

Interaction of Human IgE with Fc Epsilon RI Alpha Exposes Hidden Epitopes on IgE

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Key Words

High-affinity IgE receptor · Anti-human IgE monoclonal antibodies · Epitope exposure · Human serum IgE · Allergy

Abstract

Background: Binding of human IgE via the heavy-chain constant region domain 3 (Cε3) to the α-chain of its high affinity receptor (FcεRIα) is a key event in mediating allergic reactions. We wanted to identify epitopes within Cε3 that are stable to denaturation and to evaluate whether such structures are involved in receptor binding. The existence of stable epitopes would facilitate the generation of compounds that inhibit the IgE-FcεRIα interaction. **Methods:** Monoclonal anti-human IgE-antibodies against recombinant bacterially synthesized Cε3, which is known to be partly misfolded, were raised in mice. These antibodies were probed for binding to native, immobilized and receptor-bound IgE, respectively, providing tools for the identification of the indicated stable epitopes. **Results:** Two of the generated antibodies (8E7, 3G9) discriminate between IgE in solution and IgE attached to FcεRIα, pointing towards a steric rearrangement within Cε3 induced upon receptor binding. The described antibodies represent tools for studying the

mechanism of the Fcε-FcεRIα interaction and may be of diagnostic value since serum IgE from various human donors was differently recognized by 8E7, which is indicative for naturally occurring IgE molecules with different steric conformation. **Conclusion:** The presented data support the hypothesis of a conformational change within IgE Cε3 upon receptor binding by showing that monoclonal antibodies raised against recombinant Cε3 differently recognize soluble and receptor-bound IgE. The presence of an IgE portion in sera of human donors that is recognized by 8E7 indicates the existence of IgE molecules in different steric conformations in human blood, which may be related to pathologic parameters.

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Introduction

The attachment of human IgE (hIgE) to its high-affinity receptor (FcεRI) is considered a key event in the development of allergic reactions [1]. On mast cells and basophils, cross-linking of the FcεRI-IgE complex by an antigen results in cellular degranulation and release of a variety of preformed inflammatory mediators, e.g. histamine, serotonin and leukotriene [for a review see ref. 2]. In humans, high-affinity binding of IgE to FcεRI is accomplished by a

complex protein-to-protein interaction involving various parts of the third heavy-chain constant region domain (Cε3) of IgE and the membrane-proximal immunoglobulin-like domain α(2) of the FcεRI subunit [for reviews, see ref. 3, 4]. Although residues within Cε3 and regions belonging to the α(2) domain of FcεRIα have been identified that are important for binding, the detailed mechanism of the binding process still remains to be characterized. Experimental evidence provided by fluorescence energy transfer measurements [5, 6] as well as X-ray and neutron scattering [7] has shown that hIgE adopts a bent structure which is thought to contribute to the high affinity of IgE for FcεRI (Kd ~ 10¹⁰ M⁻¹). Moreover, this bent structure is also postulated to be responsible for the equimolar complex between IgE and cell bound or soluble FcεRIα although the IgE molecule would provide identical epitopes on the two Cε3 domains for receptor binding [8]. Based on data obtained from circular dichroism spectra [9], a conformational rearrangement affecting Cε3 upon receptor bindings has been proposed as an explanation for the 1:1 stoichiometry of the Fcε-FcεRI complex on the cellular surface. The production of biologically active recombinant Fcε and fragments thereof in eukaryotic as well as in bacterial systems has been reported by several groups [10, 11]. Although it was shown that Cε3 is the region exclusively involved in the interaction with FcεRI, the synthesis of a recombinant single Cε3 domain still being able to bind to FcεRIα with high affinity has never been successful. Basu et al. [10] have reported that the smallest human Fc fragment capable of FcεRIα binding is composed of the whole Cε3-Cε4 domains. This has led to the speculation that parts of Fcε other than Cε3, though not being directly involved in receptor binding, have to be present in these constructs in order to facilitate correct folding of the binding Cε3 domain [12–14].

In this report we show that (1) monoclonal antibodies (mAbs) raised in mice against bacterially synthesized Cε3 allow to discriminate between native and artificial parts of the recombinant Cε3 (rCε3); (2) these mAbs discriminate between hIgE in solution and IgE bound to FcεRI, indicating a conformational rearrangement of the Cε3 domain upon receptor binding and (3) that one of the mAbs (8E7) may be of clinical relevance since it discriminates between IgE molecules with different steric conformations in patient sera.

Materials and Methods

Recombinant Proteins and Control Antibodies

Recombinant human Fcε fragments (rCε3, rCε3–4, rCε1–4) were produced by DNA cloning and expression in *Escherichia coli* as previously described [15]. Recombinant soluble FcεRIα consisting of

the extracellular part (Val¹-Leu¹⁷⁹, numbering according to Shimizu et al. [16]) of FcεRIα (ecFcεRIα) expressed in Sf9 insect cells (Invitrogen) was obtained from S. Geisse, Novartis, Basel, Switzerland. Human (h) myeloma IgE, polyclonal (pc.) anti-hIgE antiserum and horseradish peroxidase (HRP)-coupled goat anti-hIgE was obtained from Sigma, St. Louis, Mo., USA. Generation and properties of mouse anti-hIgE mAb BSW17 has been described [17]. Anti-hIgE mAbs 4F4 (Cε3 specific) and 11-3 (Cε2 specific) have been described elsewhere [18]. Pc, rabbit anti-mouse IgG1 was obtained from Pharmacia, Uppsala, Sweden, and goat anti-mouse IgG was from BioRad, Hercules, Calif., USA.

Production of Hybridomas

At day 0, 100 µg of purified rCε3 in 300 µl PBS was emulsified 1:1 with complete Freund's adjuvant and injected intraperitoneally into female Balb/c mice. At days 7 and 28 after the first treatment, the mice were injected once more with 100 µg rCε3 in 300 µl PBS, emulsified 1:1 with incomplete Freund's adjuvant. On days 24, 25, 26 and 27, boosting injections were given intravenously, each containing 50 µg rCε3 in 150 µl PBS (140 mM NaCl, 1.5 mM KH₂PO₄, 2.7 mM KCl, 8.1 mM Na₂HPO₄). On day 28, the mice were sacrificed, the spleens removed and the splenocytes fused with the mouse myeloma cell line SP2 as previously described [19]. Culture supernatants of the resulting hybridomas were screened for high IgG titers and specific anti-rCε3 antibody secretion, by ELISA.

Purification of mAbs

Hybridoma supernatants were adjusted to 3 M NaCl, pH = 8.9, and loaded onto a TSK Protein A column (Merck, Darmstadt, FRG). Bound IgG was eluted with 100 mM CH₃COOH and the peak fractions were immediately neutralized and subsequently dialyzed against a buffer consisting of 10 mM NaH₂PO₄, pH = 6.8, 0.01 mM CaCl₂. Pooled peak fractions from the Protein A column were loaded onto a HP40-100 hydroxyapatite column (Pentax Asahi, Tokyo, Japan) and the antibodies were eluted with 300 mM NaH₂PO₄, pH = 6.8, 0.01 mM CaCl₂. Finally, the eluted peak fractions were pooled and dialyzed against PBS. The purity and integrity of the purified mAbs were monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and nonreducing conditions as well as by analytical size exclusion chromatography (Zorbax GF250; Rockland Technologies, USA). Isotypes of the purified antibodies were determined with the Mouse Typer Sub-Isotyping Kit (BioRad) and were found to be IgG1 κ .

ELISA Experiments

ELISA experiments were carried out in Costar Strip Plate-8 microtiter plates (Costar, Cambridge, Mass., USA). Recombinant ecFcεRIα, recombinant Cε fragments, mAbs or hIgE were immobilized in 100 µl coating buffer (0.1 M NaHCO₃, 0.01% NaN₃, pH = 9.6) in a humidified chamber at 4°C overnight in amounts indicated in the Results section for each experiment. The remaining reactive sites of the microtiter plate were blocked by incubation for 2 h at 37°C with 200 µl per well of 5% BSA (Sigma) dissolved in coating buffer and then incubated for 2 h at 37°C with 100 µl of FCS buffer ((PBS, pH 7.5, 0.05% Tween 20 (Serva, Heidelberg, Germany), 2% FCS (Bio Whittaker, Verviers, Belgium)) with the amounts of ligand specified for each experiment in the Results section. After removal of the incubation mixture, the wells were washed twice with 300 µl of washing buffer (PBS, pH = 7.5, 0.05% Tween 20). The amount of ligand bound to the immobilized proteins was determined by subsequent incubation

Table 1. Recognition of human IgE and rCε1–4 by mAbs generated against bacterially synthesized rCε3

Clone	ELISA							Western blot		
	rCε3	hIgE	hIgE 1 M urea	hIgE 3 M urea	rCε1–4	rCε1–4 1 M urea	rCε1–4 3 M urea	rCε3	hIgE	rCε1–4
2B2	+	+	+	–	+	+	–	+	–	–
1E10	++	–	–	–	+	+	–	–	–	–
8E7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3G9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5H10	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

ELISA: + = OD 405 > 0.1 < 0.5; ++ = OD 405 > 0.5 < 1.5; +++ = OD 405 > 1.5. Western blot: –, +, +++: arbitrary ranking by visual inspection of bands.

for 2 h at 37°C with HRP-conjugated antisera (1:1,000 dilution in FCS buffer), as specified for each experiment. After removal of the incubation mixture and two washing steps, a color reaction was induced using the BioRad HRP Substrate. The optical densities (OD) were determined at 405 nm (Easy Reader, SLT, Labinstruments, Austria). The OD values shown were corrected for background binding to microtiter plate wells coated with BSA only and represent the mean values of measurements made in duplicate.

IgE Determination in Cell Supernatants and Serum

Ten sera from randomly selected individuals visiting an outpatient clinic for an allergy consultation were tested. The mean IgE level was 70.9±40.4 U/ml and was measured by the Kallestad total IgE microplate kit, supplied by Sanofi Pasteur, Solothurn, Switzerland. Highly concentrated samples were diluted to 20 U/ml.

Serum IgE levels were measured by an immunodot assay. Briefly, 1 µl of 500 µg/ml anti-IgE mAb (4F4, Le27, and 11-3) or 380 µg/ml anti-IgE mAb (8E7) was dotted on nitrocellulose (Millipore) as capturing antibodies. The nitrocellulose strips were then blocked with Tris-buffered saline (TBS) containing 1.5 mg/ml casein (Fluka Chemie) for 1 h and incubated with the serum samples overnight. The strips were washed three times with TBS and developed by incubation for 6 h with HRP-conjugated sheept anti-human IgE polyclonal antibody (Binding Site, Birmingham Research Park, Birmingham, UK) diluted 1:1,000 in TBS containing 1.5 mg/ml casein. A second washing step removed the unbound enzyme-labeled antibodies. The dots were subsequently developed with 4-chloro-1-naphthol. The OD (rel. OD) of the color is directly proportional to the concentration of IgE and was determined using a hand-held reflection densitometer (Camag, Muttens, Switzerland).

Results

The interaction of hIgE with its high-affinity receptor FcεRI is known to be complex and to involve relatively large parts of the IgE Cε3 domain and the extracellular part of the FcεRI α-subunit. In this report, we intended to identify surface-exposed epitopes within Cε3 that are relatively stable to denaturation, and to evaluate whether such struc-

tures are involved in receptor binding. The existence of such regions would facilitate the generation of components that inhibit the IgE/FcεRI interaction.

We therefore raised a panel of mouse monoclonal anti-human IgE antibodies against recombinant, bacterially synthesized Cε3 that is known to be partly misfolded. Probing these mAbs for binding to native IgE should provide tools for the identification of the indicated stable epitopes.

Generation of Anti-rCε3 mAbs

Anti-rCε3 mAbs were generated by the standard hybridoma technique. After subcloning, cell supernatants of hybridoma clones selected for strong rCε3 binding were tested in ELISA for binding to bacterially produced Cε1–4 and native hIgE, respectively, each coated at 1 µg per well (table 1).

Supernatants of clones 8E7, 3G9, 5H10 and 2B2 gave positive signals not only with the original immunogen rCε3, but also reacted with immobilized hIgE and the recombinant heavy chain constant region of hIgE (rCε1–4) produced in *E. coli* [15]. Antibodies produced by clone 1E10, however, did not recognize native myeloma IgE, but was still able to bind to the recombinant IgE heavy chain. Treatment of IgE and rCε1–4 for 1 h with 1 M urea prior to immobilization on the ELISA plate had no influence on the binding pattern, whereas 3 M urea treatment resulted in loss of binding of clone 2B2 and 1E10 supernatants to immobilized native IgE as well as to recombinant Cε1–4. These results indicate that hybridomas 8E7, 3G9 and 5H10 produce mAbs directed against an epitope (or epitopes) structurally present not only on immobilized rCε3, but also on Cε1–4 and native myeloma IgE even after partial denaturation. Antibodies secreted by clone 2B2 also recognize a naturally occurring IgE epitope, which is, however, destroyed by treatment with urea at concentrations above 1 M. In con-

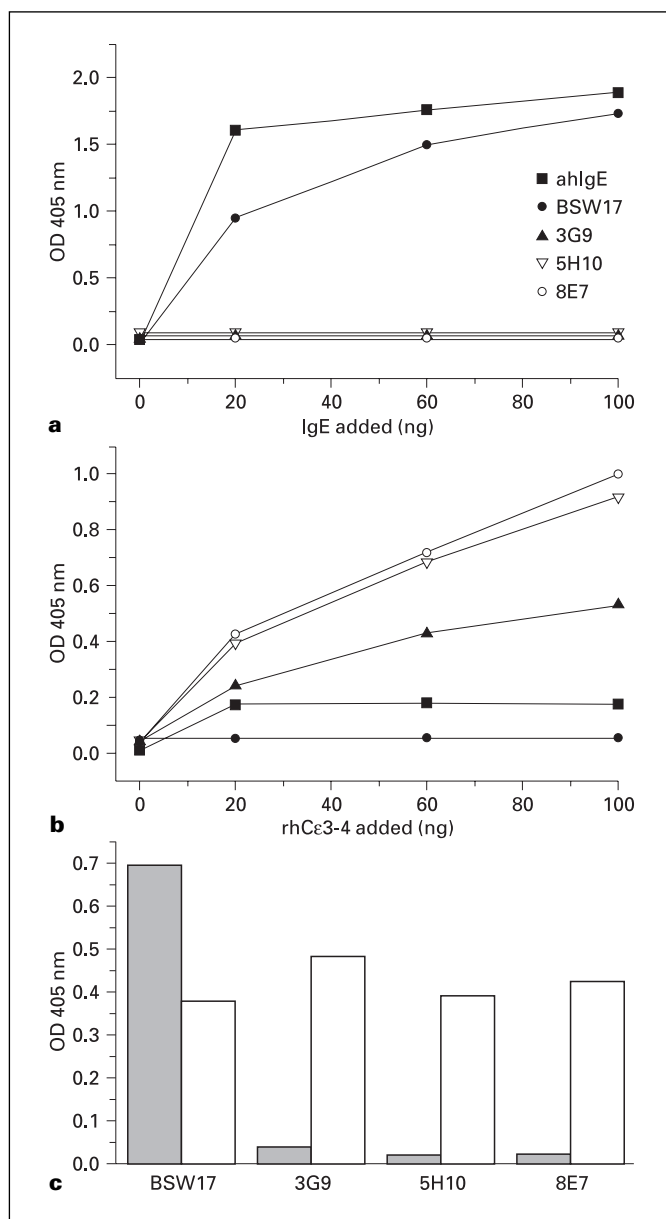


Fig. 1. Differential recognition of nonimmobilized native hIgE and rhCε3-4 by anti-rhCε3 mAbs. 1 μg of antibodies 8E7, 3G9, 5H10, BSW17 and polyclonal anti-hIgE (ahIgE) was coated to microtiter plate wells and incubated with increasing amounts of hIgE (**a**) or rhCε3-4 (**b**). Bound material was detected with goat anti-hIgE-HRP. OD₄₀₅ values were corrected for background binding to BSA-coated wells and represent mean values of duplicates. **c** 1 μg of mAbs 8E7, 3G9, 5H10, BSW17 was preincubated with 1 μg hIgE for 2 h at 25 °C; complexes were captured by immobilized polyclonal rabbit anti-mouse IgG1 and detected with polyclonal goat anti-hIgE-HRP (gray bars). The white bars represent the binding of mAbs to coated polyclonal rabbit anti-mouse IgG1 detected with polyclonal goat anti-mouse IgG-HRP. OD values were corrected for unspecific binding and represent mean values of duplicates.

trast, the epitope specific for 1E10 Ab is only presented by immobilized rCε3 and, to a certain extent, by rCε1-4, indicating an artificial structure generated by misfolding of the rCε3 domain. The signal pattern obtained from Western blots after SDS-PAGE of Cε3, hIgE, and rCε1-4 under reducing conditions are also shown in table 1: immobilized hIgE could only be detected with 8E7, 3G9 and 5H10, whereas 2B2 gave no signal. Furthermore, mAb 1E10 did not even recognize the original immunogen rCε3.

IgE isotype specificity of the anti-rCε3 domain mAbs was confirmed by ELISA (data not shown). Only 3G9 revealed partial cross-reactivity with other isotypes (IgM, IgG and IgA), whereas 8E7 and 5H10 significantly cross-reacted only with hIgG and hIgA.

Although all mAbs generated against rhCε3 except 1E10 were found to recognize epitopes that are present in immobilized native IgE, none of these mAbs was able to inhibit the binding of IgE to FcεRI (data not shown). This indicates that the epitope(s) recognized by the anti-rhCε3 mAbs is (are) not involved in the receptor-binding process although residing within the Cε3 domain of IgE.

Differential Recognition of Nonimmobilized Native hIgE and rhCε3-4 by Anti-rCε3 mAbs

Since mAbs 2B2 and 1E10 were found to recognize native IgE only weakly or not at all and the hybridoma producing 2B2 proved to be unstable, only the strongly IgE binding mAbs 8E7, 3G9 and 5H10 were used in the following experiments. As described, the binding data summarized in table 1 were obtained with native IgE and recombinant Fcε fragments immobilized in microtiter plate wells or membranes. It is common knowledge that fixation of proteins to solid surfaces or separation by SDS-PAGE leads to partial denaturation of the three-dimensional structures of native proteins. To test whether the anti-Cε3 mAbs which were found to strongly recognize immobilized natural IgE are also capable of binding to native hIgE in solution, 1 μg of 8E7, 3G9, and 5H10 were each coated to microtiter plate wells and subsequently incubated with increasing amounts of either human myeloma IgE or purified rhCε3-4 fragment, also produced in *E. coli*. As references, commercially available polyclonal anti-hIgE and mAb BSW17 were included in the experiment. Figure 1 shows binding of native myeloma IgE (fig. 1a) and rhCε3-4 (fig. 1b), respectively, to the various mAbs. Two significantly different binding patterns were observed: myeloma IgE was only able to bind to polyclonal anti-hIgE and BSW17, but was not recognized by the anti-rhCε3 mAbs. In contrast, bacterially synthesized rhCε3-4 was strongly recognized by the immobilized anti-rhCε3 mAbs but only weakly by polyclonal anti-hIgE and

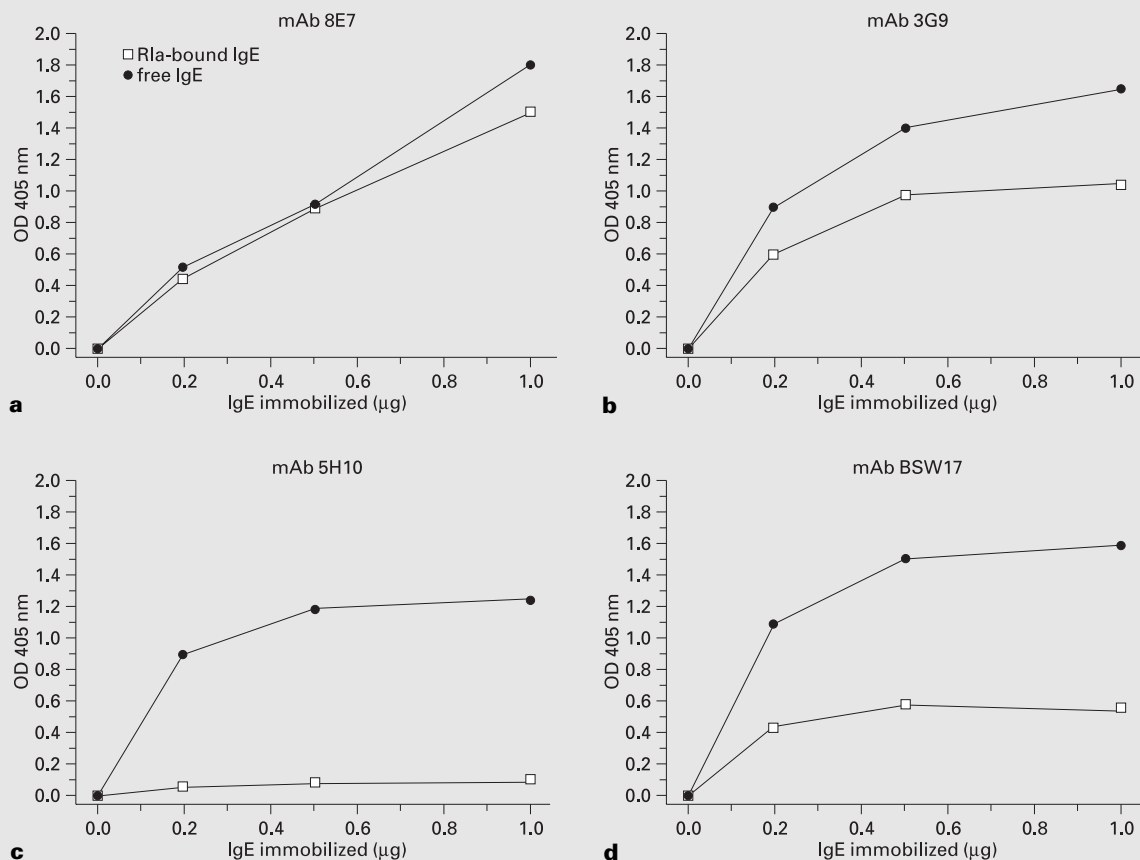


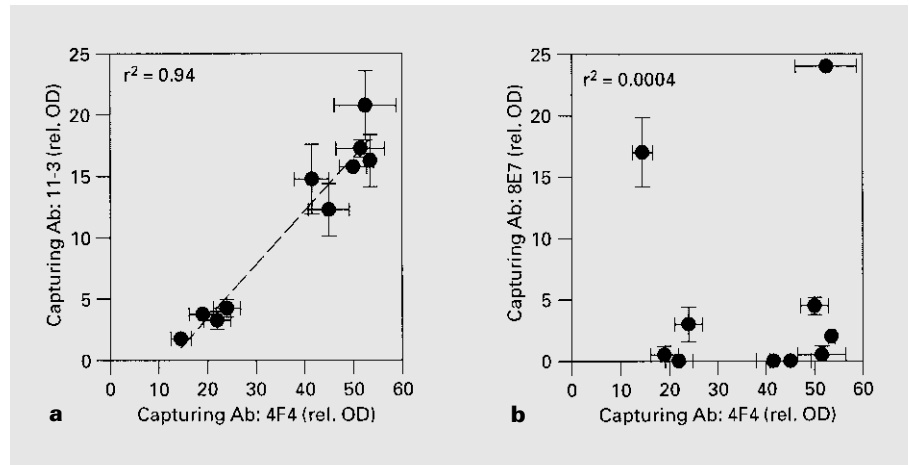
Fig. 2. Recognition of IgE by anti-rhCε3 mAbs. Increasing amounts from 0 to 1 μg of hIgE was either directly immobilized to the surface of microtiter plate wells ('free IgE') or allowed to attach to ecFcεRIα produced in insect cells ('RIα bound IgE') immobilized at a concentration of 1 μg/well. After washing, a constant amount of 5 μg/ml of the anti-Cε3 mAbs 8E7 (**a**), 3G9 (**b**), 5H10 (**c**) and BSW17 (**d**), respectively, was added in a second incubation followed by a third incubation step with a polyclonal goat anti-mouse IgG-HRP conjugate and color reaction using chromogenic substrate. OD₄₀₅ values were corrected for background binding to BSA-coated wells and represent mean values of duplicates. Each panel represents the binding curves obtained with 1 mAb as indicated.

almost not at all by BSW17. The experiment shown in figure 1a was performed with immobilized mAbs. Immobilization to solid surfaces may in principle lead to inactivation of the mAbs. Therefore we repeated the experiments with both mAbs and hIgE in solution (fig. 1c). Antibodies were preincubated with hIgE; formed complexes were captured with immobilized polyclonal rabbit anti-mouse IgG1 and detected with goat anti-human IgE-HRP. In a parallel control experiment, the ability of the mAbs to recognize immobilized rabbit anti-mouse IgG1 was tested, which is a prerequisite for the detection in the preincubation experiment. As can be seen in figure 3c, mAbs recognized rabbit

anti-mouse IgG1 almost equally well. Nevertheless, hIgE/mAb complexes could only be detected with BSW17, indicating that mAbs 3G9, 5H10 and 8E7 do not bind to hIgE when in solution. Therefore, mAb inactivation due to fixation to the microtiter plate can be ruled out in figure 1a.

These results demonstrate that the epitope(s) recognized by the anti-rhCε3 mAbs is (are) not accessible in nondenatured human myeloma IgE in solution but is (are) exposed to the surface after binding of IgE to the plastic wells (table 1). The bacterially synthesized rCε3-4 fragment (and – since it is used as immunogen – also rhCε3), however, expose the epitope region(s) also in solution.

Fig. 3. IgE levels in sera measured by mAb 8E7. Serum samples of 10 individuals were prediluted to an equal IgE level (according to a commercial assay), subsequently measured by capturing with three different mAbs (11-3, 4F4, and 8E7), and revealed by sheep anti-human IgE polyclonal antibody labeled with HRP. IgE levels measured by 4F4 were correlated to 11-3 (**a**) or 8E7 (**b**) respectively (correlation coefficient = r^2). Data represent mean values of duplicates $\pm$ SD.



Recognition of Fc ϵ RI α -Bound IgE by mAbs Raised Against Bacterially Synthesized rhC ϵ 3

It has been speculated that conformational changes of parts of the IgE molecule accompany both receptor attachment and allergen binding [7–9, 20]. Since the anti-rhC ϵ 3 mAbs described in this report were found to recognize an epitope within C ϵ 3 that is hidden in solution but accessible when IgE is coated to microtiter plate wells, we tested these mAbs also for their capability of recognizing IgE bound to Fc ϵ RI α . Microtiter plate wells were each coated with 1 μ g ecFc ϵ RI α and incubated with increasing amounts of human myeloma IgE. After attachment of the IgE to the receptor, plates were washed and treated in a second incubation with a constant amount of 5 μ g/ml of 8E7, 3G9, 5H10 and BSW17, respectively. Anti-IgE mAbs bound to ecFc ϵ RI α -attached IgE were determined by incubation with goat anti-mouse IgG-HRP and chromogenic substrate. To confirm the binding data shown in table 1, microtiter plate wells were directly coated with increasing amounts of human myeloma IgE ('free IgE') and incubated with 5 μ g/ml of the respective antibody and developed with goat anti-mouse IgG-HRP. The results of this experiment summarized in figure 2 show that mAbs 8E7 and 3G9 were able to bind to IgE which had previously been attached to ecFc ϵ RI α , whereas mAb 5H10 recognized receptor-bound IgE only weakly. Together with the results described in the previous sections, it can be concluded that binding of IgE to Fc ϵ RI leads to the exposure of the 8E7 and 3G9 specific epitope(s) that is (are) hidden in IgE in solution. The epitope recognized by 5H10, however, is not accessible for efficient antibody binding after receptor attachment of IgE. Furthermore, the results shown in figure 2 indicate that 5H10 recognizes a different epitope within C ϵ 3 as compared to 8E7 and/or 3G9.

Only Some Serum Samples Contain IgE That Reacts with mAb 8E7

Next, we investigated whether serum IgE from 10 randomly selected individuals exposes 8E7-specific epitopes. IgE levels of serum samples were determined by dot-blot sandwich immunoassays using different monoclonal anti-IgE Abs on the solid phase (fig. 3). Our data show that mAb 8E7 detected IgE only in some of the serum samples. There was no statistical correlation between IgE levels measured by 8E7 and any of the control mAbs (Le27, 11-3, and 4F4) used in this experiment (data not shown). In contrast, there was a high correlation in the IgE levels determined by the different control mAbs Le27, 11-3, and 4F4. Interestingly, mAb 8E7 did not react with IgE from culture supernatants of mononuclear cells from the same donors (data not shown). Limited shelf-life of IgE was therefore not the reason for the different IgE measurability by 8E7.

Discussion

Fc ϵ RI cross-linking via binding of polyvalent allergens to IgE attached to the receptor is part of the main route of events inducing anaphylactic reactions. Additionally, this can also be achieved by (1) binding of anti-IgE antibodies to receptor-bound IgE [17] or (2) independently of IgE by attachment of antibodies directed against Fc ϵ RI α to the receptor [21]. This has made the detailed study of the IgE-Fc ϵ RI α interaction attractive for both scientific reasons and as a potential target for the development of anti-allergic drugs. From physical measurements [9], indications for a conformational rearrangement affecting C ϵ 3 upon receptor binding can be deduced, which might contribute to the

monovalence of the IgE-FcεRIα complex on the cell surface and to the high binding affinity with a K_d of about $10^{10} M^{-1}$.

In this report we present data supporting this hypothesis by showing that mAbs raised against bacterially synthesized recombinant Cε3 (rhCε3) differentially recognize soluble and receptor-bound IgE. The recombinant protein used for immunization was recovered and refolded from bacterial inclusion bodies using a chaotropic agent and was able to bind FcεRIα only weakly (data not shown). It was thus likely to contain incorrectly folded peptide domains, resulting in the formation of both entirely artificial three-dimensional structures and structures also present in native IgE but embedded in a different three-dimensional environment which might lead to the presentation of immunogenic epitopes which are present in immobilized IgE but hidden in native IgE. Only one (1E10) of the five mAbs obtained after immunization of mice with rhCε3 was identified to recognize an (obviously artificial) epitope which is displayed only by recombinant Fcε fragments and not by native IgE. The epitope(s) recognized by the mAbs 8E7, 3G9 and 5H10 are present in both recombinant and native Fcε and remained stable even after partial denaturation of the proteins in 1 M urea indicating that these mAbs recognize a linear peptide rather than a conformational epitope within Cε3 (table 1). None of the anti-rhCε3 mAbs was found to interfere with binding of IgE to FcεRIα. Considered by itself, this observation is surprising if one takes into account that a relatively large part of the Cε3 domain involving widely separated amino acid residues is thought to take part in the receptor-binding process [5, 10, 11]. However, the results presented in figure 1a provide an explanation for the general incapability of the anti-rhCε3 mAbs to inhibit IgE-FcεRIα binding by showing that the epitope(s) specific for 8E7, 3G9 and 5H10 is (are) hidden in native IgE in solution (fig. 1c), thus preventing the formation of inhibitory immune complexes. In contrast, as a prerequisite for competitive receptor binding inhibition, such a complex was demonstrated for the control mAb BSW17. The structural differences between native IgE and recombinant Fcε fragments resulting in loss or decreased affinity of the recombinant molecules for FcεRIα are also evident from the data presented in figure 1b showing that the anti-rhCε3 mAbs readily recognized these fragments in solution. Moreover, the conformational epitope for BSW17 – a mAb that has been raised against native IgE – was found not to be accessible in rCε3–4. Since the recombinant Fcε fragments used in the experiments described in this report were generated by gene expression in *E. coli*, it cannot be ruled out that these effects are at least partially caused by the lack of gly-

cosylation of the bacterially synthesized rhFcε fragments. Most of the proposed models for the interaction between IgE and FcεRIα [22] agree on the concept of a conformation change (specifically in Cε3). Our results also point towards a conformational change within Cε3 upon binding to ecFcεRIα. This was demonstrated by showing that mAb8E7 and 3G9 are able to bind native IgE attached to FcεRIα (fig. 2) but do not recognize IgE in solution (fig. 1a, c). Thus we assume that the process of receptor binding induces a conformational change within Fcε which results in surface exposure of the 8E7 and 3G9 epitope(s), which is (are) not accessible in IgE in solution. In contrast to 8E7 and 3G9, mAb 5H10 recognizes receptor-bound IgE only very weakly, indicating that the epitope specific for 5H10 is different from that (those) of 8E7 and 3G9 and not surface exposed after receptor binding. Furthermore, the differential recognition of receptor-bound IgE by 8E7, 3G9 and 5H10 as compared to IgE immobilized in microtiter plate wells clearly distinguishes the conformational rearrangement of Fcε induced by high-affinity receptor binding from the more partial denaturation of the protein induced by binding of IgE to the hydrophobic plastic surface. The experiment presented in figure 2 does not allow to distinguish whether binding of 8E7 or 3G9 to the IgE/FcεRIα complex is affected via attachment to the Cε3 epitope(s) present on the receptor-bound heavy chain or the symmetrical Cε3 domain on the distal non-receptor-bound heavy chain. Therefore, it cannot be deduced whether the structural change after receptor binding is mainly restricted to Cε3 or rather results in an alteration of the steric positions of the constant heavy chain domains Cε2, Cε3 and Cε4 relative to each other. Experimental evidence supporting the former has been provided by circular dichroism spectroscopy [9] whereas indications for the latter exist from neutron-scattering data [7]. Recently, Keown et al. [23] reported that no change in secondary structure occurs upon IgE-FcεRI complex formation and therefore postulated that tertiary structure changes must be limited to the relative disposition of Cε3 and Cε4 of IgE-Fc or the immunoglobulin-like domains of FcεRIα.

The experimental approach described in this report leads to similar conclusions, but adds a biological dimension to the understanding of the complex structure-function relationship governing this receptor-ligand interaction which is of central importance in the immune system: the altered IgE epitope presentation may induce the formation of a special type of anti-IgE autoantibodies in vivo which primarily recognize receptor-bound IgE. This affinity for receptor-bound IgE would render such naturally occurring antibodies preferential candidates for antigen-independent IgE receptor triggering. Indeed, autoantibodies to human IgE have been

reported to be present in a variety of atopic and inflammatory disorders [24, 25] and documented as either anaphylactogenic or not [26]. Furthermore, a large proportion of circulating IgE exist in immune complexes with anti-IgE autoantibodies [27] having implications on the significance of diagnostic IgE determination [28]. This hypothesis is strengthened by demonstrating that an IgE portion is present in sera of human donors which is recognized by mAb

8E7, indicating the existence of IgE molecules in different steric conformations in human blood (fig. 3).

A correlation of the amount of such IgE variants with pathologic parameters is under investigation. If such a correlation can be demonstrated, mAb 8E7 (and 3G9) would represent valuable diagnostic tools for differential diagnostic IgE determination.

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