

A molecular model of type I allergy: Identification and characterization of a nonanaphylactic anti-human IgE antibody fragment that blocks the IgE-Fc ϵ RI interaction and reacts with receptor-bound IgE

Sylvia Laffer, PhD,^a Erik Hogbom, PhD,^b Kenneth H. Roux, PhD,^c Wolfgang R. Sperr, MD,^d Peter Valent, MD,^d Hans C. Bankl, MD,^e Luca Vangelista, PhD,^f Franz Kricek, PhD,^g Dietrich Kraft, MD,^a Hans Grönlund, MSc,^b and Rudolf Valenta, MD^a Vienna, Austria, Uppsala, Sweden, Tallahassee, Fla, and Heidelberg, Germany

Background: The IgE-mediated activation of effector cells and antigen-presenting cells through the high-affinity receptor for IgE (Fc ϵ RI) represents a key pathomechanism in type I allergy and many forms of asthma.

Objective: We sought to establish an *in vitro* molecular model for the interaction of human Fc ϵ RI, IgE, and the corresponding allergen and to identify monoclonal anti-human IgE antibodies with a therapeutic profile different from previously established anti-IgE antibodies.

Methods: Human Fc ϵ RI α chain, a human monoclonal allergen-specific IgE antibody (chimeric Bip 1), and the corresponding allergen, the major birch pollen allergen Bet v 1, were produced as recombinant proteins and analyzed by means of circular dichroism and native overlays, respectively. Using this molecular model, as well as negative stain immunoelectron microscopic analysis, and *in vitro* cultivated human basophils, we characterized mouse anti-human IgE antibodies. **Results:** We established a molecular model for the interaction of human IgE with Fc ϵ RI. Using this molecular model, we identified a nonanaphylactic anti-human IgE antibody fragment (Fab12), which blocked the IgE-Fc ϵ RI interaction and reacted with effector cell-bound IgE.

Conclusion: Fab12 represents a candidate molecule for therapy of atopy and asthma because it can be used for the depletion of circulating IgE antibodies, as well as for the depletion of IgE-bearing cells. (*J Allergy Clin Immunol* 2001;108:409-16.)

Key words: Allergy, asthma, IgE, Fc ϵ RI, competitor, therapy

Allergen-mediated crosslinking of IgE antibodies bound to effector cells (eg, mast cells and basophils) through the high-affinity receptor for IgE (Fc ϵ RI) induces the immediate release of biologically active mediators (eg, histamine and leukotrienes) and causes the acute symptoms of atopy (eg, allergic rhinitis, conjunctivitis, asthma, and anaphylactic shock).¹ When allergens are presented by means of IgE-Fc ϵ RI on antigen-presenting cells (eg, monocytes and dendritic cells), T cells become activated and release T_H2 cytokines, thus leading to chronic, delayed disease manifestations (eg, atopic dermatitis and chronic asthma).^{2,3}

Therefore the interaction of allergens, allergen-specific IgE, and Fc ϵ RI represents a key pathomechanism in atopy. Since the identification and characterization of IgE and Fc ϵ RI, considerable effort has been spent in the analysis of their interactive domains, particularly in identifying competitors of this interaction.⁴

To establish a molecular *in vitro* model for the interaction of a major allergen, its corresponding IgE, and Fc ϵ RI, we have constructed a chimeric monoclonal IgE antibody, Bip 1, with specificity for the major birch pollen allergen Bet v 1.^{5,6} We analyzed the secondary structure composition and fold of purified chimeric Bip 1 and Fc ϵ RI α chain by means of circular dichroism (CD) analysis and investigated their interaction with overlay experiments. Using the molecular system, we screened a panel of 25 mouse monoclonal anti-human IgE antibodies for mAbs that inhibit the IgE-Fc ϵ RI interaction. One of the blocking antibodies obtained, mAb 12, differed from previously characterized anti-IgE antibodies because it strongly inhibited the IgE-Fc ϵ RI interaction and recognized Fc ϵ RI α chain-bound and basophil-bound IgE antibodies. The binding sites of mAb 12 were visualized and mapped by using negative stain immunoelectron microscopy. The potential of mAb 12 to inactivate human basophils and the anaphylactic properties of mAb 12 versus an mAb 12-derived antibody fragment (Fab) were investigated by using basophil histamine release experiments. We com-

From ^athe Department of Pathophysiology, AKH, University of Vienna, Vienna; ^bPharmacia Diagnostics, Uppsala; ^cthe Department of Biological Science, Biology Unit I, Florida State University, Tallahassee; ^dthe Department of Internal Medicine I, Division of Hematology and ^ethe Department of Pathology, AKH, University of Vienna, Vienna; ^fEMBL-Structural Biology Group, Heidelberg; and ^gNovartis-Research Center, Vienna.

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Reprint requests: Rudolf Valenta, MD, Molecular Immunopathology Group, Department of Pathophysiology, AKH, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.

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Abbreviations used

CD:	Circular dichroism
Fab:	Antibody fragment
HRB:	Histamine release buffer
PMN:	Polymorphonuclear leukocyte
VH:	Variable immunoglobulin heavy chain region

ment on the usefulness of the molecular in vitro allergy model for the in vitro selection of competitors of the IgE-FcεRI interaction and discuss Fab12-based strategies for therapy of allergy and asthma.

METHODS**Generation and characterization of the chimeric Bet v 1-specific human monoclonal IgE antibody Bip 1**

The Bip 1 variable immunoglobulin heavy chain region (VH) was amplified by use of the Recombinant Phage Antibody System: Mouse ScFv Module (Amersham Pharmacia Biotech) from Bip 1 hybridoma cells.⁶ The human VH-4 leader sequence (L) was amplified with PCR from the plasmid pUCVH-4 (Hans Grönlund, unpublished data) and subsequently fused to the Bip 1 VH region by means of PCR. The LVH fragment was subcloned into the *Clal/SpeI* sites of the ϵ -expression plasmid (Hans Grönlund, unpublished data). The construct was introduced into the Bip 1 hybridoma cell line by means of electroporation (Gene Pulser, Biorad). Fusion to SP2/0 cells was performed by use of polyethylene glycol. Clones producing only IgE were identified by using ELISA and subcloned to generate monoclonal cell lines.

An affinity column was prepared by coupling 5 mg of recombinant (r) Bet v 1 to an AminoLink column (Pierce). Cell culture supernatant containing chimeric Bip 1 IgE antibodies were applied to the Bet v 1 affinity column. The column was washed with PBS, and bound chimeric Bip 1 IgE was eluted with 5 mol/L MgCl₂.

Recombinant proteins, iodine 125 labeling of purified proteins, and antibodies

Recombinant birch pollen allergen,⁵ Bet v 1, was expressed in *Escherichia coli* and purified as previously described.⁷ Recombinant baculovirus-expressed α chain of human FcεRI was purified (Novartis-Research Center).⁸ Comparable amounts (30 μg) of purified proteins were iodine 125 (¹²⁵I) labeled by using the chloramine-T method and purified through a Sephadex PD10 column (Pharmacia).⁹ ¹²⁵I-labeled anti-human IgE antibodies (RAST) were purchased from Pharmacia & Upjohn. The mouse monoclonal IgG1 anti-birch profilin antibody 4A6 is described.¹⁰ The monoclonal anti-human IgE antibodies Le27 and BSW17¹¹ were kindly provided by Novartis. A panel of 25 mouse monoclonal anti-human IgE antibodies was raised by immunization of Balb/c mice with purified IgE (ND) Fc.¹² Monoclonal antibody 12 was produced in roller bottles in Dulbecco's modified Eagle's medium. The resulting mAb 12-containing 0.45 μm-filtered medium (Millipore) was purified by using the FPLC system over a 10-cm XK50 protein A Sepharose 4FF column (Pharmacia) with a linear flow rate of 30 cm/h and eluted with 0.1 mol/L citrate buffer (pH = 5.0). The resulting mAb-containing peak was neutralized with 1.0 mol/L NaOH and concentrated in an Amicon ultrafiltration cell with a PM 30 filter to a final concentration of 5 mg/mL, followed by size exclusion chromatography on a 100-cm XK50 Superdex 200-pg column (Pharmacia) equilibrated in 0.02 mol/L PBS (pH = 7.4). Purity was determined to exceed 95% according to SDS-PAGE (Phast System, Pharmacia). Fab12 was produced from purified mAb 12 by means of papain digestion with an ImmunoPure Fab preparation kit (Pierce).

CD measurements

CD spectra were recorded on a Jasco J-710 spectropolarimeter, as previously described.⁶ Far-ultraviolet CD spectra were recorded at 20°C in 2-mm path-length quartz cuvettes (Hellma). Protein concentrations were 1.1 μmol/L (FcεRI α chain) and 1.4 μmol/L (chimeric Bip 1). All measurements were performed in 50 mmol/L NaH₂PO₄, 50 mmol/L Na₂HPO₄, and 10 mmol/L NaCl (pH 7.2).

Native blot overlays and overlay competition experiments

Screening for monoclonal anti-human IgE antibodies that can inhibit the interaction of chimeric Bip 1 and α chain was performed by using overlay competition experiments. A panel of 25 mouse monoclonal anti-human IgE antibodies and, for control purposes, an isotype-matched mAb (4A6)¹⁰ without specificity for human IgE, a mouse monoclonal antibody (BSW17) that inhibits binding of native human IgE to FcεRI,^{11,13} purified recombinant FcεRI α chain, or buffer alone were tested for their capacity to inhibit binding of chimeric Bip 1 IgE to nitrocellulose-bound FcεRI α chain. Chimeric Bip 1 IgE was preabsorbed with 50 μg/mL anti-human IgE antibodies and control reagents for 4 hours at 4°C.

The interaction of rBet v 1, chimeric Bip 1, and recombinant FcεRI α chain was studied by using native overlays.¹⁴

Immunoelectron microscopy

Immunoelectron microscopic analysis of mAbs and immune complexes was performed by using negative staining, as previously described.^{15,16} IgE was viewed alone and in complex with whole or mAb 12 F(ab')₂ fragments. Monoclonal antibody 12 was digested with pepsin-agarose (Pierce) overnight at 37°C, according to the manufacturer's instructions. The F(ab')₂ fraction was separated from other digestion products by means of HPLC. Reactants (at approximately 1 mg/mL each) were mixed in borate-buffered saline and incubated at room temperature for 30 minutes. After incubation, the reactants were allowed to bind to carbon membranes, stained with uranyl formate, and mounted on copper grids for analysis. Electron micrographs were recorded at 100,000-fold magnification on a JEOL CX 1200 electron microscope.

Combined toluidine blue/immunofluorescence staining of human basophils

Reactivity of monoclonal anti-human IgE antibodies to basophil-bound IgE was analyzed by using a double-staining technique with toluidine blue and indirect immunofluorescence, as previously described.¹⁷ Mononuclear cells enriched from the peripheral blood of 2 healthy volunteers were incubated with anti-human IgE mAb 12 for 30 minutes at 4°C. After incubation, mononuclear cells were washed twice in PBS and then exposed to fluorescein-labeled goat F(ab')₂ anti-mouse IgG for 30 minutes at 4°C. Cells were fixed in 0.025% glutaraldehyde solution for 1 minute and incubated with toluidine blue (0.0125%) for 12 minutes at room temperature. After washing, cells were analyzed with a fluorescence microscope (Olympus).

Histamine release experiments

Histamine release experiments were performed with peripheral blood polymorphonuclear leukocytes (PMNs) from allergic or healthy volunteers by using rBet v 1, anti-human IgE antibodies E-124-2-8/De2 (Immunotech), mAb 12, Le27, BSW17, and Fab12. PMNs were enriched from heparinized blood samples with Dextran T70, washed, and resuspended in histamine release buffer (HRB; 25 mmol/L TRIS [pH = 7.6], 5 mmol/L KCl, 130 mmol/L NaCl, and 0.33 mg/mL human serum albumin). HRB supplemented with 0.6

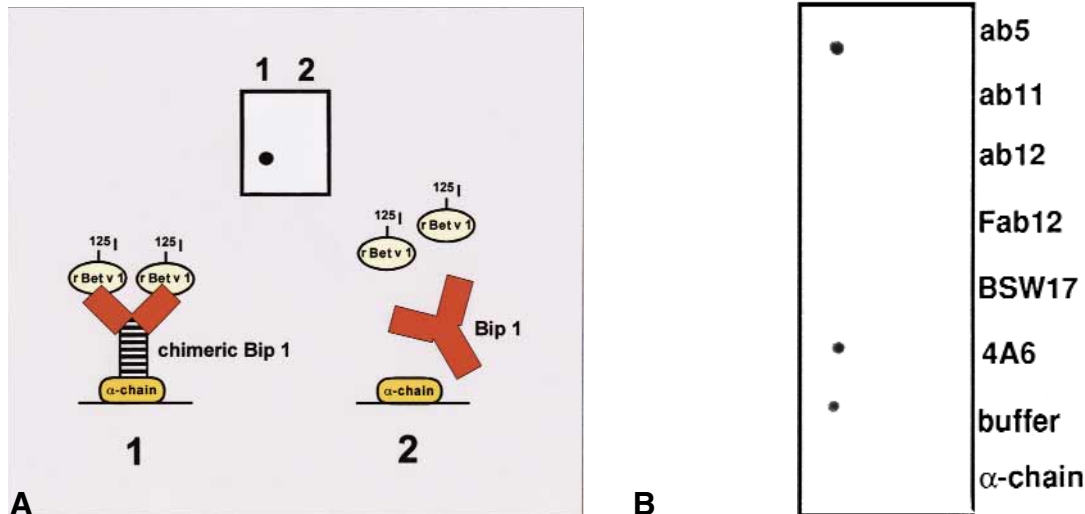


FIG 1. A, Specific interaction of rBet v 1, chimeric Bip 1 IgE, and FcεRI α chain. Nitrocellulose-bound recombinant FcεRI α chain can bind ¹²⁵I-labeled rBet v 1 through chimeric Bip 1 IgE (*panel 1*) but not through mouse-derived Bip 1 (*panel 2*). The upper part of the figure shows the result of the autoradiograph, and the lower part shows the scheme of the *in vitro* system. **B**, Identification of monoclonal anti-human IgE antibodies that inhibit IgE binding to FcεRI. Nitrocellulose-bound recombinant α chain was exposed to chimeric Bip 1 IgE, which was preincubated with monoclonal anti-human IgE antibodies (ab5, ab11, ab12, and BSW17), Fab fragments prepared from mAb 12 (Fab12), an isotype-matched control antibody (4A6), buffer alone, or recombinant α chain. Bound chimeric Bip 1 IgE was detected with ¹²⁵I-labeled recombinant Bet v 1.

mmol/L Ca²⁺ and 1 mmol/L Mg²⁺ was used in histamine release experiments. The capacity of anti-human IgE antibodies or recombinant allergens to induce basophil histamine release by means of crosslinking of FcεRI-bound IgE antibodies was tested by exposing PMNs to various concentrations of reactants at 37°C for 30 minutes and measuring liberated histamine, as previously described.¹⁸ The desensitizing effect of mAb 12 on allergen-induced or anti-IgE-induced histamine release was analyzed as previously described.¹⁹ Briefly, PMNs were first incubated either with (1) HRB containing 0.01 mol/L EDTA, (2) HRB containing 0.01 mol/L EDTA and mAb 12 (1 μg/mL), or (3) HRB containing 0.01 mol/L EDTA and an anti-human IgM antibody (control antibody, 1 μg/mL, PharMingen) for 10 minutes at 37°C. Then cells were washed and incubated with various concentrations of rBet v 1 (0.01, 0.1, 1.0, or 10 μg/mL) or mAb 12 (0.01, 0.1, 1.0, or 10 μg/mL) diluted in HRB for 30 minutes at 37°C.

RESULTS

Analysis of purified chimeric Bip 1 and α chain by means of overlay experiments and CD spectroscopy

By using native overlays, we demonstrated that nitrocellulose-bound FcεRI α chain specifically binds chimeric Bip 1 IgE antibodies (Fig 1, A, panel 1) but not mouse-derived Bip 1 IgG1 antibodies (Fig 1, A, panel 2).

Far-UV CD analysis showed that the purified molecules were folded (data not shown). The far-UV CD spectra of both chimeric Bip 1- FcεRI α chain and chimeric Bip 1-mAb 12 complexes were representative of proteins in all β conformations, but the conformational change reported to occur on binding of FcεRI α chain to IgE²⁰ was not evident in our experimental system (data not shown), a finding that is in accordance with that of

Keown et al.²¹ In addition, we could not detect a conformational change in the far-UV CD region on binding of mAb 12 to chimeric Bip 1 (data not shown).

Identification of anti-human IgE antibodies and an anti-human IgE Fab that inhibit IgE binding to FcεRI by using the *in vitro* allergy model

The usefulness of the chimeric Bip 1-FcεRI α-chain model for the identification of competitors of the IgE-FcεRI interaction was demonstrated by screening 25 mouse monoclonal anti-human IgE antibodies. As exemplified in Fig 1, B, we found antibodies that did not block binding of chimeric Bip 1 to nitrocellulose-bound α chain (eg, ab5, Fig 1, B), whereas others abolished IgE binding to the FcεRI α chain (eg, ab11 and ab12, Fig 1, B). Monoclonal antibody 12-derived Fabs were also able to inhibit IgE binding to FcεRI α chain (Fab12, Fig 1, B). As controls, we tested a monoclonal anti-human IgE antibody, BSW 17, which reportedly inhibited the IgE-FcεRI interaction,^{11,13} and FcεRI α chain to demonstrate the autoinhibition (Fig 1, B). An isotype-matched control antibody, 4A6,¹⁰ without specificity for human IgE and buffer alone failed to block the IgE-FcεRI interaction (Fig 1, B).

Negative staining immunoelectron microscopy of chimeric Bip 1 and chimeric Bip 1/mAb 12 F(ab')₂ complexes

When IgE was mixed with a molar excess of mAb 12-derived F(ab')₂, the majority of molecules formed cross-shaped figures with 2 longer pairs of arms at right

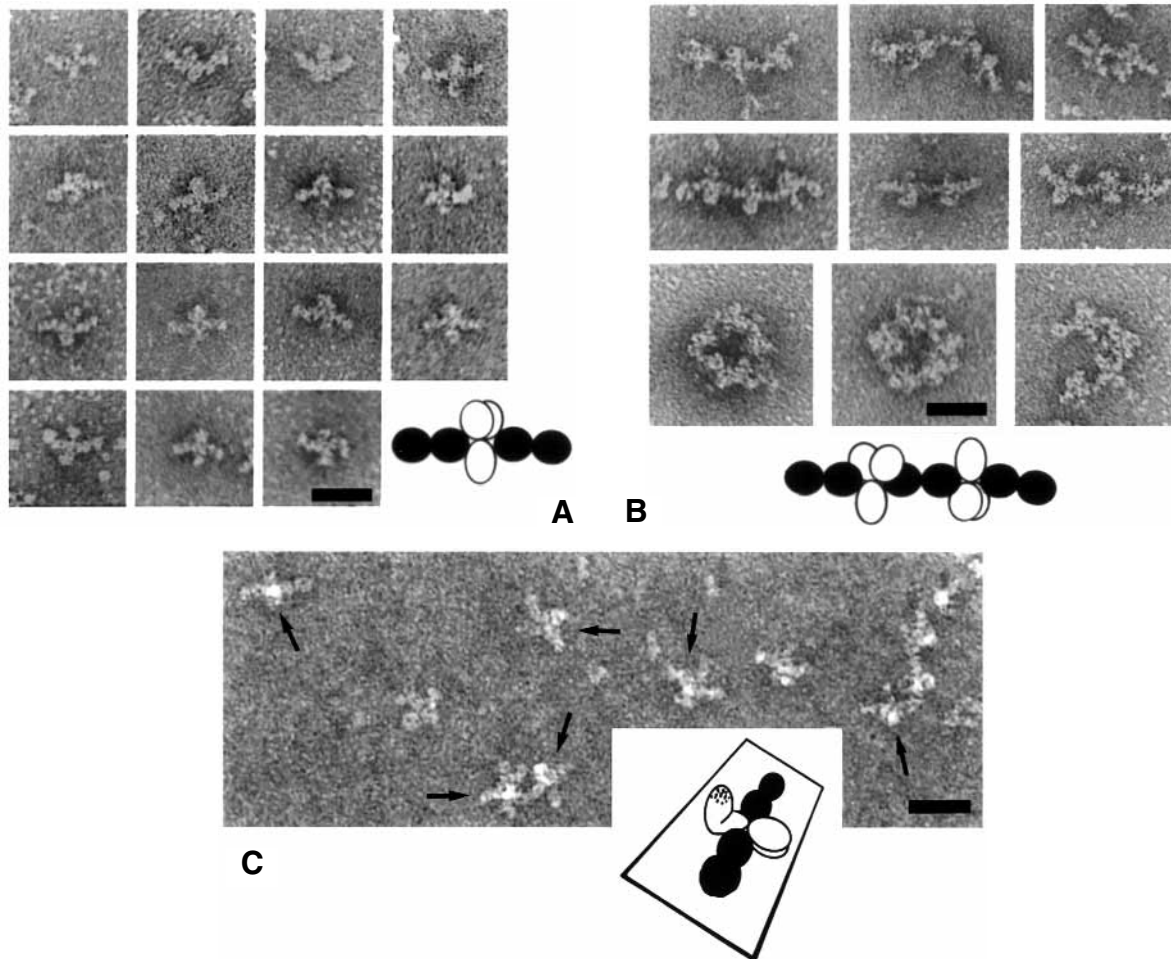


FIG 2. Electron micrographs. Electron micrographic gallery and interpretive diagram of IgE in complex with a molar excess (**A**) or with an equimolar amount (**B**) of mAb 12 F(ab')₂. **C** shows IgE reacted with a molar excess of mAb 12 F(ab')₂. An upturned C-terminal region of IgE Fc is indicated by *stipples*. *Arrows* indicate regions where the protein is thickest in the z-axis (**C**). All *bars* correspond to 25 nm. *Open figures* in the diagrams represent IgE, and *solid figures* represent mAb 12 F(ab')₂.

angles to 2 or 3 shorter arms (Fig 2, *A*). When mixed at molar equivalence, chains and, less frequently, rings of molecules were observed (Fig 2, *B*). Closer examination revealed that the longer arms are composed of 2 segments each. We interpret these images as showing 2 F(ab')₂ anti-IgE antibodies (the long arms positioned laterally in Fig 2, *A*, and the linking molecules of the chain in Fig 2, *B*) reacting with epitopes on either side of the Fc of IgE at a position very close to the geometric center of the molecule. On the basis of previously published models of IgE, this would place the epitopes near the Cε2-3 juncture. Relatively few of the complexes clearly show 3 putative IgE arms (Fig 2, *A* and *B*), yet most showed one narrower arm (Fc) and what appeared to be a pair of arms (Fabs) in close contact or partially superimposed on each other, as shown in Fig 2, *A* and *B*.

A variety of indirect physical evidence indicates that the Fc of IgE is unusual in that it is hooked in such a way that the C-terminus is in close proximity with the complementary determining region of the Fab arms.

Whereas our images do not show such a configuration, the foreshortened nature of one of the arms in the uncomplexed molecules hints at some sort of contortion. Although negative stain electron microscopy is often viewed as a 2-dimensional format, under certain conditions, 3-dimensional information can be inferred.^{22,23} For example, when overlaying stain is especially deep, those portions of the molecule with z-axis dimensions of less than the stain thickness will leave a fainter image than those portions of the molecules that completely displace the stain by spanning the distance from the carbon membrane to (or through) the surface of the stain. Fig 2, *C*, shows such an area. Many of the fainter images of IgE in complex with excess of mAb 12 F(ab')₂ can be seen to contain bright spots indicating vertically protruding protein structures. The protrusions are often near the center of the complex and can be interpreted as showing the C-terminus of the Fcε bent upward and back toward the Fab region of the molecule, as shown in Fig 2, *C*.

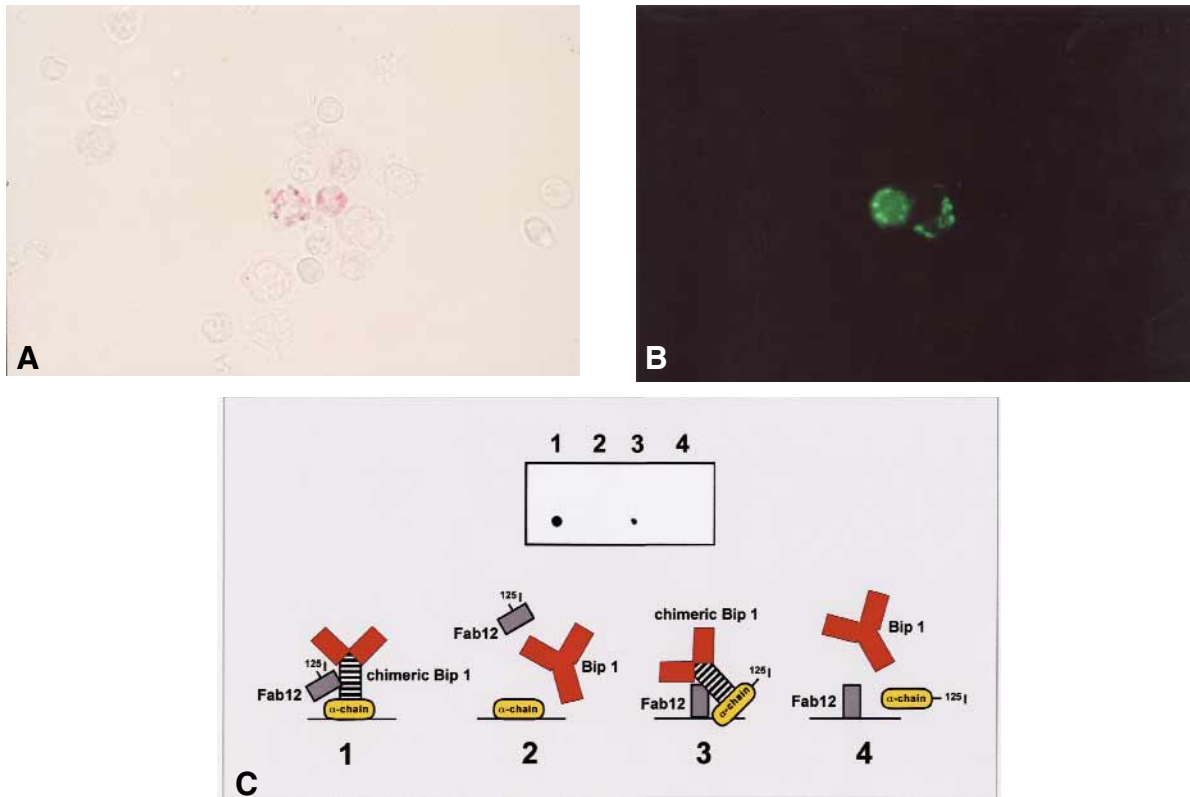


FIG 3. Monoclonal antibody 12 recognizes basophil-bound and FcεRI α chain-bound IgE. Toluidine blue-stained basophils (**A**) were exposed to mAb 12 and an FITC-labeled anti-mouse antiserum (**B**). **C**, Nitrocellulose-bound recombinant FcεRI α chain (*lanes 1 and 2*) or Fab 12 (*lanes 3 and 4*) were incubated with chimeric Bip 1 IgE (*lanes 1 and 3*) or, for control purposes, with mouse-derived Bip 1 (*lanes 2 and 4*). Bound chimeric Bip 1 IgE antibodies were detected with ¹²⁵I-labeled Fab12 (*lanes 1 and 2*) or with ¹²⁵I-labeled recombinant FcεRI α chain (*lanes 3 and 4*). The upper part of **C** shows the autoradiograph, and the lower part shows the scheme of the experimental set up.

Fab12 and mAb 12 react with α chain-bound and basophil-bound IgE antibodies

A combined toluidine blue/immunofluorescence staining technique was applied to investigate whether mAb 12 can react with basophil-bound IgE. Monoclonal antibody 12 stained the metachromatic cells (basophils) in the mononuclear cell preparations (Fig 3, A and B). By contrast, the majority of the nonmetachromatic cells were not labeled by mAb 12. The surprising finding that mAb 12, despite preventing IgE binding to the α chain (Fig 1, B) and despite its reactivity to the FcεRI-binding site on human IgE (Cε3; Fig 2), stained human basophils led us to investigate this phenomenon in greater detail. By using overlay experiments, we demonstrated that ¹²⁵I-labeled Fab12 recognizes chimeric Bip 1 IgE when bound to FcεRI α chain, whereas no reaction is observed when mouse-derived Bip 1 is used (Fig 3, C, panels 1 and 2). Furthermore, we show that nitrocellulose-bound Fab12 can bind complexes consisting of chimeric Bip 1 IgE and ¹²⁵I-labeled FcεRI α chain (Fig 3, C, panel 3). No reaction was found when mouse-derived Bip 1 was used instead of

chimeric Bip 1 IgE (Fig 3, C, panel 4). The overlay results thus confirm that Fab12 can bind to human IgE when it is bound to the FcεRI α chain. Identical results were obtained for complete mAb 12 (data not shown).

Monoclonal antibody 12 can desensitize human basophils and prevents allergen-induced, as well as anti-human IgE-induced, basophil histamine release

Preincubation of enriched human basophils with mAb 12, but not with a control antibody (anti-human IgM), in EDTA-containing HRB caused desensitization against subsequent IgE-dependent, as well as allergen (rBet v 1)-dependent, cell activation (Fig 4). A dose-dependent release of histamine was observed when basophils were exposed to mAb12 or rBet v 1 without prior desensitization (Fig 4). The possibility that incubation of basophils with anti-human IgE antibodies in HRB containing EDTA has caused release of mediators was excluded by the lack of histamine in the culture supernatants obtained from granulocytes after the preincubation (data not shown).

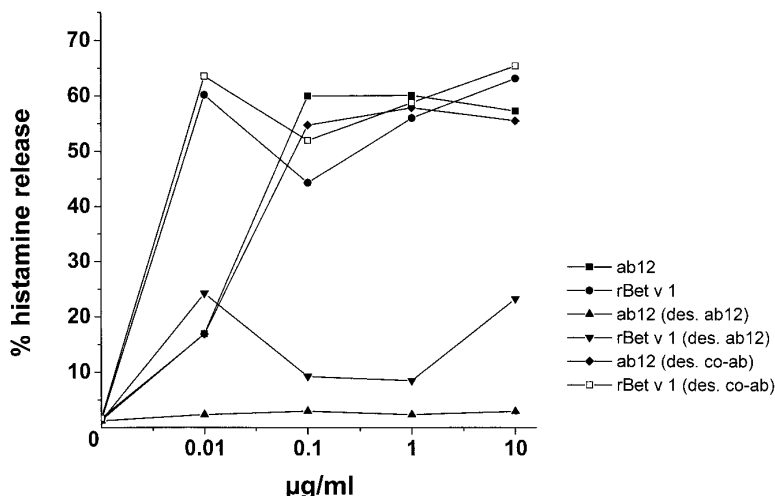


FIG 4. Monoclonal antibody 12 can desensitize human basophils from a patient with birch pollen allergy. Enriched basophils were pretreated with mAb 12 (*des. ab12*), with a control antibody (*des. co-ab*), or with buffer alone (*filled squares and dots*). Cells were then exposed to various concentrations (0.01–10 µg/mL) of mAb 12 or rBet v 1. The percentages of released histamine in comparison with total histamine are shown on the y-axis. Results represent means of triplicates.

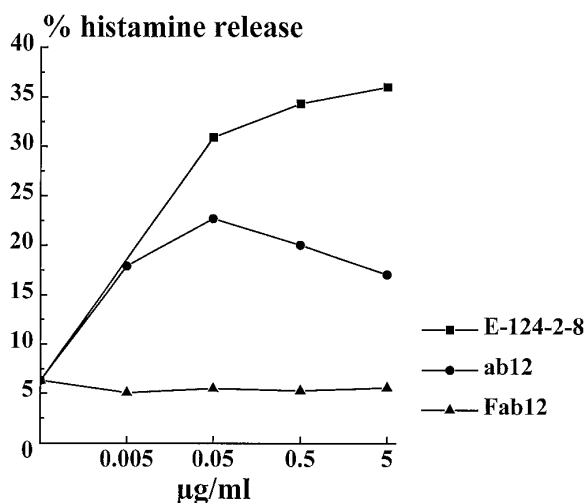


FIG 5. Fab12 lacks anaphylactic activity. Human basophils were incubated with increasing concentrations of monoclonal anti-human IgE antibodies (E-124-2-8/De2 and ab12) and Fab12 (x-axis). The percentages of released histamine in comparison with total histamine are shown on the y-axis. Results represent means of duplicate determinations.

Fab12 does not induce basophil histamine release

We incubated human PMNs with increasing concentrations of purified monoclonal anti-human IgE antibodies (mAb 12 or E-124-2-8/De2) and mAb 12-derived Fab in 6 independently performed experiments. As shown in Fig 5, we found that mAb 12 induced less histamine release than E-124-2-8/De2. The very same preparation of the mAb 12-derived Fab that had inhibited human IgE binding to FcεRI α chain (Fig 1, B) and

that had reacted with FcεRI α chain-bound IgE (Fig 3, C) did not induce any histamine release up to a concentration of 5 µg/mL in any of the experiments (Fig 5).

DISCUSSION

The interaction of allergens, allergen-specific IgE antibodies, and FcεRI present on allergic effector cells,²⁴⁻²⁶ as well as on antigen-presenting cells (eg, monocytes and dendritic cells),^{2,3,27} is one of the central events in the pathogenesis of type I allergy. We report a molecular model of this interaction consisting of a chimeric IgE antibody with specificity for the major allergen of birch (chimeric Bip 1 IgE) and the baculovirus-expressed recombinant α chain of FcεRI. The correct folds of chimeric Bip 1 IgE and recombinant FcεRI α chain were demonstrated by means of CD analyses. Negative stain immunoelectronmicroscopy of chimeric Bip 1 IgE showed that, in contrast to other monomeric Ig forms, it did not display a clear 3-armed Y-shaped configuration, a fact that was also recently observed for the Fc of Fab-tagged IgE.²⁸ The latter study, together with the data obtained for chimeric Bip 1, supports the notion that IgE may be contorted differently from the thus far proposed hook-shaped model in which both Fab arms and the Fc are bent into a U-shaped configuration. Instead, we would propose that the Fc is hooked either toward only one Fab arm or that the Fc brings its C-terminus up near the bases of the Fab arms so that both complementary determining regions are equidistant from the Fc base.

By using native overlays, we demonstrated the correct interaction of the trimeric complex consisting of rBet v 1, chimeric Bip 1, and recombinant FcεRI α chain. Our hypothesis that the molecular in vitro allergy model described can be used to screen and identify competitors of the IgE-FcεRI interaction was tested by screening a

panel of mouse monoclonal anti-human IgE antibodies for their capacity to inhibit the IgE-FcεRI interaction. Of the 25 monoclonal anti-human IgE antibodies, we isolated one with previously undescribed properties. Monoclonal antibody 12 strongly inhibited the binding of chimeric Bip 1 IgE to the FcεRI α chain and reacted with FcεRI α chain-bound IgE. Furthermore, complete mAb 12 showed a reduced capacity to induce basophil histamine release compared with other mouse monoclonal anti-human IgE antibodies (eg, E-124-2-8Dε2), which may be attributed to the fact that these antibodies recognize different epitopes on the IgE molecule, leading to differences in cell activation.

As exemplified for mAb 12, use of the *in vitro* allergy model for the screening of compounds (eg, antibodies, Fabs, peptides, drugs, nucleic acids, lipids, and carbohydrates) that interfere with the IgE-FcεRI interaction is suggested. Mouse-derived anti-human IgE antibodies were recently developed for a general therapy of atopy and asthma.²⁹

However, mAb 12 differs substantially from the previously described anti-human IgE antibodies (eg, BSW17)^{11,13} because, despite its ability to block the binding of human IgE to FcεRI and its reactivity to the FcεRI-binding region of human IgE, mAb 12 was able to recognize FcεRI α chain-bound and basophil-bound IgE antibodies. At least 2 explanations may be considered for this behavior. First, it is possible that mAb 12, on binding to IgE, can induce a change in the IgE conformation that prevents its interaction to the receptor but, on the other hand, can recognize receptor-bound IgE. However, CD analyses of chimeric Bip 1 and mAb 12 alone, as well as of complexes of chimeric Bip 1 IgE and mAb 12, gave no evidence for such conformational changes (data not shown). The second and more likely explanation is that mAb 12 interacts exactly with or very close to the receptor binding site of human IgE and, because of the 1:1 stoichiometry of the IgE-FcεRI interaction,^{30,31} binds to one of the IgE-constant domains, whereas the second domain can interact with the FcεRI α chain. This assumption is supported by results obtained through 2 types of experiments: (1) mAb 12-derived Fabs, despite reacting with receptor-bound IgE and preventing IgE binding to the receptor, did not induce histamine release from human basophils and (2) negative stain immunoelectron microscopy showed that mAb 12 F(ab)² bound on either side of the Fc of IgE near the Cε2-3 juncture. The latter result is also supported by the fact that mAb 12 reacted with recombinant Cε3. However, it was not possible to map its binding site with synthetic overlapping 15-mer peptides spanning Cε3 (Valenta, unpublished data).

Fab12 is a nonanaphylactic competitor of the IgE-FcεRI interaction with a rather low molecular weight. If produced as a recombinant Fab fragment containing humanized framework regions, it may be administered to atopic patients, such as rhuMAB-E25, to complex and remove circulating IgE.²⁹ Compared with the previously described anti-human IgE antibody (rhuMAB-E25),²⁹ Fab12 has what we believe to be an important advantage:

in addition to the lack of anaphylactic activity, Fab12 reacts with IgE antibodies that are bound through FcεRI to basophils and thus presumably also to mast cells, eosinophils, and antigen-presenting cells containing FcεRI-bound IgE. Fab12 may therefore not only be used for the depletion of IgE antibodies from the circulation but also to target effector and inducer cells of atopy for therapeutic intervention at the cellular level. The latter assumption is supported by the desensitization experiments, which show that desensitization of human basophils with mAb 12 renders the cells unresponsive to subsequent allergen stimulation (Fig 4). On the other hand, Fab12 will deplete IgE antibodies, as well as IgE-bearing cells, without requiring administration into patients if used for selective extracorporeal plasmapheresis.³²

In conclusion, we have established a molecular *in vitro* system of type I allergy that allowed us to identify Fab12, a competitor of the IgE-FcεRI interaction, as a candidate molecule for therapy of atopy.

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