

Antigen-Specific Inhibition of IgE Binding to the High-Affinity Receptor¹

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Since the beginning of this century, allergen immunotherapy has been widely used to treat allergic disorders. In addition to the long-term clinical efficacy of this therapy, there are immediate beneficial effects as observed in rush immunotherapy for which there is no clear mechanism. We investigated the direct impact of an Ag on its specific IgE in terms of IgE measurability in immunoassays and subsequent binding of IgE to the high-affinity receptor for IgE. As a model we used a chimeric IgE specific for NIP that exhibits similar biologic properties as serum or myeloma IgE. To mimic particulate and soluble allergens we coupled 15 NIP molecules to BSA. Using this "artificial allergen" we could show that the presence of the Ag reduced the IgE measurability in immune assays. Furthermore, IgE binding to the FcεRI was 60% inhibited in the presence of the Ag shown with an optical biosensor that monitors molecular interactions. In normal basophils passively sensitized with IgE that was preincubated with the Ag, no sulfidoleukotriene release could be induced. Rush immunotherapy may invoke a similar phenomenon, resulting in the short-term alteration of symptoms by blocking or substantially reducing binding of IgE to its high-affinity receptor. Thus, our result may explain some of the short-term beneficial effects observed in rush immunotherapy. *The Journal of Immunology*, 1995, 155: 2948–2954.

Although IgE represents the least frequent of all the Igs, particular interest has been focused on its regulation and biologic properties due to its key role in allergic disorders (1, 2). Different approaches have been chosen to treat allergy, primarily to interfere with the activity of allergic mediators such as histamine or leukotrienes or to modulate IgE synthesis by cytokines (3) or Abs either directed against cytokines (4) or IgE (5, 6). The most common treatment for allergic disorders, namely allergen immunotherapy, was first described in the early decade of this century (7).

In the last 25 years several placebo controlled trials (8) showed that allergen immunotherapy is clinically effective providing that appropriate extracts are used. Although, a variety of clinical changes have been described in the course of allergen immunotherapy (9), little is known how immunotherapy causes its beneficial effects. On the other

hand several mechanisms have been postulated by which allergen immunotherapy exerts its symptom amelioration in allergic disorders. The concept of blocking Abs argued that allergen immunotherapy induced protective IgG Abs (10). Special attention has been devoted to IgG subclasses, namely IgG1 and IgG4. However, the results were controversial. Several groups claimed that an increase in IgG4 is associated with improvement (11–14), others associated an increase with failure of allergen immunotherapy (15). Some even claimed that the increase in IgG does not necessarily relate to the mechanism of allergen immunotherapy but reflects the expected immune response to repeated immunization with an Ag (16). Finally, Jarolim et al. (17) showed that changes of Ab patterns in immunoblot do not relate to the success or failure of allergen immunotherapy. Alternatively, a shift in the cytokine pattern produced by T cells was postulated to be the cause for the beneficial effects of allergen immunotherapy (18, 19). Tolerance induction (20) and the shift in cytokine pattern could explain late phase changes in allergen immunotherapy, e.g., attenuation of allergen-induced CD4⁺ T cell and eosinophil recruitment or attenuation of late-phase response in skin, nose, and asthma. "Protective Abs" and a shift in cytokine pattern would exert their beneficial effect only in the long-term range. However, both hypotheses fail to explain the immediate improvement observed in rush allergen immunotherapy.

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Our aim was to investigate the effect of a given Ag binding to its specific IgE. As a model we used a hapten-specific IgE (21). We could show that the Ag interfered with the measurement of specific IgE in immunodot assays. This inhibition was Ag specific and dose dependent. More importantly, less hapten-specific IgE was bound to the FcεRI in the presence of an excess of the Ag. Furthermore, in cells derived from dextran sedimented whole blood, no sLT release could be induced if cells were passively sensitized in the presence of the Ag. Thus, our findings suggest a direct effect of the Ag on specific IgE being eventually responsible for the short-term beneficial effects of allergen immunotherapy.

Materials and Methods

Cell lines

IgE-producing cell lines U266 and JW8 (21) were maintained in RPMI 1640 (Seromed, Basel, Switzerland) supplemented with 10% FCS (FCS), 2 mM glutamine and 100 U/ml penicillin-streptomycin at 37°C in 5% CO₂.

Abs, Ag, and recombinant FcεRI α-chain

The following mouse monoclonal anti-human IgE Abs were used in this study: Le27 and BSW17 generated in our laboratory (22), 11-5 was obtained from Dr. R. Vasilov, NPO Biotechnologia, Moscow, Russia; 4F4 was obtained from Dr. M. P. Samoilovich and Dr. V. B. Klimovich, Hybridoma Technology Lab., Central Research Institute for Roentgenoradiology, St. Petersburg, Russia; IT was purchased from Immunotech (Marseille, France). All mouse monoclonal anti-IgE Abs were domain mapped using recombinant IgE domains as published recently (23). The pentaglobulin preparation was purchased from Biotest, Othmarsingen, Switzerland.

The following IgE were used in this study: chimeric hapten-specific IgE (JW8) (21) and human myeloma IgE (ND). Both Abs were purified from cell supernatants by affinity chromatography using an anti-IgE mAb (Le27) bound to affinity adsorbents as described by the supplier (Boehringer Mannheim GmbH, Mannheim, Germany).

Recombinant FcεRI α-chain expressed in a baculo virus system was kindly provided by Dr. H. Kocher, Sandoz AG, Basel, Switzerland. NIP₁₅-BSA (*M_r* 70,000) was prepared in our laboratory according to a standard protocol (24). 8 mg of 4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP) (Sigma Chemical Co., Buchs, Switzerland) were preactivated with 3 mg *N*-hydroxysuccinimide (NHS) (Fluka Chemie AG, Buchs, Switzerland) and 5 mg *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide (Fluka Chemie AG, Buchs, Switzerland) dissolved in 0.3 ml *N,N*-dimethylformamide for 2 h at room temperature to form a succinimide ester. The activated NIP was coupled to 60 mg BSA in 3 ml 0.05 M borate-buffer for 18 h at room temperature. The reaction mix was purified by chromatography using a Biogel P-60 column (Bio-Rad, Zürich, Switzerland). The grade of substitution was determined by the extinction at 436 nm.

IgE measurement by immunodot assay

IgE determinations were conducted in standard immunodot assays (25). Briefly, 1 μl of 500 μg/ml anti-IgE mAb was dotted on nitrocellulose (Milipore Corp., Bedford, MA). The nitrocellulose strips were then blocked with TBS containing 1.5 mg/ml casein (Fluka Chemie AG) and incubated overnight with either hapten-specific IgE or control IgE (ND). The strips were washed three times with TBS and incubated 6 h with horseradish peroxidase (HRP)-conjugated anti-IgE mAb or sheep anti-human IgE Ab (The Binding Site Ltd, Birmingham, U.K.) diluted 1:1000 in TBS containing 1.5 mg/ml casein. After washing three times with TBS the strips were developed with 4-chloro-1-naphthol and read with a hand-held reflection densitometer (Gretag Limited, Regensburg, Switzerland).

Binding of IgE to FcεRI α-chain (IASys cuvette system)

One hundred microliters of 20 μg/ml recombinant FcεRI α-chain in 10 mM sodium acetate buffer pH 4 was covalently coupled to the dextran matrix of an IASys cuvette according to the manufacturer's protocol (Fisons Applied Sensor Technology, Cambridge, U.K.).

Binding studies were performed at 25°C. The IASys cuvette was regenerated with 20 mM HCl. Analysis of association and dissociation was performed using FASTfit, a data analysis software included in the IASys system.

Ultracentrifugation

JW8-IgE and NIP-BSA or their respective immune complexes were subjected to gradient centrifugation. Four hundred microliters of each preparation were layered on 7 ml of 10 to 40% (W/W) sucrose gradient in polycarbonate thickwall tubes (Kontron Instruments, Zürich, Switzerland) and spun at 100,000 × *g* using a TST 41-14 swing-out rotor (Kontron) in a T-1170 ultracentrifuge (Kontron) at 4°C for 16 h. After centrifugation fractions were collected from the bottom of the tubes. One microliter of each fraction was dotted onto nitrocellulose. After blocking with TBS containing 1.5 mg/ml casein (Fluka Chemie AG), dots were either revealed with alkaline phosphatase-conjugated sheep anti-human IgM or IgG Ab (The Binding Site Ltd.), HRP-conjugated anti-IgE Ab (The Binding Site Ltd.) or indirectly with hapten-specific IgE (JW8-IgE) and HRP-labeled anti-IgE Ab, respectively. After washing three times with TBS, alkaline phosphatase-conjugated Abs were developed with naphthol AS-MX as described elsewhere (26). HRP-conjugated Abs were sequentially developed using enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, England) according to the manufacturer's protocol and 4-chloro-1-naphthol and read with a hand-held reflection densitometer.

Measurement of sulfidoleukotriene synthesis

Newly formed sulfidoleukotrienes (LTC₄, LTD₄ and LTE₄) were measured in cell supernatants using the commercial CAST ELISA (CAST ELISA, EK-SLT, Bühlmann Laboratories AG, Allschwil, Switzerland) after Ag (NIP-BSA) or anti-IgE mAb challenge.

Cells derived from dextran-sedimented whole blood were passively sensitized with hapten-specific IgE (JW8) or control IgE (ND) for 90 min at 37°C. Subsequently, cells were challenged with Ag (NIP-BSA) or anti-IgE mAb in the presence of IL-3 without further washing. After incubation for 40 min at 37°C cell supernatants were harvested and sulfidoleukotriene (sLT) synthesis was assayed. Cell number and concentration of IL-3 used in the assay were according to the manufacturer's protocol.

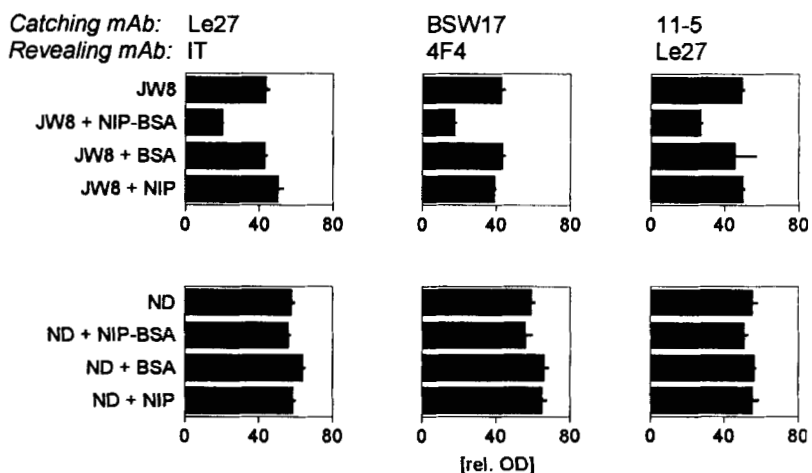
Results

Ag interferes with IgE detection

To investigate whether an Ag interferes with the determination of specific IgE we used as a model a hapten-specific chimeric IgE produced by a cloned pSV-V_{NP}He transfectant of J558L (JW8) (21). The NIP-specific JW8-IgE (100 U/ml) was incubated either with 10 μg/ml NIP coupled to BSA (15 NIP residues per BSA molecule, m.w. 70 kDa) as the Ag, with 10 μg/ml carrier protein (BSA, m.w. 66 kDa) or with 1 μg/ml hapten (NIP, m.w. 323 D) at 37°C overnight. Ag and carrier protein were used at a 100 times molar excess compared with JW8-IgE, whereas the hapten was used at a 2500 times molar excess compared with JW8-IgE. As a control we included a myeloma IgE (ND) (100 U/ml) of unknown specificity.

After overnight incubation these IgE samples containing either Ag, carrier protein or hapten, were measured by immunodot assays using different tandems of monoclonal anti-IgE Abs (6). Figure 1 shows that NIP-BSA reduced

FIGURE 1. Measurement of hapten-specific IgE in the presence of Ag. Hapten-specific IgE (JW8-IgE) (100 U/ml) and control IgE (ND-IgE) (100 U/ml) were incubated with either 10 μ g/ml NIP-BSA, 10 μ g/ml carrier protein, 1 μ g/ml hapten or buffer alone at 37°C overnight. The different IgE samples were subsequently measured with three different tandems of catching/revealing anti-IgE mAb (Le27/IT, BSW17/4F4 and 11-5/Le27). Values displayed represent mean and SD of duplicate measurements.



the measurable amount of JW8-IgE by three different tandems of monoclonal anti-IgE Abs. Average reduction was 50% compared with JW8-IgE alone. The observed inhibition was Ag specific, as neither the carrier protein nor the hapten interfered with IgE detection. Furthermore, inhibition was not due to the fine specificity of the anti-IgE mAb used since NIP-BSA interfered also with JW8-IgE detection in 12 other tandems based on 6 different anti-IgE mAb (data not shown). NIP-BSA had no influence on the measurement of control IgE (ND). Therefore, the observed signal reduction with JW8-IgE was not due to a nonspecific interaction of NIP-BSA with the constant ϵ region of the IgE molecule.

Ag-specific interference with IgE determination is dose dependent

The observed inhibition of IgE determination was achieved with an excess of Ag. Thus, we investigated whether this reduction could also be observed in the equimolar range of hapten-specific IgE and Ag. Different concentrations of NIP-BSA were incubated with either hapten-specific IgE (JW8) (100 U/ml) or the control IgE (ND) (100 U/ml) at 37°C overnight. The concentrations of NIP-BSA ranged from 10 μ g/ml to 0.01 μ g/ml. It should be noted that 10 μ g/ml NIP-BSA corresponds to approximately a 100 times molar Ag excess, whereas 0.01 μ g/ml NIP-BSA corresponded to a hundred times molar IgE excess. Figure 2 shows that inhibition of the immunodot assay was observed in the range of 10 μ g/ml to 0.01 μ g/ml NIP-BSA. Similarly, we used tandems purely based on monoclonal anti-IgE Abs (seven tandems based on four different anti-IgE mAbs) where we observed a comparable reduction in IgE measurability (data not shown). Concerning the actual binding sites, 0.01 μ g/ml NIP-BSA correspond to 0.75 NIP per IgE binding sites; thus, our data show that at least one NIP per binding site was required for reduction of IgE measurability. Control IgE (ND) showed no reduced measurability in the presence of NIP-BSA, confirming that the observed inhibition was Ag specific.

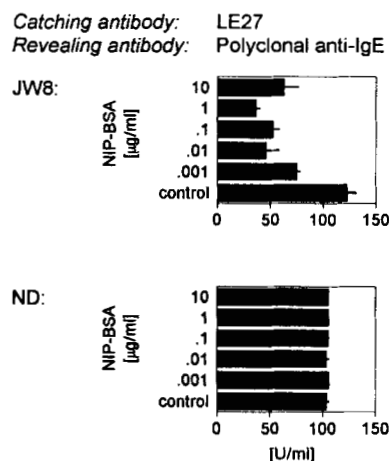
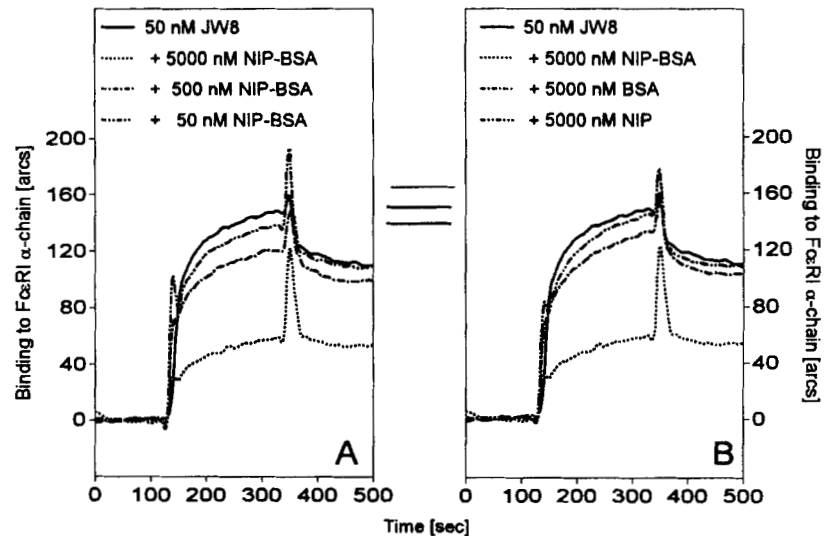


FIGURE 2. Dose-dependent inhibition of hapten-specific IgE measurement by Ag. Hapten-specific IgE (JW8-IgE) (100 U/ml) and control IgE (ND-IgE) (100 U/ml) were incubated with different concentrations of NIP-BSA ranging from 10 to 0.001 μ g/ml NIP-BSA at 37°C overnight. These IgE samples were then measured with two different tandems of catching/revealing anti-IgE Ab, namely Le27/polyclonal anti-IgE and 11-3/polyclonal IgE. Values displayed represent mean and SD of duplicate measurements.

Binding of specific IgE to immobilized Fc ϵ RI α -chain in presence of Ag

Next, we addressed the question whether Ag might also interfere with the binding of IgE to the high-affinity receptor for IgE (Fc ϵ RI). For this purpose we used the IAsys system, an optical biosensor that allows monitoring of biospecific molecular interactions without the need for labeling of ligands or agonists. Recombinant Fc ϵ RI α -chain was covalently linked to the dextran matrix of the IAsys cuvette. Binding of IgE was monitored by the change in refractive index in the boundary layer that is detected by the IAsys as a change in the resonance angle of incident light.

FIGURE 3. Binding of hapten-specific IgE (JW8-IgE) to immobilized FcεRI α-chain in presence of NIP-BSA in the IA-sys cuvette system. JW8 was incubated with different concentrations of NIP-BSA, with carrier protein and hapten alone over night at 37°C before addition to the measuring device. *A* shows binding of JW8-IgE to the FcεRI α-chain (solid line) or in presence 5000, 500 or 50 nM NIP-BSA as indicated in the figure legend. *B* shows binding of JW8-IgE to the FcεRI α-chain (solid line) or in presence of 5000 nM NIP-BSA, 5000 nM BSA or 5000 nM NIP as indicated in the figure legend.



To assess binding of hapten-specific IgE in the presence of Ag, 50 nM JW8-IgE was incubated with different concentration of NIP-BSA ranging from 5000 nM to 50 nM at 37°C overnight. Figure 3*A* shows that binding of JW8-IgE to the FcεRI α-chain was dose-dependently inhibited by NIP-BSA. At a 100 times molar excess of NIP-BSA we observed a 60% inhibition of JW8-IgE binding to the FcεRI α-chain. Although reduced binding was observed, neither the association rate ($\sim 0.0165 \text{ s}^{-1}$) nor the dissociation rate ($\sim 0.0261 \text{ s}^{-1}$) were altered as determined from the FASTfit program provided by the IA-sys. If NIP-BSA and JW8-IgE were added to the IA-sys cuvette without preincubation, a 40% inhibition of binding was observed (data not shown). Again inhibition was Ag specific, as neither the hapten alone nor the carrier protein by itself influenced binding to the FcεRI α-chain (Fig. 3*B*). Since optical biosensors monitor binding directly as it happens, the observed reduced binding is due only to the interaction between the hapten-specific IgE and the Ag.

Size estimation of JW8-IgE/NIP-BSA complexes by ultracentrifugation

Next, we addressed the question whether the observed reduced binding of hapten-specific IgE (JW8-IgE) in presence of the Ag to anti-IgE Ab and FcεRI α-chain was due to the formation of large JW8-IgE/NIP-BSA aggregates.

Fifty nanomoles JW8-IgE plus 5000 nM NIP-BSA and controls 50 nM JW8-IgE, 5000 nM NIP-BSA were incubated at 37°C overnight. The immune complexes so formed were analyzed by ultracentrifugation on a sucrose gradient. As a standard we included a pentaglobulin preparation giving the relative position of pentameric IgM (M_r 900 kDa) and IgG (M_r 150 kDa). Figure 4 shows that the JW8-IgE/NIP-BSA complexes are significantly smaller than pentameric IgM. Thus, the JW8-IgE/NIP-BSA complexes have a m.w. smaller than 900 kDa. NIP-BSA exists as monomers, dimers, and

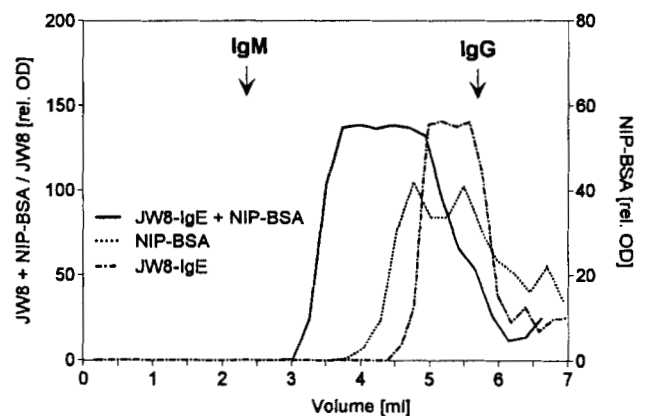


FIGURE 4. Size estimation of newly formed immune complexes. After overnight incubation at 37°C, 400 μ l of 50 nM JW8-IgE plus 5000 nM NIP-BSA, 50 nM JW8 or 5000 nM NIP-BSA were fractionated by ultracentrifugation on sucrose gradients. Arrows indicate peak positions of pentameric IgM and monomeric IgG as described in *Materials and Methods*.

trimers judged on the relative position to IgG, whereas IgE exists as monomers. Neither the carrier protein (BSA) nor the hapten (NIP) on its own produced a shift in the m.w. when incubated with JW8-IgE (data not shown).

Passive sensitization of basophils with Ag-specific IgE

It has been shown that JW8-IgE can be used to passively sensitize human basophils (21). Figure 5 shows that NIP-BSA induced a strong sulfidoleukotriene (sLT) release in cells derived from dextran-sedimented whole blood after passive sensitization with JW8-IgE. The release was NIP-BSA specific, as neither the carrier protein (BSA) nor the hapten (NIP) induced sLT release. Furthermore, untreated control cells or cells passively sensitized with a myeloma

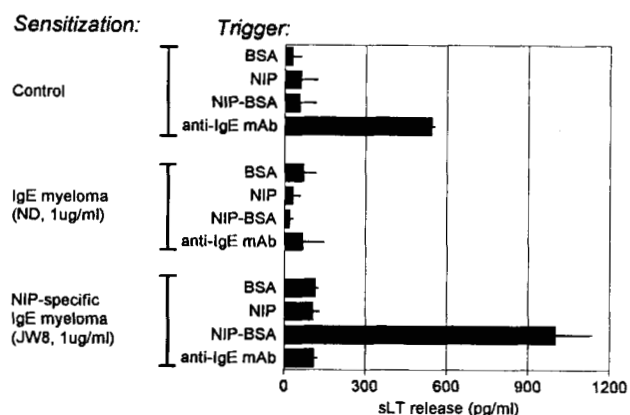


FIGURE 5. Passive sensitization of human basophils with hapten-specific IgE. Dextran-sedimented whole blood was passively sensitized with 1 μ g/ml JW8-IgE or 1 μ g/ml ND-IgE for 90 min. Without washing, cells were triggered with either 100 ng/ml NIP-BSA, 100 ng/ml BSA, 100 ng/ml NIP, or 100 ng/ml Le27. Sulfidoleukotriene release was assayed with the commercial CAST ELISA. Values displayed represent mean and SD of duplicate.

IgE (ND) of unknown specificity showed no sLT release after NIP-BSA challenge.

As an internal control for the CAST ELISA, we used an anti-IgE mAb (Le27). Le27 (0.1 μ g/ml) induced a strong sLT release in the untreated control cells indicating that the basophils were sensitized *in vivo* as expected. However, in cells additionally sensitized with JW8-IgE or ND-IgE (1 μ g/ml), no sLT release was induced by the same anti-IgE mAb (0.1 μ g/ml) unless lower concentrations of IgE (0.1 μ g/ml or 0.01 μ g/ml) were used for passive sensitization (data not shown). This implies that a ten times excess of added IgE in relation to added anti-IgE is capable of absorbing the triggering anti-IgE mAb.

Passive sensitization of basophils with Ag-specific IgE is inhibited by Ag

Next, we intended to investigate whether the observed reduced binding of JW8-IgE to Fc ϵ RI in presence of its Ag has an impact on Ag mediated sulfidoleukotriene release from human basophils. For this purpose, we preincubated 0.3 μ g/ml hapten-specific IgE (JW8-IgE) with different concentrations of NIP-BSA at 37°C for 4h. The concentrations of NIP-BSA ranged from 3 μ g/ml to 0.03 μ g/ml, whereby 3 μ g/ml NIP-BSA corresponds to approximately a 25 times molar Ag excess, whereas 0.03 μ g/ml NIP-BSA corresponded to a 4 times molar IgE excess. These JW8-IgE samples containing different concentrations of NIP-BSA were then added to cells derived from dextran-sedimented whole blood. Figure 6 shows that hapten-specific IgE at all JW8-IgE/NIP-BSA ratios tested was unable to directly induce sLT release, suggesting that even though IgE/Ag complexes were generated they were not anaphylactogenic. A subsequent stimulus with NIP-BSA could

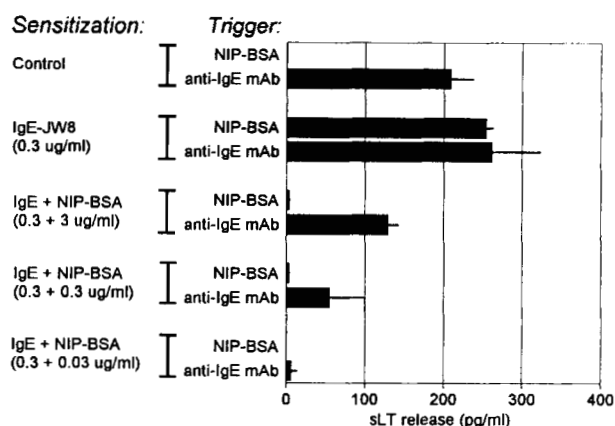


FIGURE 6. Passive sensitization of human basophils with hapten-specific IgE in the presence of Ag. Dextran-sedimented whole blood was passively sensitized with 0.3 μ g/ml JW8 containing different concentrations of NIP-BSA. Cells were triggered either with 10 ng/ml NIP-BSA or 300 ng/ml Le27. Sulfidoleukotrienes were assayed using the commercial CAST ELISA. Values displayed represent mean and SD of duplicate.

not induce sLT release. Furthermore, an anti-IgE mAb (0.3 μ g/ml) induced sLT release in untreated control cells and cells passively sensitized with JW8-IgE. Cells passively sensitized using JW8-IgE preincubated with different concentrations of NIP-BSA showed reduced sLT release after a subsequent trigger with anti-IgE mAb. Inhibition was dose dependent, since at the highest NIP-BSA concentration used, sLT release was approximately 40% inhibited, whereas at the lowest concentration of NIP-BSA tested complete abrogation of sLT release was observed.

Discussion

Most of the current hypotheses on the mechanism of allergen-induced allergen immunotherapy fail to explain the short-term beneficial effects as observed in rush immunotherapy. Therefore, we intended to investigate possible direct effects of the allergen on the biologic activity of specific IgE. Since allergen-specific IgE in pure form is not readily available, we used as a model a chimeric IgE specific for the hapten NIP (JW8-IgE). It was shown by Neuberger et al. (21) that JW8-IgE has similar biologic properties as serum derived IgE or myeloma IgE.

Since multiple and identical antigenic determinants are present on particulate allergens, we covalently linked approximately 15 NIP molecules to a carrier protein (BSA). Thereby, we created an "artificial allergen" with multiple antigenic determinants. A soluble allergen may not display multiple identical epitopes, but several different antigenic determinants. Since in serum there are IgE molecules of different specificities resulting in potentially more than one IgE molecule binding to a soluble allergen. Thus, our "artificial allergen" also represents a model for soluble allergen, as more than one IgE molecule can bind. Furthermore, as

shown by the size estimation in Figure 4, both the coupling procedure and storage produced NIP-BSA dimers and trimers. Thus, NIP-BSA can bind several JW8-IgE molecules on the surface similar to natural IgE where multiple IgE of different allergen-epitope specificities can bind to the allergen. Therefore, NIP-BSA represents an interesting tool to investigate direct specific interactions between the IgE molecule and its Ag.

We were interested to study the effect of Ag on IgE measurability because it is well-documented that certain types of allergens enter the immune system (27) and must interact with Abs. Indeed, we could show that the presence of Ag interfered with the measurement of hapten-specific IgE in immunoassays. Regarding the number of antigenic determinants of the Ag and binding sites of the IgE molecule, we observed inhibition from a 750 times excess of NIP to one NIP molecule per IgE binding site. The significant inhibition observed in the immunoassays was not inherent to the assay reagents used, as the same results were found using different tandems of monoclonal anti-IgE Ab or polyclonal anti-IgE Ab. Thus, we show that the measurement of purified hapten-specific IgE is disturbed by the presence of Ag. Whether this observation holds true for naturally occurring IgE can only be speculated. Clearly, serum levels of allergens are most likely to be low—or even absent—to interfere with IgE detection except in the case of food allergens and eventually certain contact allergens or professional allergens that might be taken up in larger quantities. Thus, one might speculate that the relative difficulty for IgE determination in food allergy could eventually be related to a similar mechanism as observed with our “artificial allergen.”

Malling et al. (28, 29) reported that in the course of allergen immunotherapy levels of specific serum IgE were increased whereas cell bound specific IgE did not alter. These authors speculated that allergen immunotherapy may favor the production of IgE with low affinity to the FcεRI. Our results clearly demonstrate that hapten-specific IgE showed a reduced binding to the FcεRI α-chain in the presence of its Ag. This was very clearly demonstrated using the IAsys system where FcεRI α-chain was coupled to the solid phase. The IAsys system represents a direct approach where bimolecular interactions are detected without the need for revealing bound IgE. Thus, based on our results, the allergen alone may alter the binding capacity of IgE without interfering with IgE regulation as has been proposed in the past (29). Our data might also represent an explanation for the immediate and beneficial effects observed during allergen immunotherapy.

One of the obvious questions to be solved remains whether the reduced measurability of IgE and the reduced binding capacity of IgE to FcεRI were due to the formation of immune complexes or to a conformational change in the IgE molecule. Evidence for immune complexes arises from the fact that detection of IgE was reduced with all anti-IgE mAb tested, implying that the corresponding epitopes were hidden

within immune complexes. The same reasoning applies to the binding of IgE to FcεRI. However, analysis of the formed immune complexes by ultracentrifugation on sucrose gradients revealed that the size of the newly formed immune complexes were in a size range that is composed of maximally four IgE molecules and one monomeric NIP-BSA. However, the actual size distribution obtained shows that the mean size of the immune complexes is even smaller. We have shown earlier that IgE can be measured by immunoassays consisting of monoclonal anti-IgE mAb even in the presence of a third interfering anti-IgE mAb (6). Thus, it is not likely that steric hindrance by the NIP-BSA should occur but not for anti-IgE mAb recognizing the IgE molecule in the second, third, or fourth domain. Alternatively, one might speculate that binding of the Ag with either one or both Ag binding sites of the IgE molecule induces or stabilizes an IgE conformation that is unfavorable for binding to FcεRI. This hypothesis would be in line with the observation that IgE bound to the FcεRI is in a bent or “banana shape” form (30). Thus, binding of the Ag by IgE would favor a more planar form, a conformation that may not bind to FcεRI. Which of the two hypotheses may finally apply cannot be decided at present. We know that certain anti-IgE mAb (Lc27 and 4F4) cannot detect heat-denatured (56°C, 240 min.) IgE in a sandwich assay and that some of our anti-IgE mAb (BSW17) only recognize native IgE but do not bind to single recombinant ε domains, suggesting that a conformational change in the IgE molecule leads to a loss of antigenic sites. Thus, both effects, namely hiding of epitopes within immune complexes and loss of epitopes due to a conformational change in the IgE molecule, may contribute to reduced measurability in immune assays and decreased binding to FcεRI. The fact that NIP-BSA by itself is forming to a certain degree dimers and trimers might imply that these polymers may eventually be more active than the monomeric form. Purification of monomeric NIP-BSA could have been used to address the question of whether it could be prevented that polymers are formed again during the *in vitro* assays. Nevertheless, here we report the finding that an Ag can alter the biologic activity of IgE. To reproduce our data with specific IgE from patients and natural allergens, it would be mandatory to use sera from patients with very high specific IgE levels. Since a large proportion of specific IgE is hidden within immune complexes in sera, it is not possible to isolate specific IgE from human sera (31, 32). Thus, we are trying at present to generate allergen-specific hybridomas secreting human IgE. As soon as human allergen-specific IgE becomes available, our experiments can be reproduced with natural allergens. However, the problem will still be that the basophils to be used for such experiments should not have Abs of the same allergen specificity on their surface. Procedures such as stripping surface IgE by low pH (33) are never complete and human basophilic cell lines that express the FcεRI and secrete significant amounts of mediators do not exist. Thus, in the past it was not possible to observe our phenomenon that IgE in immune complexes is no longer capable of sensitizing, because either the reagents

(monoclonal IgE) or target cells with unoccupied receptors were not available.

Interestingly, in the presence of excess IgE, anti-IgE mAb could not trigger sLT release from human basophils. This observation might be explained by absorbance of anti-IgE mAb by IgE. The resulting number of free anti-IgE mAb would be too low to trigger sLT release and the formed IgE/anti-IgE mAb immune complexes are not anaphylactogenic as seen for JW8-IgE and NIP-BSA as well. Thus, if IgE forms similar immune complexes with natural allergens, it can be assumed that soluble IgE by itself is a blocking Ab.

Acknowledgments

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