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Antigen Interaction and Heat Inactivation Expose New Epitopes on Human IgE

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Key Words

Antigen binding
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Abstract

It is well established that heat-denatured IgE is no longer capable of binding to FcεRI. We have found an antibody that interacts with heat-denatured IgE. Interestingly, this antibody can also be used to detect some serum IgE, but not IgE synthesized *de novo* in vitro. However, native IgE can be transformed into an IgE that is recognized by this antibody, if antigen is added. Our data indicate that physiological mechanisms exist that biologically inactivate IgE which might still be mistaken for 'functional' IgE by assays based on polyclonal antibodies.

Introduction

The involvement of IgE in atopic diseases is undisputed. However, IgE concentrations are still only estimated by immunological assays and expressed as international units. Furthermore, total IgE levels have only a weak clinical correlation with allergic diseases. It has even been suggested that non-IgE-dependent mechanisms may give rise to anaphylactic reactions [1] or inhibit them [2].

Heterogeneous results were also found in an international study where different monoclonal antibodies (mAbs) were compared using established IgE determinations based on polyclonal anti-IgE antisera [3]. IgE fragments may be mistaken for complete IgE in determination assays [4], and autoantibodies, namely IgM rheumatoid factor [5] or anti-IgE autoantibodies [6, 7], may be responsible for some differences in IgE determinations. Furthermore, we have previously shown a decrease in IgE binding to the high-affinity receptor in the presence of antigen [8] and we speculated that IgE may be hidden in immune complexes or altered in its conformation by antigen-Ab reactions leading to a less

efficient recognition of IgE by FcεRI. Thus, similar to antigens, one might speculate that the binding of an anti-IgE Ab could cause a similar change or a destabilization in the three-dimensional structure of the IgE molecule. Moreover, since the discovery of IgE it is known that IgE changes its biological properties upon heat treatment over 56 °C. It loses its capacity to sensitize cells expressing the high-affinity receptor for the ε part of the IgE (FcεRI) but can still be recognized by some anti-IgE Abs. One of our anti-IgE Abs recognized only the heat-denatured form indicating recognition of a new epitope formed during heat denaturation.

In this study, we intended to address the question whether IgE exposes this epitope under other conditions than after heat treatment. We could show that a similar epitope occurs in myeloma IgE preparations as well as in patient sera whereas it is not present on *de novo* synthesized IgE. Interestingly, a similar functional and antigenic change in the IgE molecule was also induced after interaction with the antigen. The less NIP-specific IgE-antigen complexes were binding to FcεRI, the more they were recognized by the antibody specific for denatured IgE.

Materials and Methods

Sources of IgE

The following IgE preparations from patients suffering from IgE myeloma were selected for this study: PS-IgE (a kind gift from Drs. T. and K. Ishizaka, La Jolla, Calif., USA), Sav-IgE (a kind gift from Dr. V. Savazal, Pilsen, Czech Republic) and WT-IgE (a kind gift from Dr. D. Stanworth, Birmingham, UK). All were purified on a Mono Q ion exchange column using the Pharmacia FPLC system.

Chimeric JW8-IgE [9] consisting of a mouse V_H and a human C ϵ H region and the human plasmacytoma ND-IgE [10, 11] were obtained from supernatants of the IgE-producing cell lines JW8 and U266, respectively. Cell lines were maintained in RPMI-1640 (Seromed, Basel, Switzerland) supplemented with 10% FCS, 2 mM glutamine and 100 U/ml penicillin-streptomycin at 37°C in 5% CO₂. SUS11-IgE is a human IgE-producing hybridoma clone. Cell preparation, culture, fusion and cloning were carried out as described elsewhere [12]. All cell culture supernatant IgE was purified by affinity chromatography using an anti-IgE mAb (Le27, specific for C ϵ H4) bound to affinity adsorbents as described by the supplier (Boehringer Mannheim, Mannheim, Germany).

Heat denaturation of IgE was carried out in Eppendorf tubes (Vaudaux, Schönenbuch, Switzerland) in a water bath (Brouwer, Lucerne, Switzerland) at 36, 46, 56, 66 or 76°C for 2, 4, 8, 16, 30, 32, 64, 128, 256 or 360 min.

Anti-IgE Abs, Antigen and Fc ϵ RI α -Chain

Monoclonal mouse anti-human IgE Ab Le27 (C ϵ H4 specific) and BSW17 (C ϵ H3 specific) were generated in our laboratory [13]. 11-3 (C ϵ H2 specific) was obtained from Dr. R. Vasilov, NPO Biotechnology, Moscow; 4F4 (C ϵ H3 specific) and 1C1 (C ϵ H2 specific) were obtained from Dr. M.P. Samoilovich and Dr. V.B. Klimovich, Hybridoma Technology Laboratory, Central Research Institute for Roentgeno-Radiology, St. Petersburg, Russia. All anti-IgE mAb were domain mapped using recombinant IgE domains as previously published [14]. A sheep anti-human IgE polyclonal Ab was purchased from the Binding Site (Birmingham Research Park, Birmingham, UK). A mAb, 8E7/H8, generated against the recombinant C ϵ H3 domain, was kindly provided by the Sandoz Research Center, Vienna, Austria.

NIP₁₅-BSA (M_r 70,000) was prepared in our laboratory according to a standard protocol [15]. 8 mg of NIP (Sigma, Buchs, Switzerland) were preactivated with 3 mg N-hydroxysuccinimide (Fluka Chemie, Buchs, Switzerland) and 5 mg N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (Fluka Chemie) 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 mixture 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.

Recombinant α -chain of the high-affinity IgE receptor, expressed in a baculovirus system, was kindly provided by Dr. H. Kocher, Sandoz, Basel, Switzerland. Anti- α -chain mAb (22E7) [16] was a kind gift from J. Kochan.

Patient Sera and Cell Cultures

Sera from 21 randomly selected individuals (9 women and 12 men, aged 14–54 years, visiting our outpatient clinic for an allergy consultation) were included in this study (mean IgE level =

208.9 $\pm$ 58.3 U/ml). From an additional 10 patients, mononuclear cells were isolated from heparinized blood by density gradient centrifugation on Lymphoprep (density 1.077 g/ml) at 400 g for 30 min, washed twice in HBSS without Ca²⁺ and Mg²⁺ and suspended in complete Iscove's modified Dulbecco medium as described elsewhere [17]. Peripheral blood mononuclear cells were cocultured with CDw32/Fc γ RII-transfected Ltk – cells [18] in the CD40 system [19, 20] in TC 24 wells at a concentration of 1.5 $\times$ 10⁶ cells/ml (Falcon 3047, Becton Dickinson, Heidelberg, Germany) at 37°C in 5% CO₂.

Anti-CD40 mAb G28-5 [21] and human recombinant IL-4 were kindly provided by the Sandoz Research Center and used at a final concentration of 100 ng/ml and 100 U/ml, respectively.

Gel Electrophoresis

IgE preparations were fractionated on an SDS-polyacrylamide gel with separating zones of 6% (w/v) acrylamide/bisacrylamide (40:1). The running buffer consisted of 0.1% (w/v) SDS (Fluka Chemie) in 24.8 mM Tris (Merck, Darmstadt, Germany) and 191.8 mM glycine (Merck), pH 8.2. Samples containing 1 μ g SUS11-IgE, 0.5 μ g ND-IgE and 5 μ g of Sav- or PS-IgE were applied to the gel and run at room temperature at 110 V using precolored molecular weight markers (Amersham, Amersham, UK). The gel was either silver stained or transferred to nitrocellulose (Millipore, Bedford, Mass., USA) for Western blotting and incubated overnight with peroxidase-labeled polyclonal anti-IgE Ab.

Protein Determination

Protein. Protein concentrations were determined by a commercial protein assay (Bio-Rad Laboratories, Hercules, Calif., USA). The measurement is based on a differential absorbance maximum of a dye reagent in response to various protein concentrations. Absorbance of light (λ = 595 nm) by Coomassie brilliant blue G-250 was measured in the presence of the different IgE preparations.

IgE: Commercial Assay. Quantitative measurement of IgE in units was carried out by the Kallestad total IgE microplate kit, supplied by Sanofi Pasteur, Solothurn, Switzerland.

IgE: Dot Assays. IgE measurements were carried out in standard immunodot assays [22]. Briefly, 1 μ l of 500 μ g/ml anti-IgE mAb was dotted on nitrocellulose (Millipore) as capturing Ab. The nitrocellulose strips were then blocked with Tris-buffered saline (TBS) containing 1.5 mg/ml casein (Fluka Chemie) and incubated overnight with different myeloma IgEs. The strips were washed three times with TBS and developed by incubation for 6 h with peroxidase-conjugated anti-human IgE Ab diluted 1:1,000 in TBS. A second washing removed the unbound enzyme-labeled Abs. The strips were subsequently developed with 4-chloro-1-naphthol. The intensity of the color is directly proportional to the concentration of IgE and was determined using a hand-held reflection densitometer (Camag, Muttenz, Switzerland).

When Fc ϵ RI α -chain was used as a capturing agent, an anti- α -chain Ab was first dotted on nitrocellulose at a concentration of 500 μ g/ml. Strips were blocked with TBS containing 1.5 mg/ml casein and incubated for 6 h with Fc ϵ RI α -chain at a concentration of 5 μ g/ml. The following incubation with IgE was performed as described above.

To determine specific IgE concentrations, titration curves were performed for each type of IgE and for each sandwich immunoassay. In order to compare the resulting 'standard' curves, an identical optical density (OD) value was chosen in the linear range of the curves and its reciprocal dilution expressed as relative specific units (rel U/ μ l).

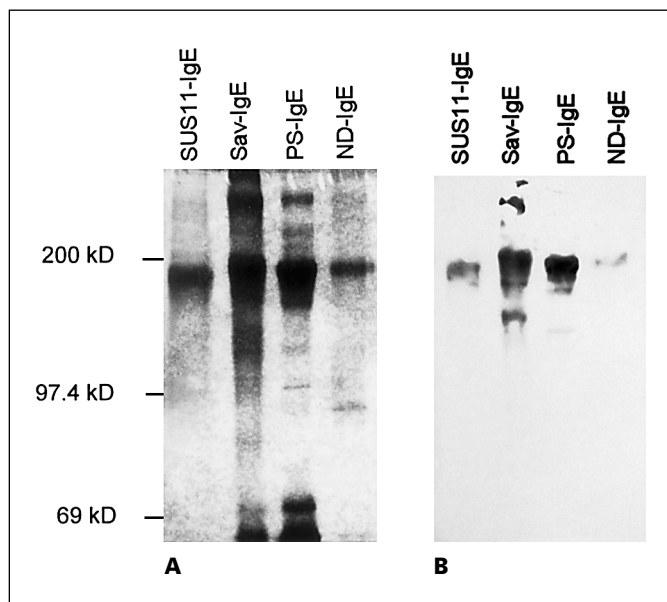


Fig. 1. SDS-PAGE and Western blot analysis of different semi-purified IgE preparations. **A** Silver-stained SDS-PAGE of IgE preparations. SUS11-IgE and ND-IgE were purified by affinity chromatography using an anti-IgE mAb (Le27), Sav-IgE and PS-IgE on a Mono Q ion exchange column. Samples were applied on a gel containing 6% bisacrylamide/acrylamide: SUS11-IgE at 1 μ g IgE, ND-IgE at 0.5 μ g, Sav-IgE and PS-IgE at 5 μ g estimated protein concentration per lane. **B** For Western blotting, the proteins of a gel applied as described in A were transferred onto nitrocellulose. IgE was detected by a peroxidase-labeled polyclonal anti-IgE Ab.

Results

We have purified four different IgE preparations by different procedures for our studies on IgE quantification. Figure 1 shows that the human hybridoma SUS11-IgE and ND-IgE, harvested from the U266 cell line, produced bands at the expected molecular weight, and displayed a similar purity. The two myeloma IgEs (Sav-IgE and PS-IgE) contained several bands that were detected by a polyclonal anti-IgE Ab in a Western blot (fig. 1B).

In order to compare these different samples for the amount of detectable IgE, we determined IgE levels either by a commercial ELISA or by in-house sandwich immunoassays based on different monoclonal anti-IgE Abs as shown in figure 2. Based on the results shown in figure 1, one would expect a high proportion of intact IgE in the SUS11-IgE and the ND-IgE preparations. The relatively pure SUS11-IgE preparation with a measured protein concentration of 63.8 μ g/ml should yield theoretically an IgE concentration of 27 kU/ml (according to WHO standard:

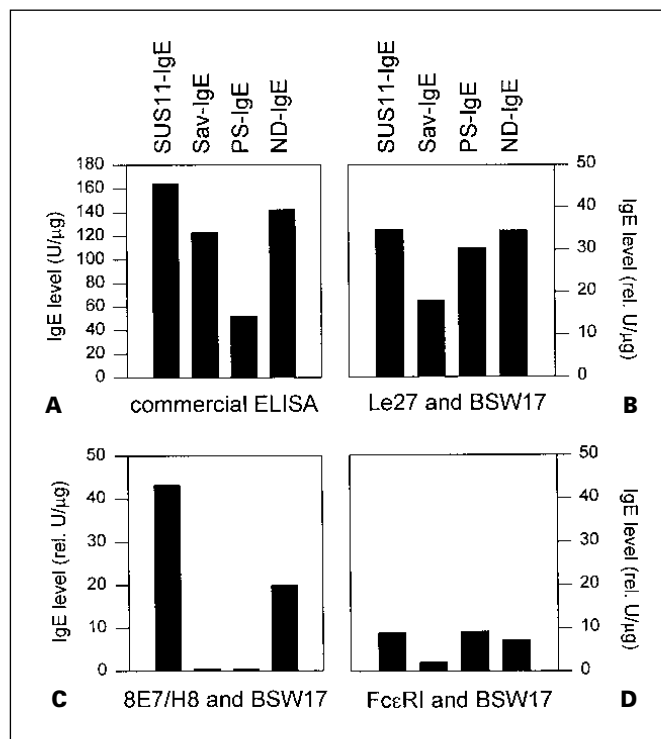


Fig. 2. Estimation of purified IgE preparations by different sandwich immunoassays. IgE concentration was determined by a commercial ELISA (Kallestad; **A**) or by sandwich immunoassays: IgE was incubated with a capturing agent [Le27 (**B**) 8E7/H8 (**C**) and FcεRI α -chain (**D**)] and detected by the peroxidase-labeled mAb BSW17. The levels of IgE measured by the different sandwich immunoassays are expressed as relative specific units (dilution factors to yield equal relative OD per protein concentration).

1 U/ml = 2.4 ng/ml) and a specific IgE concentration of 417 U/ μ g, but the commercial assay only measured 164 U/ μ g, i.e. not even half of the expected quantity. Furthermore, ND-IgE and SUS11-IgE are of nearly the same purity according to electrophoresis, but our different immunoassays detected different amounts of IgE in these two samples. Moreover, if immunoassays (fig. 2B, D) were used for detection, the PS-IgE preparation had to be diluted higher than the Sav-IgE preparation to yield the same signal (relative OD), while an opposite pattern was seen using the commercial assay.

In order to investigate whether IgE may expose 'new' epitopes after heat denaturation, different IgE samples were heat treated. Figure 3A shows that exposure of IgE to 66 or 76 $^{\circ}$ C completely abrogated detection of IgE by the anti-IgE mAbs used. Interestingly, at 56 $^{\circ}$ C (Fig. 3B) ~30% of the IgE were still detectable after 4 h of heat treatment in the assay with 11-3 as catching and BSW17 as revealing Ab,

Fig. 3. Measurement of heat-treated IgE by dot assay. **A** Affinity-purified IgE from the supernatant of the IgE-producing B cell line U266 (ND-IgE) was treated for 10 min. **B** ND-IgE was heated at 56°C for different periods of time. Different capturing anti-IgE mAbs were used on the solid phase. □ = Le27, an anti-CεH4 Ab; ○ = an anti-CεH3 Ab; △ = BSW17, an anti-CεH3 Ab; ◇ = 11-3, an anti-Cε H2 Ab). Peroxidase-labeled mAb BSW17 was used as revealing mAb.

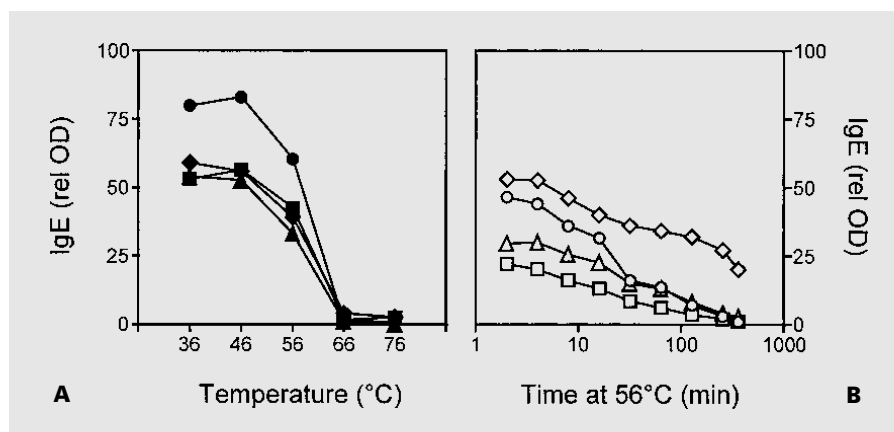


Fig. 4. Detection of heat-denatured IgE by sandwich immunoassays. ND-IgE (black columns) and PS-IgE (white columns) were adjusted to the same amount of IU and were subsequently denatured at 56 or 76°C for 30 min and incubated with solid-phase bound FcεRI α-chain or anti-IgE mAbs (8E7/H8 or Le27). Detection was performed by a polyclonal anti-human IgE sheep Ab labeled with peroxidase.

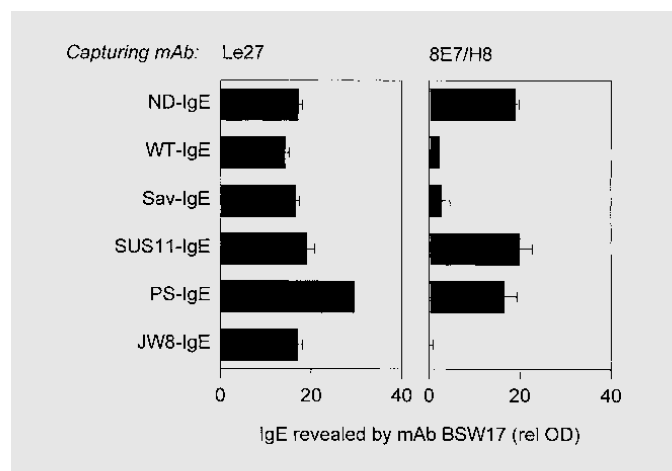
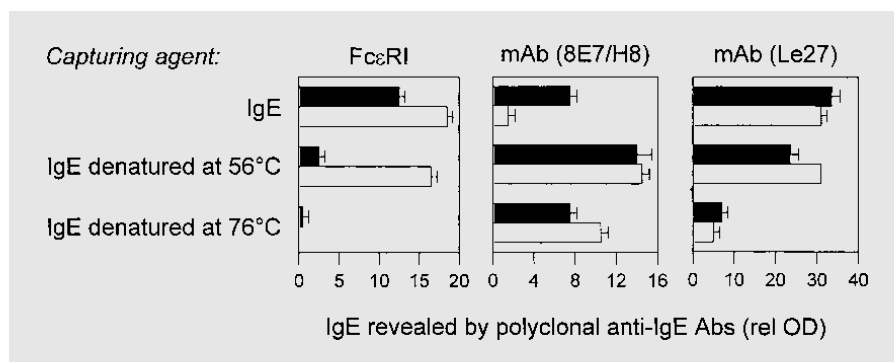


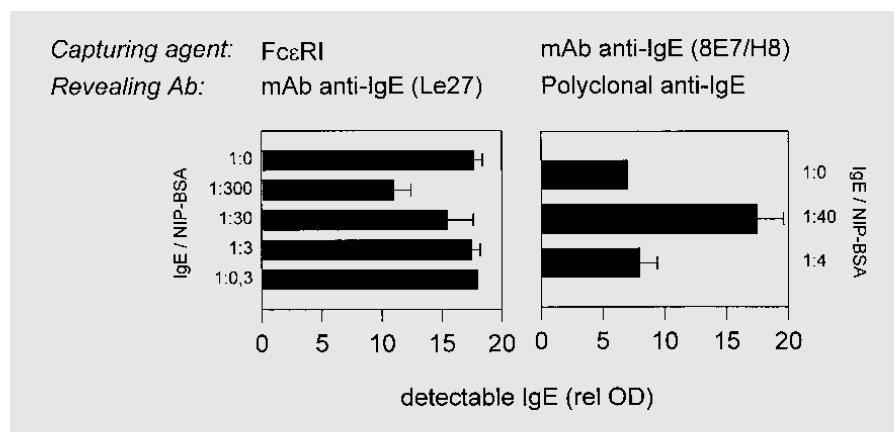
Fig. 5. Detection of IgE in 'native' IgE preparations by an Ab (8E7/H8) recognizing denatured IgE. Different IgE preparations (ND-IgE, WT-IgE, Sav-IgE, SUS11-IgE, PS-IgE and JW8-IgE) were adjusted to 25 U/ml by a commercial assay and subjected to measurement using either the mAb Le27 or 8E7/H8 on the solid phase and peroxidase-labeled BSW17 as revealing mAb.

whereas in the other assays IgE could not be detected any longer.

Next, we investigated the effect of heat denaturation on IgE binding to the FcεRI α-chain. In addition, we used a mAb (8E7/H8) generated against a recombinant CεH3 domain. This Ab reacted strongly with different recombinant IgE preparations containing the third domain (data not shown), but only weakly with native IgE preparations. For this study, we arbitrarily chose two myeloma IgE preparations, namely the myeloma proteins ND-IgE and PS-IgE. Interestingly, heat denaturation at 56°C led to an increased signal using the mAb 8E7/H8, while a gradual loss of IgE binding was observed for the mAb Le27 and also for FcεRI α-chain (fig. 4). These results suggested that there are Abs that discriminate predominantly between two antigenic states of IgE, namely a native and a heat-denatured form with new antigenic properties. At 76°C, Le27 and FcεRI α-chain had lost their capacity to recognize IgE while 8E7/H8 was still capable of capturing the same amount of IgE as in the non-denatured sample or recognized even more IgE.

If tested in a sandwich immunodot assay (fig. 5), the mAb 8E7/H8 also differs strongly in its capacity to recog-

Fig. 6. Measurement of hapten-specific IgE in the presence of its antigen (NIP-BSA). Hapten-specific IgE (JW8-IgE) was incubated with different concentrations of NIP-BSA overnight. IgE/NIP-BSA indicates the ratio between IgE binding sites and epitopes on NIP-BSA. The samples were then measured with two different pairs of capturing/revealing reagents, namely FcεRI α-chain/Le27 and 8E7/H8/polyclonal anti-IgE Ab.



nize different IgE preparations from mAb that recognizes the fourth domain of the IgE molecule. Interestingly, SUS11-IgE, stored for the shortest time and purified using affinity chromatography, contained a significant amount of IgE that could be measured with 8E7/H8 which recognized denatured IgE. Based on our data, we concluded that the different IgE preparations contained various amounts of 'denatured' IgE that were differentially detected by different anti-IgE Abs. Since the mAb 8E7/H8 recognized heat-denatured IgE in all preparations, it can also be excluded that an allotypic marker was responsible for the observed differences.

Indeed, after heat denaturation, every IgE preparation that we have examined so far, contained a certain amount of IgE detectable by 8E7/H8. Some myeloma IgE preparations contained this epitope already without heat treatment. Thus, we addressed the question of whether there is a physiological mechanism that would lead to the appearance of this epitope, e.g. IgE binding to its antigen. For this purpose, we preincubated the hapten-specific IgE (JW8-IgE) with its antigen (NIP) before measuring in assays based on either the FcεRI α-chain or 8E7/H8. As shown in figure 6, detection can be influenced dose dependently by NIP-BSA. At an epitope excess of more than 10 times of NIP-BSA relative to the amount of binding sites of the IgE, we observed a decreased binding to the FcεRI α-chain while an opposite pattern was seen when the IgE was captured by 8E7/H8.

Since one could argue that myeloma and chimeric IgEs are not 'normal' IgEs, serum samples from arbitrarily chosen allergic individuals were collected and adjusted to identical IgE units by a commercial IgE determination kit. The samples were then measured by several different dot assays (fig. 7). We used mAbs against the second constant domain of IgE (mAb 11-3), the third (mAb 4F4), and mAb Le27 against the fourth domain. The results of these three Abs

substantially differed with regard to the amount of IgE detected in the sera. These findings are in accordance with the results presented in figure 3, where IgE detection by these Abs showed quantitative differences depending on the duration of heat denaturation.

In order to generate a reagent similar to the traditionally used poly- or oligoclonal antisera, a mixture of five mAbs was included in the experiment. This resulted in a detection pattern similar to the one by the Ab against the fourth domain (Le27), except that sera Nos. 2 and 12 were less pronounced in comparison to the remaining sera. Interestingly, when using the FcεRI α-chain as a capturing reagent, a pattern different from that obtained by the mAbs was observed. Serum No. 8, for example, seems to contain an IgE that binds very strongly to the FcεRI α-chain while no IgE is detected by 8E7/H8 in this specimen. Sera Nos. 2 and 12 containing the highest IgE levels determined by the mAb Le27 (anti-CεH4 domain) also seemed to contain a substantial amount of IgE that can be bound by the mAb 8E7/H8. On the other hand, 8E7/H8 generated a pattern of IgE detection different from that obtained by the other mAbs or by the FcεRI α-chain, again supporting the view that 8E7/H8 recognized an epitope of IgE that is already exposed on some IgE molecules without in vitro heat denaturation.

This finding raises the question of whether this epitope may already be present at the moment of IgE secretion by a B cell. In order to address this question, we compared de novo synthesized IgE from mononuclear cells with serum IgE from the same donors in dot assays. For this purpose, we isolated mononuclear cells from 10 individuals and determined plasma IgE levels with a commercial assay. Mononuclear cells were stimulated with IL-4 and anti-CD40 mAb, and the supernatants harvested after 12 days. Then, culture supernatant IgE and serum IgE were adjusted to the same IgE levels, and again measured with different

Fig. 7. Assessment of IgE by different dot immunoassays in sera of atopic donors. Peroxidase-labeled anti-IgE mAb (BSW17) was used to detect IgE captured by solid-phase mAbs (11-3, Le27, 4F4, or 8E7/H8), by a mixture of mAbs (ICI, 11-3, 8E7/H8, Le27 and 4F4), or by FcεRI α-chain.

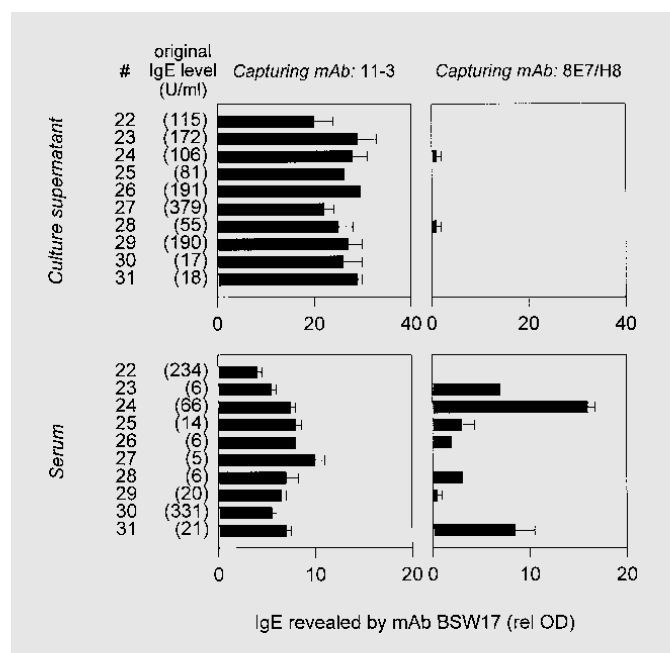
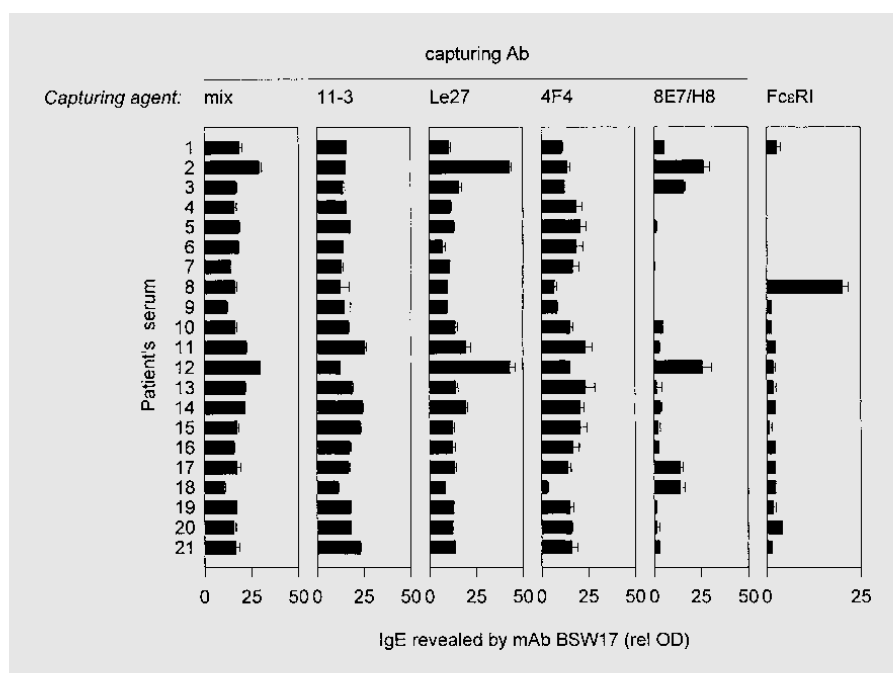


Fig. 8. Detection of IgE in cell supernatant or in serum. IgE from sera as well as from cell supernatant were measured with two different sandwich immunoassays based on different capturing/revealing anti-IgE mAbs (8E7/H8/BSW17 and 11-3/BSW17). After the sample number, values (in units) measured by a commercial assay before dilution of the highly concentrated samples are given in parentheses.

sandwich immunoassays. The results are presented in figure 8. Our data show that the mAb 11-3 (against the second IgE domain) detects more IgE in the cell culture supernatants than in the corresponding sera (relative to the commercial assay). However, the mAb 8E7/H8 generated against the recombinant CεH3 domain does not detect any IgE in the culture supernatants, but measures IgE in the serum samples. The serum samples (Nos. 22–31) are listed in chronological order of collection documenting that the storage of IgE was not the reason for generating IgE measurable by 8E7/H8. Thus, IgE synthesized de novo in vitro does not seem to be recognized by 8E7/H8.

Discussion

Since the discovery of IgE, it has been known that reaginic Abs are heat labile. Here we show that most mAbs that we have generated, or obtained from other laboratories, seem to recognize epitopes that disappeared during heat denaturation. Nevertheless, some Abs partly recognized heat denatured IgE, and one antibody (8E7/H8) only interacted with the denatured form of IgE, which was no longer capable of binding to the FcεRI α-chain. Many commercially distributed total IgE determination assays are based on polyclonal antisera. Thus, it is likely that some of these reagents will also recognize IgE with a similar antigenic change or simply denatured IgE, resulting in an overestima-

tion of reaginic, biologically active IgE. Indeed, when we mimicked a polyclonal antiserum by mixing different monoclonal anti-IgE Abs, including the one that recognizes heat-denatured IgE, we obtained IgE levels that were relatively close to those obtained by a commercial assay based on polyclonal Abs. Thus, it may be possible to mimic a polyclonal antiserum with a collection of mAbs, but its use may be questionable. If monoclonal anti-IgE Abs are used to measure IgE, their epitope specificity is crucial: as shown here, Abs against the CεH4 domain even recognized IgE that was heat inactivated at 56°C, and, as shown previously, also IgE fragments [23]. Epitope specificity is also crucial, if soluble α-chain [24] is present in the serum, as it could block the detection of IgE by Abs directed against the binding site for the FcεRI α-chain.

Our results indicate that heat treatment of IgE induces the formation or exposure of new epitopes on the IgE molecule. Using a mAb recognizing such a heat-induced epitope, we could detect IgE in some IgE-myeloma-derived samples as well as in certain patient sera. The different biological and immunological activities of the various IgE preparations could not be attributed to storage conditions or to the purification methods, as both, affinity purification and other standard biochemical methods have been used, resulting in no preferential type of IgE due to a particular method.

Thus, the question arises as to how the conformation of IgE has been altered. As shown recently, preincubation of hapten (NIP)-specific chimeric IgE (JW8-IgE) with carrier protein (BSA)-bound epitopes (NIP) led to a reduced binding capacity of IgE to FcεRI if the epitopes were in excess relative to the binding sites on IgE [8], but it was not clear whether the reduced measurability and the reduced binding capacity of IgE were due to immune complexes effectively hiding IgE or to an actual conformational change in the IgE molecule. However, here we show that in the same experimental setup the binding of IgE to an anti-IgE Ab that detects heat-denatured IgE is increased, thus excluding the immune complex hypothesis. Our data support the hypothesis that antigen binding induces a change or stabilization of the IgE molecule in a conformational state with lower affinity to the FcεRI rather than forming large, inaccessible immune complexes. Since allergen-specific IgE in pure form is not available at present [12, 25], we used the chimeric IgE (JW8-IgE) specific for the hapten NIP as a model [9]. Thus, it can only be speculated whether our conclusion may also be true for IgE specific for natural allergens.

Our data show that no significant levels of altered IgE were detected in culture supernatants of human mononuclear cells stimulated with IL-4 and anti-CD40 Ab. This de

novo synthesized IgE has never encountered its antigen because it resulted from a polyclonal stimulation that primarily favored the outgrowth of naive B cells switching to IgE [12]. Therefore, the postulated conformational change or stabilization upon antigen binding which possibly transforms or fixes IgE in a three-dimensional structure similar to denatured IgE is unlikely to have occurred in this type of IgE derived from cell cultures.

Antigen-induced conformational change in IgE may play a role in allergy diagnosis. According to our data, it is likely that allergens in RAST assays induce the formation or exposure of new epitopes on the IgE molecule. Such a change in the immunogenicity of IgE may result in an underestimation of specific IgE depending on the epitope specificity of the revealing agent. Nevertheless, it is interesting to speculate that eventually the difficulty in determining food-specific IgE may lie in the fact that antigen and specific IgE have formed immune complexes *in vivo*. This would not only make it difficult to capture the specific IgE via the antigen in a RAST assay but also to recognize conformationally altered IgE by some of the commercially available reagents.

In rush hyposensitization therapy an immediate beneficial effect can be observed for which there is no clear explanation. One might speculate that the injected antigen binds to IgE and therefore reduces the binding of IgE to FcεRI. Interestingly, our findings support a theory by Malling et al. [26], namely that immunotherapy induces IgE with reduced or lower affinity for binding to the FcεRI.

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