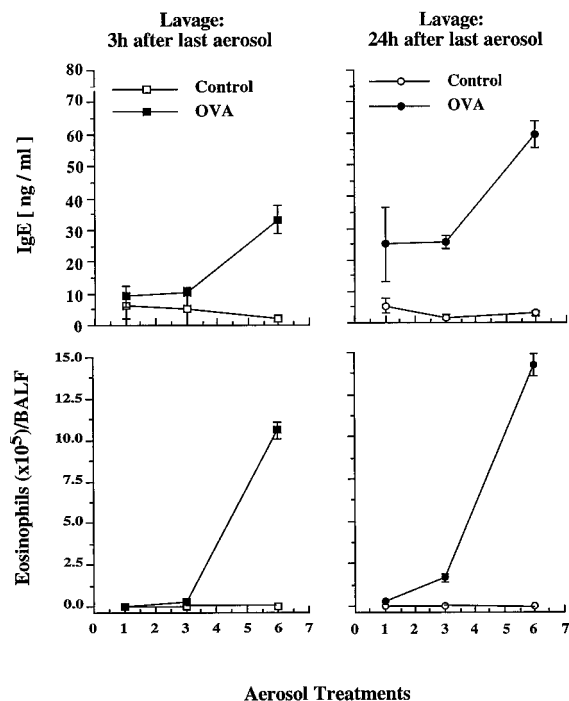


FIGURE 1. Detection of IgE and eosinophils in BAL fluid (BALF) from immunized mice with airway inflammation. BALB/c mice were immunized with OVA in alum and 14 days later were challenged with nebulized OVA 1, 3, or 6 times, 30 min each day. Control mice received aerosolized PBS challenge. The BAL fluid was collected either 3 h or 24 h after aerosol challenge, and the numbers of eosinophils and levels of IgE were determined as described in *Materials and Methods*. Data are presented as mean $\pm$ SEM from three mice for each data point, and results are representative of more than five experiments.

IgE peaked at 3 days after the end of the last Ag exposure; the levels declined afterward, but significant amounts could still be detected 15 days after the challenge. In general, OVA-specific IgE was 50% of total IgE in the airways. Three days after the last aerosol, OVA-specific IgE reached peak levels and accounted for 50–90% of the total IgE. In contrast, although significant numbers of eosinophils could be detected in BAL fluid obtained from mice 1–3 days after the last aerosolized Ag challenge, the numbers declined precipitously afterward and reached a background level 9 days after the last Ag exposure.

IgE-OVA immune complexes are naturally formed in the airways in mice challenged with nebulized OVA

The Ag-specific IgE present in the airway secretions likely recognizes the same Ag to which the animals are exposed, thus forming

Table I. OVA-specific IgE in BAL fluid of BALB/c mice^a

No.	Adsorbent (μ l)	IgE (ng/ml) (unabsorbed)		% OVA-Specific IgE
		BSA-Sepharose	OVA-Sepharose	
1	12.5	56	33	42
2	25	58	25	57
3	50	56	26	54

^a BSA and OVA were separately coupled to CNBr-activated Sepharose 4B (Pharmacia-Upjohn) at 2 ml protein (5 mg/ml) to 0.4 g Sepharose. The BAL fluid from OVA-sensitized and -challenged mice from a representative experiment of three experiments was pooled. The IgE concentration in the BAL fluid was 60 ng/ml. Then, 100- μ l aliquots of the BAL fluid were adsorbed with 12.5, 25, or 50 μ l of Sepharose 4B beads coupled to Ag (OVA) or control (BSA). The unbound IgE in the supernatants was quantitated by ELISA. The percentage of OVA-specific IgE present in BAL fluid was calculated as a reciprocal of percent value of IgE not absorbed by OVA-Sepharose/IgE not absorbed by BSA-Sepharose.

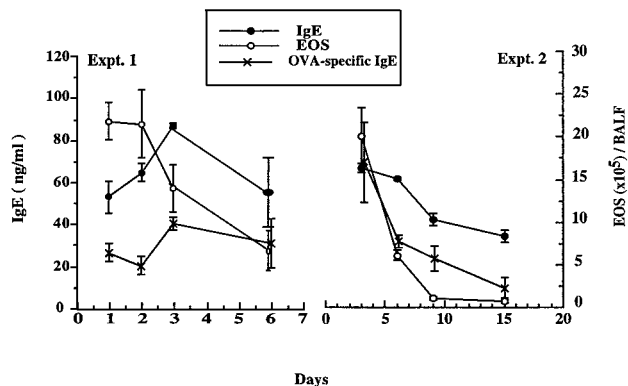


FIGURE 2. Detection of IgE and eosinophils (EOS) in BAL fluid (BALF) from mice with airway inflammation over various intervals after termination of airway Ag exposure. BALB/c mice were sensitized with OVA and then challenged with nebulized OVA six times. The BAL fluid was collected from mice 1, 2, 3, and 7 (Experiment 1) and 3, 6, 9, and 15 (Experiment 2) days after the last challenge. The numbers of eosinophils and levels of total and OVA-specific IgE were determined as described in *Materials and Methods*. Data are presented as mean $\pm$ SEM from three mice for each data point.

immune complexes. To definitively establish the presence of such immune complexes, we challenged the OVA-immunized mice with nebulized OVA six times, obtained BAL fluids at various time points after the last challenge, and quantitated the amounts of the IgE immune complexes in the fluids. As shown in Fig. 3, significant levels of IgE-OVA complexes could be detected in BAL fluid harvested immediately after the Ag challenge. The levels in fluids obtained at later time points declined progressively, but the immune complexes remained readily detectable in the fluid obtained 30 min after the Ag challenge.

Administered IgE immune complexes are more potent than the Ag alone in inducing airway inflammation

Ag-Ab complexes are better recognized by lymphoid cells than Ag alone. We therefore decided to determine whether there is a difference in airway inflammation induced by Ag alone vs IgE-Ag

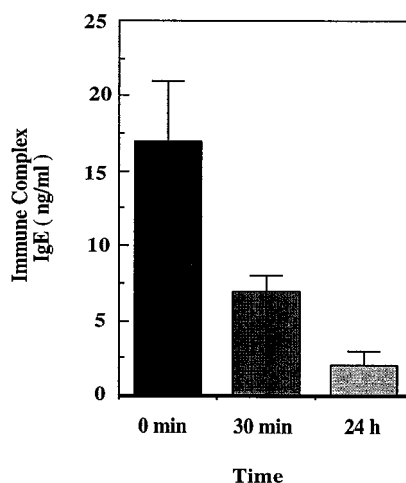


FIGURE 3. Detection of IgE-OVA immune complexes in BAL fluid from mice challenged with nebulized OVA. OVA-immunized BALB/c mice were challenged with nebulized OVA six times as described in Fig. 1. BAL fluids were obtained immediately (0 min), 30 min, or 24 h after the last challenge. The amounts of the IgE-OVA complexes were determined as described in *Materials and Methods* and are expressed as the quantity (nanograms per milliliter) of IgE in the complexes. Data are presented as mean $\pm$ SEM, from three mice.

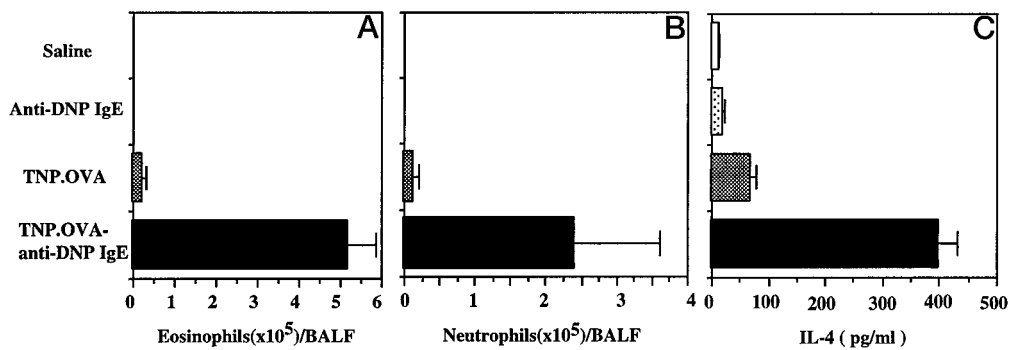


FIGURE 4. Comparison of airway inflammation induced by IgE immune complexes and Ag alone. OVA-immunized BALB/c were challenged with either 2 μ g TNP-OVA alone or 20 μ g anti-DNP IgE three times intranasally. The BAL fluid was collected 3 h after last challenge, and the numbers of leukocytes and levels of IL-4 were determined as described in *Materials and Methods*. Each data point represents mean $\pm$ SEM of data from three mice, and the results are representative of five separate experiments. The *p* values of TNP-OVA vs TNP-OVA-anti-DNP IgE in eosinophil (A), neutrophil (B), and IL-4 (C) determinations are all <0.001 .

complexes. For this purpose, we used TNP-OVA as the immunogen, which can form immune complexes with a previously well-characterized mouse monoclonal anti-DNP IgE Ab. To conserve the amount of IgE to be used, we administered the Ag or the immune complexes intranasally, rather than giving them in an aerosolized form. First, we established that OVA-alum-immunized mice developed similar levels of airway inflammation (cellular infiltration and IL-4 levels) on intranasal challenge with either OVA or TNP-OVA (data not shown). We then compared airway inflammation induced by intranasally administered TNP-OVA and IgE-TNP-OVA, in mice previously immunized with OVA. As shown in Fig. 4, the BAL fluid from mice challenged with IgE-TNP-OVA contained significantly higher numbers of eosinophils (Fig. 4A) and higher levels of IL-4 (Fig. 4C), as compared with that obtained from mice challenged with TNP-OVA alone. In addition, BAL fluid from mice challenged with IgE immune complexes contained significantly higher numbers of neutrophils as compared with those from mice challenged with the Ag alone (Fig. 4B). To ascertain that it was the presence of immune complexes rather than the mere addition of IgE that contributed to the augmented inflammatory response, we also challenged mice with TNP-OVA plus ragweed Ag-specific monoclonal IgE. As shown in Fig. 5, only

TNP-OVA mixed with anti-TNP IgE, and not with anti-ragweed Ag IgE, induced heightened inflammatory responses.

IgE-Ag complexes and Ag produce comparable airway inflammatory responses in Fc ϵ RI-deficient mice

To better understand the mechanisms by which the IgE-Ag complexes affect heightened airway inflammatory responses over the Ag alone, we compared the responses induced by intranasal administration of TNP-OVA-IgE complexes vs TNP-OVA in OVA-sensitized Fc ϵ RI(–/–) mice. As a control, we used a mouse strain that is also deficient in the murine Fc ϵ RI- α chain but expresses the human Fc ϵ RI- α chain (mast cells in these mice contain functional Fc ϵ RI, that recognizes endogenous murine IgE). As shown in Fig. 6, the BAL fluid IL-4 levels in Fc ϵ RI(–/–) mice challenged with either IgE immune complexes or Ag alone were similar. In contrast, in Tg1101 mice, the immune complexes induced higher levels of IL-4 than Ag alone.

Discussion

The studies presented herein demonstrate that: 1) significant amounts of both free IgE as well as IgE immune complexes are

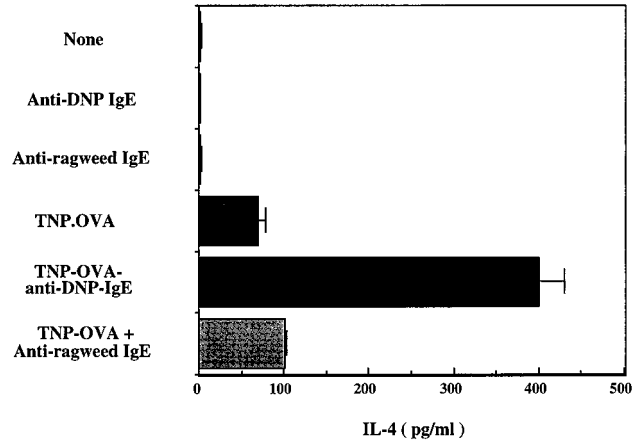


FIGURE 5. Comparison of airway inflammation induced by TNP-OVA in combination with anti-DNP IgE or anti-ragweed IgE. Mice immunized with OVA were challenged 14 days later intranasally with 2 or 4 μ g TNP-OVA mixed with 20 μ g anti-DNP IgE or TNP-OVA mixed with 20 μ g anti-ragweed IgE. The results are mean $\pm$ SD of data from three mice. Similar results were obtained in two separate experiments.

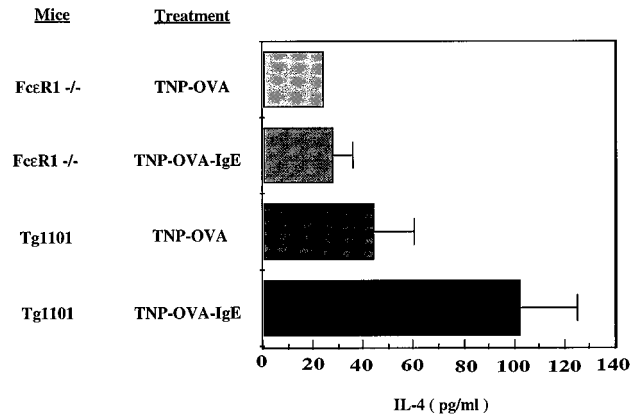


FIGURE 6. Comparison of airway inflammation induced by TNP-OVA-IgE and TNP-OVA in Fc ϵ RI(–/–) mice. Fc ϵ RI(–/–) mice and Tg1101 mice, which express functional human Fc ϵ RI in the place of murine Fc ϵ RI, were immunized with OVA in alum. After 14 days, they were challenged with TNP-OVA alone or TNP-OVA-IgE immune complexes intranasally for 3 consecutive days. Three hours after the last challenge, BAL fluid was collected from each mouse, and IL-4 levels were determined by ELISA. The data are average values obtained from three mice $\pm$ SEM. Similar results were obtained in two independent experiments.

present in airway secretions of mice that are sensitized to OVA in alum i.p. and later challenged with the same Ag in a nebulized form through the airways; 2) the IgE persists in the airway secretions for >15 days after termination of the Ag exposure; 3) intranasally administered TNP-OVA-IgE immune complexes induce significantly more pronounced airway inflammation over that induced by the Ag (TNP-OVA) alone, as evidenced by the higher levels of IL-4 and larger numbers of eosinophils in the BAL fluid; and 4) the higher potency of the IgE immune complexes over the Ag is dependent on FcεRI, given that it was not observed in FcεRI-deficient mice. In addition, airway inflammation induced by the IgE immune complexes differs qualitatively from that induced by the Ag alone in that significant numbers of neutrophils are detected in BAL fluid from mice treated with the IgE immune complexes. The detection of IgE in the BAL fluid in the mouse model used in this study is consistent with the fact that the airway inflammation induced in this model is a Th2 response, as amply documented by other investigators studying the model. The mouse model of airway inflammation has many features observed in human allergic airway inflammation. The accumulation of IgE in airways reported here represents yet another, because IgE is also detected in airway secretions present in human asthmatics (5).

There are several possible explanations for the presence of IgE in the airway secretions. It has been reported that in mice subjected to airway Ag challenge, germinal centers are developed within the parenchyma of the inflamed lung, and plasma cells producing Ag-specific Igs, including IgE, are present (28). These IgE-secreting plasma cells could migrate across the airway epithelia, together with other inflammatory cells, and then gather in the secreted fluid. Alternatively, IgE secreted by plasma cells present in the lung parenchyma may diffuse through the interstitial space and the epithelial barrier and eventually accumulate in the secreted fluid. Finally, it is also possible that the circulating IgE, which is elevated in these mice, is the source for some of the IgE found in the BAL fluid entering the airways via increased vascular permeability that occurs during inflammation.

There are also several possible mechanisms for the enhanced response induced by Ag-IgE complexes as compared with Ag alone. First, it may be mediated through type II IgE receptor (FcεRII). This receptor is expressed by APC and shown to have a role in Ag presentation (29–31). Thus, the IgE immune complexes could be more effectively captured through this receptor. Also, alveolar macrophages express FcεRII, which has been shown to mediate the production of cytokines and chemokines (32). Thus, IgE immune complexes may be more effective than the Ag in activating these cells via this receptor.

The possible involvement of FcεRI is obvious. First, the IgE immune complexes are more effective in inducing mast cell activation through cross-linking of FcεRI, whereas Ag can cross-link only the receptor occupied by IgE specific for the given Ag. Augmented mast cell activation may directly contribute to the more pronounced inflammatory responses that are observed, or indirectly contribute to the augmented inflammatory responses through the cytokines or chemokines that they produce. In this context, it has been shown that IgE can up-regulate mouse mast cell FcεRI (33). This in turn makes the mast cells more responsive to IgE-Ag immune complexes, perhaps passing the critical threshold for mast cells to release cytokines such as IL-4. The IL-4 released by mast cells can in turn up-regulate IgE synthesis by B cells in bronchus-associated lung tissue (BALT) areas of lung tissue independent of T cells (34). These events can lead to a domino effect where more IgE produced by B cells may become involved in forming more

immune complexes resulting in further exacerbation of airway inflammation. Second, FcεRI is expressed by some types of APC, including monocytes (35), dendritic cells (36), and alveolar macrophages (37), and the IgE immune complexes may be more readily captured by these cells and processed for Ag presentation. Our results with FcεRI-deficient mice indeed support a critical role for FcεRI in the augmented responses induced by IgE immune complexes.

The observation that BAL fluid from sensitized mice challenged with IgE immune complexes intranasally contains significant numbers of neutrophils is noteworthy. Because anti-DNP-IgE or TNP-OVA alone fails to induce an influx of neutrophils into airways, a simple explanation such as the presence of endotoxin in either preparation can be ruled out. It has been shown recently that mast cell protease 6 is effective in attracting neutrophils into the peritoneal cavity of mice injected with this protease i.p., probably through induction of an IL-8-like cytokine production by endothelial cells (38). Therefore, our finding can be explained at least in part by the IgE immune complexes being more potent in activation of mast cells. The heightened neutrophil response could also be due to other mediators from mast cells or other inflammatory cells, triggered by IgE immune complexes. Detection of neutrophils in BAL fluid has clinical relevance, because neutrophils are frequently present in nasal secretions or BAL fluid from patients with allergic rhinitis or asthma (39, 40).

The significance of our findings, i.e., the presence of IgE in airway secretions and IgE immune complexes as potent inducers of airway inflammation, must be discussed in the context of repeated Ag exposure. It has recently been reported that in mice primarily sensitized with OVA and subsequently challenged with the same Ag through the airways, a reexposure to OVA 1 wk after the first airway challenge results in an augmented allergic response (41). Although various factors could contribute to the augmented response, considering our finding that IgE persists in the airways for >2 wk after last Ag exposure, it is possible that IgE immune complexes, which are formed in the airways when the mice are reexposed to the Ag, contribute to the potentiated secondary response.

IgE is believed to play an important role in human allergic inflammation. However, the role of IgE in the mouse model of allergic airway inflammation has not been definitively established. It has been shown that treatment of mice with nonanaphylactic anti-IgE Ab results in an attenuated response to airway challenge, supporting a contributory role of IgE in airway inflammation in this mouse model (42). On the other hand, IgE-deficient mice generated by gene targeting showed eosinophilia and airway hyperresponsiveness after airway challenge that are comparable to the wild-type mice (43). It is possible that several pathways are involved in the overall response observed in the mouse model of allergic airway inflammation and that IgE is involved in one or more of these pathways. Genetically IgE-deficient mice might be able to mount a full allergic response comparable to that found in the wild-type mice through IgE-independent pathways only. Also relevant to the present discussion is the role of mast cells in allergic airway inflammation. Based on their localization in airway tissues and their production of various potent inflammatory mediators, mast cells are believed to play an important role in allergic airway inflammation (44). However, there are studies that show that mast cell-deficient mice develop airway inflammation comparable to their normal congenic mice upon airway Ag challenge (45), although other studies found that mast cell-deficient mice developed significantly reduced eosinophilia in the airways as compared with the normal congenic mice (46). Again, the results demonstrate the complexity and redundancy in the mechanisms

responsible for airway inflammatory responses and the relative contribution of mast cell-dependent pathway may be dependent on the procedures used to induce airway inflammation (47).

In summary, our data suggest that IgE immune complex-mediated enhanced airway inflammation in the mouse model is likely a result of a series of events, involving, among other possible mechanisms, lung mast cell activation, and provide new evidence for a central role for IgE and mast cells in airway inflammation. Our findings should be relevant to chronic allergic inflammation in humans repeatedly exposed to allergen. In allergic human individuals, in which allergen-specific IgE Abs may be present in airway secretions, IgE immune complexes could be formed on exposure to the specific allergens, which could contribute to augmented allergic responses. Thus, allergen-specific IgE present in the airway secretions may facilitate the perpetuation of allergic airway inflammation.

Acknowledgments

We thank Dr. Wai-Ping Fung-Leung of R.W. Johnson Pharmaceutical Research Institute for providing FcεRI(−/−) and Tg1101 mice, Dr. Jian Gong for preparation of TNP-OVA and anti-DNP IgE, Lan Yu for maintaining the transgenic mice, Ellenie Tuazon for differential cell counts, Dr. John Nedrud at Case Western Reserve University for advice on intranasal administration of proteins, Dr. J. Ohara for providing purified IL-4, Dr. Bruce Zuraw for reviewing the manuscript, and Jane Gentry for assistance in preparation of the manuscript.

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