

Effect of Anti-IgE Antibodies on FcεRI-Bound IgE¹

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Anti-IgE mAbs have always been used to trigger mediator release from basophils or mast cells. Now nonanaphylactogenic anti-human IgE Abs are in clinical evaluation as a therapeutic agent against atopic disease. We have found a mAb that is nonanaphylactogenic but recognizes receptor-bound IgE. Interestingly, the Ab prevents the association of IgE with its receptor if immune complexes were formed between IgE and anti-IgE mAb. This explained the phenomenon that addition of anti-IgE Ab to receptor-bound IgE resulted in a decrease of receptor-bound IgE, because IgE dissociating from the receptor was complexed, altering the thermodynamic balance of receptor-bound vs free IgE. Our data show that there are nonanaphylactogenic Abs that do not directly interfere with the receptor binding site on IgE and are capable of preventing the association of IgE with its high affinity receptor. This unique feature would render such an anti-IgE Ab a possible candidate for immunotherapy of atopy. *The Journal of Immunology*, 1996, 157: 5646–5652.

The potential biologic role of anti-IgE autoantibodies in allergic diseases such as asthma or rhinitis has been debated for a long time (1–5). Circulating IgE molecules are also found as IgE/anti-IgE immune complexes in the serum (4, 6–8), as well as in association with the high affinity receptor FcεRI-bound IgE (6, 9). Similarly, therapeutic anti-IgE Abs were found to produce immune complexes in vivo (10). Such anti-IgE Abs may influence IgE synthesis by eliminating IgE-producing cells (5), presumably due to cross-linking of surface IgE leading to apoptosis (11–13). In addition, anti-IgE Abs can inhibit the binding of IgE to the low affinity receptor FcεRII (CD23) or even remove surface-bound IgE, and may thereby have a regulatory function on the immune system (14, 15).

Naturally occurring Abs or therapeutic anti-IgE Abs have a beneficial effect according to their epitope specificity for IgE (16). Namely, it has been shown that most heterologous anti-IgE Abs are in vitro anaphylactogenic (17), but some Abs that either interfere with the binding site of IgE for the FcεRI, or recognize epitopes nearby the binding region are not anaphylactogenic and may be used therapeutically (18–22).

In this work, we describe a mouse anti-human IgE mAb, BSW17, as a possible candidate for immunotherapy of atopy. BSW17 was found to inhibit IgE synthesis in vitro (13) and was the only anti-IgE mouse mAb from a panel of 15 anti-IgE Abs that was not anaphylactogenic (23–27). Using a transfectant Chinese hamster ovarian (CHO)³ cell line expressing the human α-chain and γ-chain from the high affinity receptor FcεRI (28), we show in

this work that BSW17 nevertheless interacted with receptor-bound IgE. But BSW17 reduced in a time-dependent manner the amount of labeled IgE detectable on the cell surface. This effect was not due to internalization of receptor-bound immune complexes since the same results were obtained using IgE bound to human rFcεRI α-chain in a cellfree assay. Moreover, using an optical biosensor, we were able to demonstrate that BSW17 was not interfering with the binding site, but recognized a distinct epitope within the Fcε C region. The presence of BSW17 did not change the normal dissociation rate of bound IgE from the FcεRI α-chain. However, preformed immune complexes of IgE and BSW17 were no more binding to the high affinity receptor α-chain expressed on CHO cells nor to the recombinant α-chain. Thus, the reduction of cell surface-associated IgE in the presence of BSW17 was due to a shift in the thermodynamic balance of bound vs free IgE toward the free IgE/BSW17 immune complex. This unique feature of recognizing and reducing the amount of receptor-bound IgE without triggering effector cells renders BSW17 a candidate for immunotherapy.

Materials and Methods

Reagents

Rat anti-mouse Ig-conjugated peroxidase (anti-mouse Ig-POX) was purchased from Nordic Immunology (Tilburg, The Netherlands). Goat anti-mouse Ig was obtained from Cappel/Copper Biomedical (Malvern, PA).

Mouse anti-human IgE mAbs

mAbs Le27 and BSW17 are described elsewhere (24). The anti-IgE mAbs 11-1, 11-3, 11-4, and 11-5 were obtained from Dr. R. Vasilov (NPO Biotechnologia, Moscow, Russia), and 4F4, 4F12, 5D4, 2B9, 5B9, 1E10, 4F1, and ICI were obtained from Drs. Samoilovich M. P. and Klimovich V. B. (Hybridoma Technology Lab., Central Research Institute for Roentgenoradiology, St. Petersburg, Russia). mAb IT is a commercial Ab from Immunotech International (Marseilles, France). The domain specificity recognized by the mAbs has been established by using recombinant ε heavy chain peptides (14). In summary, the domain specificity and isotype are shown in Table I.

Peroxidase (horseradish peroxidase; Sigma Chemical Co., St. Louis, MO)-coupled Abs (Le27-POX, BSW17-POX, and IT-POX) were prepared by the periodate method, as described earlier (25). These peroxidase-conjugated anti-IgE Abs were used for detecting cell-bound IgE after appropriate dilution (900–1000× dilution). FITC-labeled Le27 and BSW17 were prepared in our institute.

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³ Abbreviations used in this paper: CHO, Chinese hamster ovary; CFC, PBS containing 1% FCS and 1% sodium azide; ¹²⁵I-IgE-SUS11, ¹²⁵I-labeled immunoglobulin E-SUS11; POX, peroxidase; HA, 20 mM HEPES, 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, 0.025% BSA; RT, room temperature.

IgE Ab

Human myeloma IgE-PS was affinity purified from serum of a patient with an IgE myeloma (a kind gift of Dr. K. Ishizaka, La Jolla, CA), as described elsewhere (26). IgE-PS-biotin conjugate was prepared in our institute. IgE-SUS11 is an in-house-produced human hybridoma cell line (29).

Human rFcεRI α-chain

Human rFcεRI α-chain expressed in a baculovirus system was kindly provided by Dr. H. Kocher (Sandoz AG, Basel, Switzerland). Shortly, recombinant human α-chain was expressed in SF9 cells grown in TC100 medium + 10% FCS for 48 h as the extracellular part of the human α-chain. The recombinant protein was purified by affinity chromatography on immobilized anti-human FcεRI mAb 5H5/F8 coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at 10 mg/ml gel, according to manufacturer's instructions. Cell supernatants were purified on a 5-ml column in PBS at 0.5 ml/min, and eluted with 50 mM citric acid and 140 mM NaCl, pH 2.7, and immediately afterward corrected to pH 7 with NaOH. Protein concentration of the eluted fraction was estimated by means of UV absorption. The purity and quality were assessed by SDS-PAGE.

IgE binding to solid-phase rFcεRI α-chain

Soft RIA plates were coated with 20 μg/ml α-chain or 20 μg/ml mAb in 0.1 M carbonate buffer, pH 9.6, and afterward blocked with TBS containing 1.5 mg/ml casein (Fluka Chemie AG, Buchs, Switzerland). IgE-SUS11 was ¹²⁵I-labeled using the chloramine-T method (30). A quantity amounting to 10⁵ cpm per well was used. All experiments were performed at 37°C, and plates were washed with 0.9% NaCl + 0.05% Tween-20 and cut in pieces, and each well was measured in a gamma counter for 1 min.

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

One hundred microliters of 20 μg/ml human rFcεRI 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 in PBS at 37°C. The IASys cuvette was regenerated with 20 mM of HCl. Analysis of association and dissociation was performed using FASTfit, a data analysis software included in the IASys system.

CHO cells transfected with human FcεRI (α- and γ-chains)

The transfectoma (28), kindly provided by Dr. J. F. Kinet, was maintained in Iscove's modified Eagle's medium containing 2 mM glutamine, 100 U/ml penicillin-streptomycin, 15% FCS, and 1.25 mg/ml geneticin (G418; Life Technologies, Inchinnan, U.K.).

Basophil-containing human PBMC

Human peripheral blood, anticoagulated with 10 mM of EDTA, was mixed with 0.25 vol of 6% dextran (Pharmacia) for sedimentation at room temperature (RT). Leukocytes were pelleted by centrifugation (150 × g, 20 min, at RT) and suspended in HA buffer (20 mM HEPES, 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, and 0.025% BSA (Boehringer Mannheim Corp., Mannheim, Germany)). Cells were further fractionated by Ficoll-Hypaque density centrifugation (500 × g, 40 min, at RT). PBMC were harvested, washed three times (300 × g, 10 min, at 4°C) in HA buffer, and finally suspended in HACM buffer (HA buffer supplemented with 1 mM CaCl₂ and 1 mM MgCl₂) or fresh serum- and phenol red-free MEM (T038; Biochrom-Seromed, Berlin, Germany) supplemented with BSA (100 μg/μl) at a cell density of 10⁷ cells/ml.

Anti-IgE-induced mediator release from human basophils

After warming up to 37°C for 10 min in flat-bottom tissue culture grade 96-well plates (Falcon), PBMC (10⁶/well) were stimulated by the addition of anti-IgE for 40 min at 37°C. The reaction was stopped by cooling plates on ice. After centrifugation (500 × g, 5 min, at 4°C), the amounts of histamine and sulfidoleukotrienes of the supernatant were assayed by a histamine RIA kit (Immunotech International, Marseillies, France) and a conventional fluid-phase sulfidoleukotrienes RIA using a mouse anti-sulfidoleukotrienes mAb (1A-LDR₁) (31) and [14, 15(n)-³H]leukotriene C₄ (Amersham, Bucks, U.K.). Sulfidoleukotriene measurement was based on Amersham's leukotriene C₄/D₄/E₄ [³H] assay system. Briefly, the supernatant (100 μl/tube) and a fixed quantity of [14, 15(n)-³H]leukotriene C₄ (final 2500× dilution) were competed for binding to a limited quantity of 1A-LDR₁ (25 ng/tube) in 400 μl of RIA buffer (PBS containing 0.01% gelatin). After overnight incubation at 4°C, the Ab-bound leukotrienes

Table I. Characterization of monoclonal anti-IgE antibodies

mAb	Isotype	Domain Specificity	Histamine Release ^a
Le27	IgG 1	CεH4	+++
11-4	ND	CεH4	++
BSW17	IgG 1	CεH3	—
11-1	ND	CεH3	+++
4F4	IgG 2a	CεH3	+++
4F12	IgG 1	CεH3	+++
1T	IgG 2a	CεH2	+
11-3	ND	CεH2	++
11-5	ND	CεH2	+
5D4	IgG 2a	CεH2	+++
2B9	IgG 1	CεH2	++
5B9	IgG 1	CεH2	+++
1E10	IgG 2a	CεH2	+++
4F1	IgG 2a	CεH2	++
1C1	IgG 2a	CεH2	+

^a +++, 40–80% histamine release; ++, 20–40% histamine release; +, <20% release; —, no release. In the case of Le27 and BSW17, crosses represent several years of experience using more than 600 blood donors. For the other Abs, the range is based on 5–10 different experiments.

were separated by adsorption of free leukotrienes on dextran-coated charcoal (RIA buffer containing 1.6% charcoal and 0.32% dextran), followed by centrifugation (2000 × g, 15 min, at 4°C). The concentration of leukotrienes in the sample supernatant was calculated from a standard curve by the measurement of radioactivity in the supernatant.

IgE-binding assay by ELISA system using CHO cells

CHO transfectant cells (10⁵/well) in 10% FCS and RPMI 1640 were adhered to flat-bottom tissue culture grade 96-well plates by overnight incubation at 37°C. After washing with Hank's solution, human myeloma IgE-PS was added for 2 h at 37°C. Following three further washes, bound IgE on CHO cells was detected by mouse monoclonal anti-human IgE coupled with peroxidase for 2 h at 37°C or 4°C. After washing, bound anti-IgE-POX was visualized with 250 μl of 150 μg/ml 2,2'-azino-bis-(3-ethyl-benz-thiazoline-sulfonate) (ABTS; Sigma Chemical Co.) in 0.05 M of citrate buffer, pH 4, containing 0.01% hydrogen peroxidase. After leaving for 60 min at RT, the color was read at 414 nm in an ELISA reader (Titertek Multiskan MC; Flow Laboratories, McLean, VA).

IgE-binding assay by immunofluorescence using CHO cells

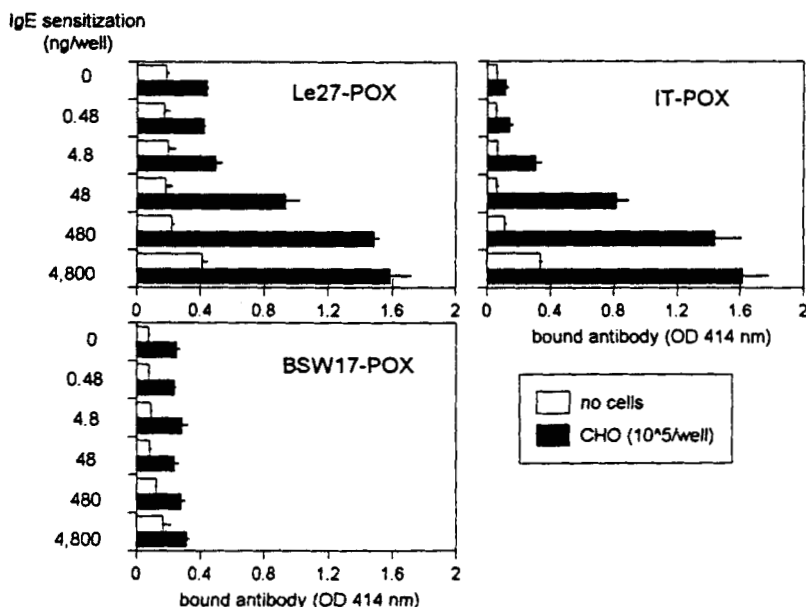
CHO transfectant cells (5 × 10⁵/well) in 10% FCS and RPMI 1640 were incubated overnight at 37°C in flat-bottom Teflon 24-well plates to avoid adherence. Without washing, human myeloma IgE-PS-biotin conjugate (final concentration, 2.4 μg/ml) was added for 2 h at 37°C. Cells were transferred into tubes and washed once in PBS containing 1% FCS and 0.1% sodium azide (CFG-PBS), resuspended in 500 μl of CFG-PBS, containing 2 μg of avidin-R-phycoerythrin (Pierce, Rockford, IL) for 30 min on ice. After washing twice in CFG-PBS, cells were fixed in 1 ml of 1% paraformaldehyde and analyzed on a flow cytometer (EPICS PROFILE II; Coulter Corp., Hialeah, FL).

Results

Histamine-releasing activities of different anti-human IgE mAbs from basophils

A panel of 15 anti-IgE mAbs was tested for their capacity to induce histamine release from human blood basophils. With one exception, namely BSW17, all of them were anaphylactogenic (Table I). The epitope specificity of these mAbs is summarized in Table I, as previously determined with rFcε heavy chain peptides (14), but no correlation was observed between their domain specificity and their histamine-releasing activity.

FIGURE 1. Binding capacity of anti-IgE Abs to FcεRI-bound IgE in direct ELISA. Adherent CHO transfectoma cells expressing FcεRI (10^5 /well) were incubated with IgE-PS for 2 h at 37°C. After washing, peroxidase-conjugated anti-IgE Abs (Le27-POX, BSW17-POX, or IT-POX) were added for 2 h at 37°C. After washing and staining with substrate for 60 min, OD was measured at 414 nm. Each bar represents the mean $\pm$ SD of triplicates.



Binding capacity of anti-IgE Abs to IgE on FcεRI in a cell ELISA

To investigate whether BSW17 is nonanaphylactogenic because it does not recognize receptor-bound IgE, we used a CHO transfectoma cell line expressing the α - and γ -chains of the human high affinity FcεRI. For comparison, we chose two anaphylactogenic anti-IgE Abs named Le27 (anti-CεH4) and IT (anti-CεH2). Peroxidase conjugates of these three anti-IgE Abs (Le27-POX, BSW17-POX, and IT-POX) were able to recognize IgE dotted onto nitrocellulose to the same degree (data not shown). But in the cell ELISA with CHO transfectoma cells previously sensitized with IgE-PS, no significant signal could be detected with BSW17-POX, while Le27-POX and IT-POX showed a clear binding to IgE present on the receptor (Fig. 1).

These data cannot be interpreted yet to determine whether BSW17 has interacted or not with surface IgE, as it has been described that surface-bound immune complexes such as IgE/anti-IgE complexes can be internalized at 37°C (32). Thus, the same experiment as in Figure 1 was performed in the presence of cytochalasin B and D, drugs that disrupt microfilaments at 4°C, as well as 37°C. Although the binding of Le27-POX and IT-POX to surface-bound IgE was slightly reduced, cytochalasin B and D did not affect recognition of surface IgE (data not shown). Obviously, this transfected CHO cell line did not internalize cell surface-bound IgE/anti-IgE immune complexes and monomeric IgE during the incubation period. In the case of Ab BSW17, it was not clear whether this Ab was actually not capable of recognizing receptor-bound IgE or whether IgE had already been dissociated in the presence of BSW17 from the receptor.

Binding capacity of FITC-labeled anti-IgE Abs to IgE on FcεRI and effect of anti-IgE Abs on the binding of IgE to FcεRI

To investigate whether BSW17 can bind to IgE on FcεRI expressed on CHO cells, we chose a different assay system and prepared Le27-FITC and BSW17-FITC conjugates. Nonadherent CHO transfectoma cells, previously sensitized with IgE-PS-biotin, were incubated with the FITC-labeled anti-IgE Abs for 2 h at 37°C, and the amount of bound IgE was then detected using avidin-R-phycoerythrin by incubation on ice for 30 min. Under these

conditions, both anti-IgE Abs were binding to receptor-bound IgE, and the extent of bound IgE did not change significantly during the experiment (data not shown).

To further investigate the influence of the anti-IgE Abs on the binding of IgE to the high affinity receptor, Le27-FITC or BSW17-FITC were added to CHO transfectoma cells immediately before the addition of IgE-PS-biotin, and the experiment was conducted under the same conditions as described above. BSW17 inhibited IgE binding to FcεRI in a dose-dependent manner, and a 10× excess of BSW17 completely inhibited IgE binding; meanwhile, the anaphylactogenic anti-IgE Ab Le27 only marginally inhibited IgE binding at very high concentrations (Fig. 2). The same inhibition experiments were also performed using the CHO cell ELISA, with identical results to those obtained by immunofluorescence (data not shown). Thus, using the more sensitive approach with FITC-labeled Abs, it was clear that, for both anti-IgE Abs, their specific epitopes were accessible on receptor-bound IgE, but the presence of BSW17 influenced the binding capacity of IgE to the receptor.

Dissociation of bound IgE from FcεRI in the presence of anti-IgE

Therefore, we investigated whether anti-IgE Abs may not only interfere with binding, but may also dissociate IgE bound to the receptor expressed on CHO cells. For this purpose, the α -chain expressing CHO cells, sensitized with IgE-PS-biotin, was incubated with Le27-FITC or BSW17-FITC for longer periods at 37°C, and the amount of cell surface-associated IgE was detected as described above. As shown in Figure 3, the anti-IgE Abs Le27 and, to a greater degree, BSW17 were reducing the signal of cell surface-associated IgE.

Direct binding studies on recombinant human α -chain

To better understand the effect of the BSW17 Ab, we used the IAsys system, an optical biosensor that allows monitoring of bio-specific molecular interactions without the need for labeling of ligands or agonists. Purified human rFcεRI α -chain was covalently linked to the dextran matrix of the IAsys cuvette. Binding of IgE

FIGURE 2. Binding of IgE to FcεRI in the presence of anti-IgE Abs analyzed by flow cytometry. CHO transfectoma cells (5×10^5 /Teflon well) pre-incubated overnight at 37°C were incubated with IgE-PS-biotin and Le27-FITC or BSW17-FITC anti-IgE mAbs for 2 h at 37°C. After washing, bound IgE was detected by avidin-R-phycoerythrin. Each bar represents the mean channel of fluorescence.

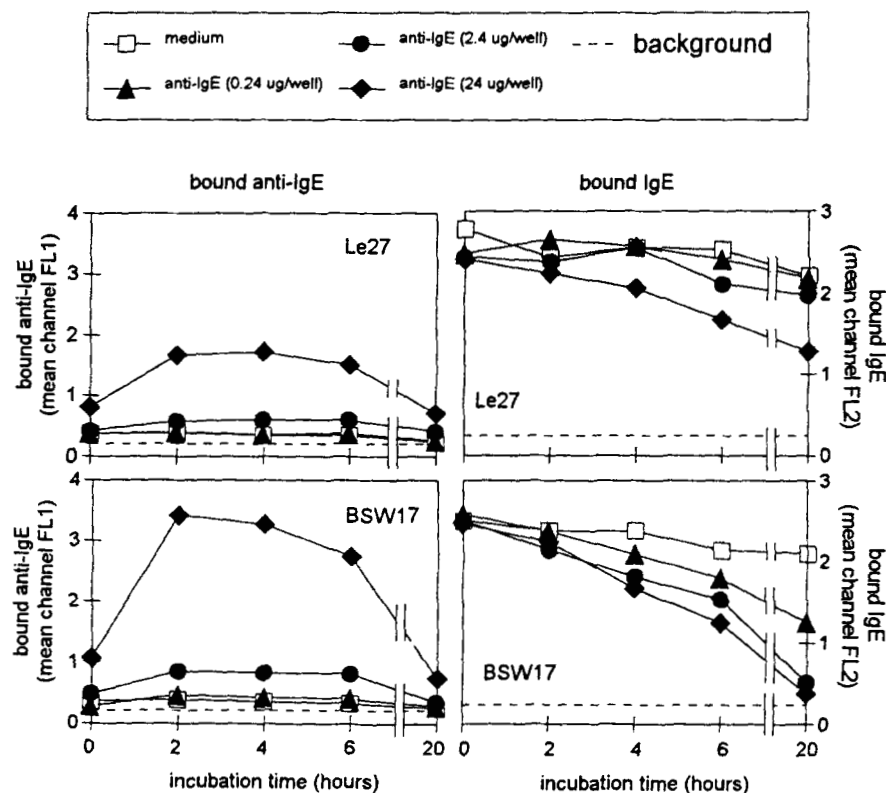
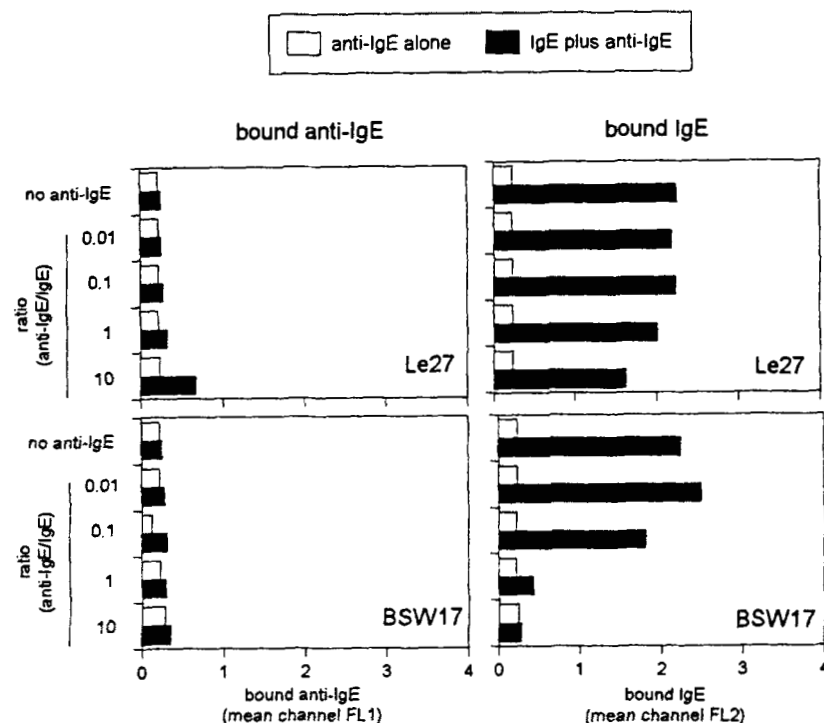


FIGURE 3. Fate of FcεRI-bound IgE in the presence of anti-IgE Abs analyzed by flow cytometry. After preincubation of CHO transfectoma cells (5×10^5 /Teflon wells) overnight at 37°C, IgE-PS-biotin was added for 2 h at 37°C, washed, and incubated with Le27-FITC or BSW17-FITC at 37°C. At the indicated time points, bound IgE was detected by avidin-R-phycoerythrin. Each time point shows the mean channel of fluorescence. Background indicates spontaneous fluorescence of cells alone.

at 37°C 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 (Fig. 4). With polyclonal human IgG, no binding to the α -chain was detected, nor with polyclonal

human IgA and IgM (data not shown). To study the interaction of α -chain-bound IgE with anti-IgE, the α -chain-coated cuvette was incubated with 50 nM of IgE-SUS11 at 37°C. At the time point indicated (180 s after the addition of IgE into the cuvette), BSW17

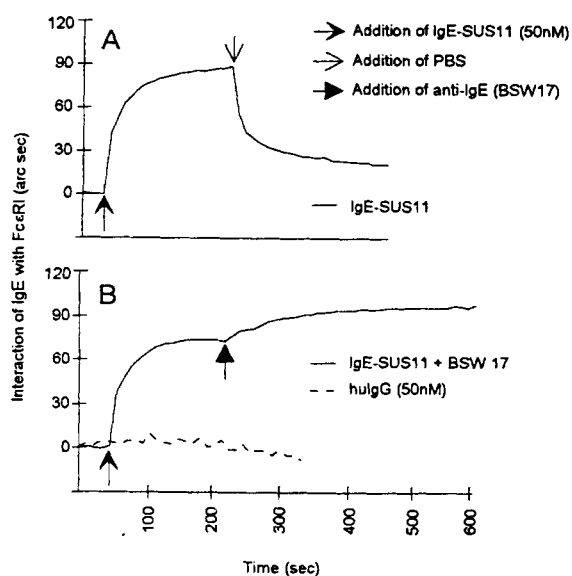


FIGURE 4. Direct measurement of IgE binding to human rFcεRI α-chain. For measurement by the IAsys system, purified human rFcεRI α-chain (20 μg/ml) was immobilized by covalent binding to a dextran-coated cuvette. At time points indicated, 50 nM of IgE-SUS11 was added, and the binding to the FcεRI α-chain was measured as alteration of refractive index of the surface, expressed as arc seconds. After 180 s (A), the cuvette was either washed to determine the normal off rate, or BSW17 (100 nM) (B) was directly added into the cuvette.

was added directly into the cuvette to a final concentration of 100 nM (corresponding to 16 μg/ml of BSW17) without prior removal of unbound IgE-SUS11. As shown in Figure 4, BSW17 interacted with α-chain-bound IgE, but the amount of α-chain-bound IgE remained constant even after 600-s incubation time. The time range of the experimental procedure on the IAsys system, which cannot be meaningfully extended, is probably too small to observe a change in the amount of α-chain-bound IgE. Nevertheless, from these data we could exclude a direct competition for the binding site between FcεRI and BSW17 or a sterical hindrance for the binding of BSW17, nor could we observe a direct binding of BSW17 or the control Ab Le27 to the α-chain.

Dissociation of labeled IgE from human recombinant α-chain

To clarify whether the observed effect of reducing receptor-associated IgE on the transfectant CHO cells was due to BSW17 interacting with receptor-bound IgE, or due to an anchorage of the IgE receptor, only to be observed on cells, we chose a cellfree system using human rFcεRI α-chain. For this purpose, we used ¹²⁵I-labeled IgE-SUS11 (¹²⁵I-IgE-SUS11) to determine the amount of IgE bound to recombinant α-chain after incubation with anti-IgE Abs. Figure 5 shows that the amount of α-chain-bound ¹²⁵I-IgE-SUS11 was decreased in the presence of BSW17, confirming the results of the experiments using CHO transfectoma cells. Again, the control Ab Le27 had no effect on the dissociation of FcεRI α-chain-bound IgE. For control, we coated plates with Le27 anti-IgE Ab as a catcher for IgE. No change in the amount of bound IgE was observed. Thus, our results clearly demonstrate that the decrease in receptor-bound IgE was due to BSW17, both in the cell-based assays as well as in the assay using rFcεRI α-chain.

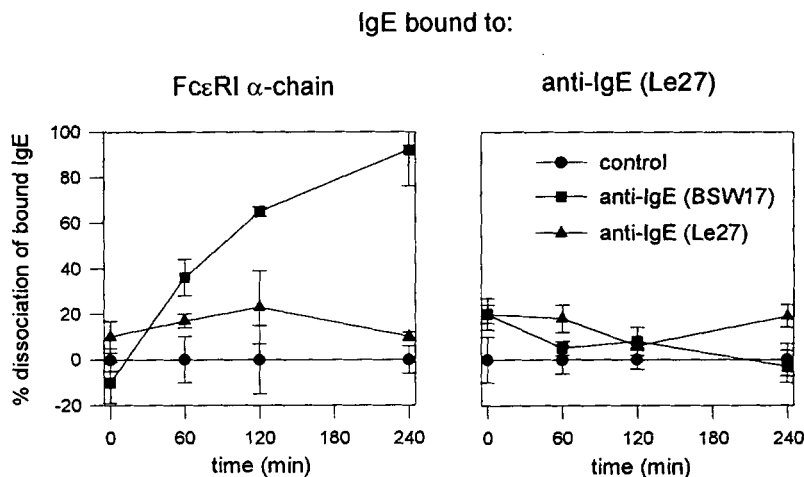
Binding of preformed immune complexes to recombinant human α-chain

Furthermore, similar experiments as outlined in Figure 2 were performed using human rFcεRI α-chain instead of the FcεRI α-chain transfectant CHO cells. Immune complexes were performed using labeled ¹²⁵I-IgE-SUS11 and either anti-IgE mAb BSW17 or Le27, and then incubated on plates coated with recombinant human α-chain or Le27 (Fig. 6). As observed in the cell assay, the binding of IgE to the α-chain was abolished completely in the presence of BSW17, while the association of IgE with the α-chain was not influenced in the presence of the control Ab Le27. The presence of BSW17 in immune complexes with IgE affected only the binding site of the α-chain and not the epitope for the anti-CεH4 anti-IgE Le27, as could be demonstrated with Le27-coated plates. Only at high concentration of BSW17 was a slight reduction observed (Fig. 6, right panel).

Discussion

Theoretically, nonanaphylactogenic Abs against IgE might interfere with the binding site of IgE for its receptor. Indeed, such Abs have been generated and are presently in clinical trials for treatment of atopic diseases (10, 20–22). Our anti-IgE Ab, called BSW17, recognized an epitope within the third constant domain of the Fcε part (14). This led to our initial assumption that this Ab

FIGURE 5. Dissociation of FcεRI α-chain-bound IgE with anti-IgE Abs. Human rFcεRI α-chain (20 μg/ml) or mAb Le27 (20 μg/ml) was coated to RIA plates, and 10⁵ cpm/well of ¹²⁵I-IgE-SUS11 was added overnight at 37°C. The plates were washed, and anti-IgE mAbs BSW17 or Le27 were added at 10 μg/ml and incubated at 37°C. At the time points indicated, wells were measured in a gamma counter for 1 min. The binding of IgE is indicated as percentage of total bound IgE (control). Each time point represents the mean ± SD of triplicates.



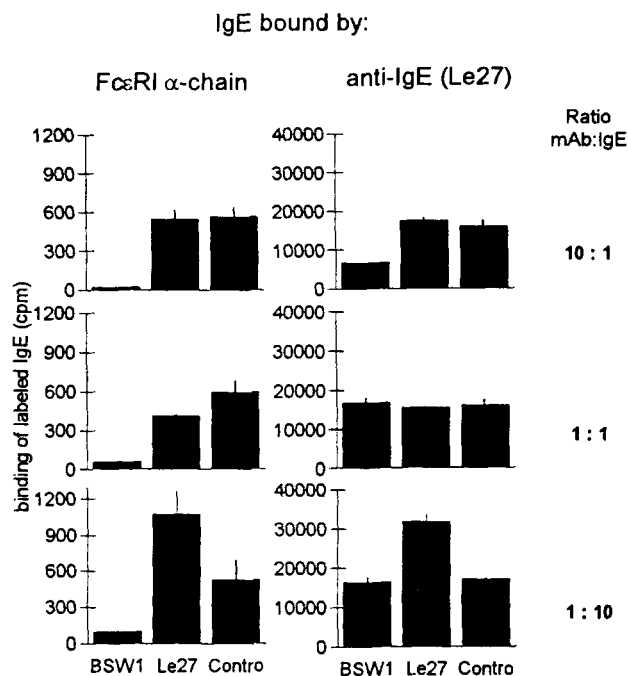


FIGURE 6. Binding of preformed IgE/anti-IgE mAb immune complexes to FcεRI α-chain. Plates were coated with human rFcεRI α-chain (20 μg/ml) or Le27 mAb (20 μg/ml). Immune complexes were preformed at 37°C at the indicated ratios of ¹²⁵I-IgE-SUS11 (10⁵ cpm) and anti-IgE mAb (BSW17 or Le27) overnight. Immune complexes were added into the plates at 37°C for 6 h, then wells were measured in a gamma counter for 1 min. Each time point represents the mean ± SD of triplicates.

might interfere with the IgE binding site. Nevertheless, our data presented in this work clearly show that the nonanaphylactogenic property of BSW17 was not due to its incapacity to bind IgE on the receptor. Additionally, there was no direct competition between BSW17 and the α-chain of FcεRI for the binding site on the Fcε part of IgE.

Since the FcεRI α-chain alone is sufficient for binding IgE (33), human rFcεRI α-chain alone was used for receptor-binding studies in a cellfree system. Based on data obtained from our IgE-binding experiments using the optical biosensor IAsys, we determined an affinity of $2 \times 10^{-8} \text{ M} \pm 8 \times 10^{-9} \text{ M}$ for the α-chain, which is about 10 times lower than published for the intact FcεRI (18, 19, 34). The reason for this discrepancy might be the covalent linking of the α-chain to the dextran matrix of the system, probably influencing the structure of the α-chain.

However, the advantage of the system is the absence of cells, in which it has been reported that chemically stabilized or cross-linked IgE oligomers are quickly internalized (32, 35–39). Such internalization was also reported for FcεRI-transfected COS cells (39). In this respect, our results from the cellfree system completely confirmed the experiments using CHO transfectoma cells in the presence of cytochalasin D. Thus, the decrease of the cell surface-associated IgE was not due to internalization of the immune complexes of IgE and BSW17.

The binding of the anti-IgE Ab BSW17 to its epitope within the third constant ε domain probably induced a change in the conformation of the Fcε constant domains similar to the effect induced by Ag. Namely, we have shown recently that preformed immune complexes of IgE-JW8 and NIP-BSA, the specific Ag for IgE-

JW8, showed a reduced binding to the α-chain compared with the uncomplexed IgE-JW8 (40).

From our data, we conclude that BSW17 recognized receptor-bound IgE, but did not influence the normal off rate of IgE from the receptor. Once the immune complex of IgE and BSW17 has dissociated from the receptor, BSW17 effectively prevented reassociation of IgE with its receptor. Therefore, the thermodynamic balance of bound vs free IgE is shifted toward the unbound immune complex. Nevertheless, it cannot be excluded completely that the inhibited binding of complexed IgE to the receptor is due to a sterical hindrance, but the fact that BSW17 recognized receptor-bound IgE favors the hypothesis that it induced a conformational change in the IgE molecule. Furthermore, we have shown earlier that BSW17 cannot be used in immunoassays as catching and revealing Ab at the same time (13). If used as a catching Ab on the solid phase, it even hindered Abs with specificity to the Fcε domains 2 or 4, again suggesting that BSW17 induced a conformational change in the constant part of IgE. Furthermore, it cannot be excluded completely that the epitope for BSW17 only contains a part of the binding site for the FcεRI α-chain (18–20), allowing a weak binding of BSW17 to receptor-bound IgE. A precise epitope mapping is undertaken currently to clarify this point.

The additional property of recognizing surface IgE without being anaphylactogenic and neutralizing IgE discriminates this Ab from others presently in clinical trials (10, 21, 22). Moreover, we have shown that this Ab is capable of inhibiting in vitro IgE synthesis (13) and also of inhibiting binding of IgE to the low affinity receptor for IgE, CD23 (14). Thus, this Ab might not only be capable of desensitizing an individual by neutralizing IgE, but it may also inhibit de novo IgE synthesis.

The concept that BSW17 neutralizes IgE by inducing a conformational change may also be of importance for other molecular interactions with IgE, such as Ags or allergens (40). Thus, the conformation of IgE must be crucial for its binding to FcεRI, and either Ag or anti-IgE Abs may influence its conformation. This may represent a potential role for naturally occurring anti-IgE Abs in vivo (41).

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