

Original article

A high-affinity monoclonal anti-IgE antibody for depletion of IgE and IgE-bearing cells

Background: We have identified a monoclonal anti-human immunoglobulin E (IgE) antibody, which recognizes FcεpsilonRI-bound IgE and prevents binding of IgE to FcεpsilonRI. In this study, we assessed the binding kinetics and affinity of monoclonal antibody 12 (mAb12) for IgE and investigated whether mAb12 can be used for depletion of IgE and isolation of IgE-bearing cells from peripheral blood.

Methods: Binding kinetics and affinity for IgE were studied using Biacore surface plasmon resonance technique experiments. IgE antibodies were depleted from serum using sepharose-coupled mAb12 and IgE-bearing cells were enriched from heparinized blood samples with mAb12. The extent and biological relevance of IgE depletion were studied by quantitative IgE measurements and basophil histamine release experiments. Specific binding of mAb12 to IgE-bearing cells (basophils, mast cells, IgE-secreting plasma cells) was demonstrated by FACS.

Results: Monoclonal antibody 12 shows rapid association ($k_a = 5.46 \times 10^5/\text{Ms}$) with IgE, almost no dissociation ($k_d = 8.8 \times 10^{-5}/\text{s}$) and an affinity for IgE ($K_D = 1.61 \times 10^{-10} \text{ M}$), which is as high as that of FcεpsilonRI. Immobilized mAb12 could be used to deplete IgE antibodies and isolate IgE-bearing cells from peripheral blood in a single-step procedure.

Conclusions: Monoclonal antibody 12 is a high affinity anti-human IgE antibody, which efficiently removes IgE and IgE-bearing cells from peripheral blood and may thus be used for extracorporeal depletion of IgE and IgE-bearing cells.

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Allergen-mediated crosslinking of immunoglobulin E (IgE) antibodies bound to effector cells (e.g., mast cells, basophils) via FcεpsilonRI induces the immediate release of biologically active mediators as histamine and leukotrienes that cause the acute symptoms of atopy (e.g., allergic rhinitis, conjunctivitis, asthma, and anaphylactic shock) (1). When allergens are presented via FcεpsilonRI

receptors on antigen-presenting cells (e.g., monocytes, dendritic cells), T cells become activated and release proinflammatory cytokines, leading to chronic manifestations of allergy such as atopic eczema and chronic asthma (2–4). As IgE-mediated activation of immune cells represents a key mechanism in allergic inflammation, considerable effort has been spent on the molecular characterization of IgE and the development of therapeutic reagents inhibiting IgE-mediated effects (5, 6).

Passive treatment with anti-IgE antibodies as well as active vaccination against IgE have been considered strategies for the treatment of IgE-mediated diseases (7–10). The humanized monoclonal anti-IgE antibody (rhuMab)-E25, designated omalizumab or Xolair, is the

Abbreviations: 7 AAD, 7-amino-actinomycin D; BSA, bovine serum albumin; chim Bip1, a human monoclonal IgE specific for the major birch pollen allergen Bet v 1; CML, chronic myeloid leukemia; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; mAb12, monoclonal anti-human IgE antibody 12; NHS, N-hydroxysuccinimide; RU, resonance unit.

best-characterized therapeutic antibody. (rhuMAb)-E25 binds to circulating IgE but does not react with mast cell- or basophil-bound IgE and hence lacks anaphylactic activity (11). Administration of (rhuMAb)-E25 to allergic patients leads to reduction of circulating IgE and, interestingly, to reduced IgE-receptor expression on basophils (12). Several clinical studies indicate the safety and clinical efficacy of (rhuMAb)-E25 for the treatment of various manifestations of allergy (13–17).

We previously identified an anti-human IgE antibody (mAb12) that profoundly differed from previously characterized therapeutic anti-IgE antibodies [e.g., (rhuMAb)-E25] because mAb12 inhibited the IgE-FcεpsilonRI interaction, lacked anaphylactic activity when applied as Fab but, unlike (rhuMAb)-E25, reacted with effector cell-bound IgE (18).

Here we studied by surface plasmon resonance-based assays the affinity and binding kinetics of the interaction of mAb12 with IgE. With the aim to develop therapeutic strategies based on the extracorporeal removal of IgE and reduction of IgE-bearing cells, we investigated whether mAb12 can be used for the targeting and depletion of IgE as well as for the reduction of IgE-bearing and secreting cells in peripheral blood.

Methods

Reagents, characterization of allergic patients

The identification and purification of mAb12 and of a human monoclonal IgE antibody specific for the major birch pollen allergen, Bet v 1, have been reported earlier (18). Recombinant alpha-chain of the human high affinity receptor for IgE is described (19). MoAb 4A6, a mouse IgG₁ antibody specific for birch pollen profilin was used as isotype-matched control antibody (20). For IgE-depletion experiments, sera were obtained from patients with a wide range of total serum IgE levels and sensitization profiles (i.e., 179–12 620 kU/l). The diagnosis of allergy was based on clinical characterization, skin prick testing and determination of specific IgE antibodies (UniCAP, Phadia, Uppsala, Sweden) (Table 1).

Biacore assays

Biacore measurements were carried out using a Biacore 2000 instrument at 25°C (BIAcore AB, Uppsala, Sweden). The surface

of a C1 sensor chip (BIAcore AB) was activated by injection of a 1 : 1 mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide at a flow rate of 5 µl/min for 7 min. MAb12, which had been diluted in 10 mM sodium acetate, pH = 5.8, at a concentration of 6 µg/ml, was injected (5 µl/min) into flow cell 2 to obtain an immobilization level of 50 RU, giving rise to an R_{max} of 100 RU. Then, the surface of the sensor chip was deactivated by injection of 1 M ethanolamine-HCl, pH = 8.5 (5 µl/min, 7 min). The final immobilization level was 57 RU. Flow cell 1 was activated and deactivated without coupling of protein to serve as a reference cell.

Interaction of mAb12 with a purified monoclonal human IgE (chimeric Bip1) (18) specific for the major birch pollen allergen, Bet v 1, was assessed by injection of chimeric Bip1, diluted in HBS-EP buffer [0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% (v/v) surfactant P20, pH = 7.4], at twofold increasing concentrations (starting concentration: 1 nM; top concentration: 64 nM) into the flow cells at a flow rate of 30 µl/min for 5 min. Dissociation of IgE from immobilized mAb12 was investigated by injection of HBS-EP at 30 µl/min for 30 min. The sensor chip surface was regenerated by injection of 10 mM glycine-HCl, pH = 2, at 20 µl/min, for 30 s.

Association- (k_a) and dissociation-rate constants (k_d) of the interaction of a single IgE-binding domain of mAb12 with a single IgE molecule (monovalent interaction) were calculated with BIAEVALUATION 3.2 software (BIAcore AB), the affinity constant (K_D) was calculated from k_d/k_a.

On a second C1 sensor chip, FcεpsilonRI alpha-chain, dissolved in 10 mM sodium acetate, pH = 5, at a concentration of 13 µg/ml, was immobilized in flow cell 2 using the same procedure as described above. The immobilization level was 100 RU.

Rate constants and affinity of the interaction of the alpha-chain with monoclonal IgE were determined as described above.

To study binding of mAb12 to alpha-chain bound IgE, purified monoclonal IgE (32 nM in HBS-EP) was injected (5 µl/min, 3 min) into the flow cell containing immobilized alpha-chain and, after a stabilization time of 10 min (injection of HBS-EP at 5 µl/min), mAb12 or an isotype-matched control antibody, 4A6 (20), (both 64 nM in HBS-EP, 20 µl/min, 3 min) were applied. Dissociation of alpha-chain bound material (mAb12, IgE), was assessed by injection of HBS-EP at 20 µl/min for 30 min.

Inhibition of the interaction between IgE and alpha-chain by mAb12 was studied by preincubation of chimeric Bip 1 (32 nM) with mAb12 (128 nM) or an isotype control [4A6 (20)] (128 nM) at room temperature for 4 h and subsequent injection (20 µl/min, 3 min) into the flow cell containing immobilized alpha-chain.

Depletion of IgE from sera of allergic patients using mAb12

Five milligram mAb12 or control antibody [4A6 (20)] were coupled to 1 g CNBr-activated Sepharose 4B by the cyanogen bromide

Table 1. Demographic, clinical and serological characterization of allergic patients

Patients	Gender	Age	Allergen source	Symptoms	Total IgE (kU/l)	Specific IgE (kUA/l)					
						Birch	Timothy grass	Mites	Cat	Dog	Mugwort
1	m	49	t, g, a, mi, mo	ad, rc	1626	14.1	46	39.8	nd	nd	nd
2	m	28	t, g, w, f, a, mi, mo, bee, wasp	ad, rc	12 620	192	234	218	32.2	18.9	15.2
3	m	28	t, g, w, a, mi	ad, rc	280	8.5	7.1	3	5.8	4.3	nd
4	m	25	t, g, a, mi	as, rc	427	1.2	0.6	3.7	0.5	nd	nd
5	m	33	t, g, w, a	as, rc	179	9.5	64.5	nd	1.13	nd	3.3

a, animals; f, food; g, grass; mi, mites; mo, molds; t, trees; w, weeds; ad, atopic dermatitis; as, asthma; rc, rhinoconjunctivitis; nd, not determined.

method as recommended by the manufacturer (GE Healthcare, Fairfield, CT, USA).

Twenty milliliter aliquots of patients' sera, diluted with 20 ml phosphate-buffered saline (PBS), were passed once over the mAb12- or the control-column containing 3.5 ml gel each. Total and specific IgE were measured before and after application to the column by UniCAP (Phadia) and by ELISA.

Enzyme-linked immunosorbent assay – ELISA

Purified recombinant allergens from birch pollen (rBet v 1, rBet v 2), from timothy grass pollen (rPhl p 1, rPhl p 2, rPhl p 5) and from house dust mite (rDer p 2) were dissolved in coating buffer (0.1 M NaHCO₃, pH = 9.6) at a concentration of 4 µg/ml. Hundred microliter aliquots of dissolved recombinant allergens were coated to ELISA plates overnight at 4°C. Plates were washed five times with Tris-buffered saline (pH = 7.5), 0.05% (v/v) Tween-20 (TBST) and were blocked for 2.5 h at 37°C with TBST containing 1% bovine serum albumin (BSA). The ELISA plate-bound proteins were incubated overnight at 4°C with sera from allergic patients, diluted 1 : 10 in TBST, and, for control purposes with serum from a non-allergic individual. Bound human IgE was detected with an alkaline phosphatase-conjugated mouse monoclonal anti-human IgE antibody (PharMingen, San Diego, CA, USA). IgG subclass responses specific for allergens were determined before and after depletion for control purposes by incubation with mouse anti-human IgG₁₋₄ antibodies (PharMingen), diluted 1 : 1000, at 4°C overnight. Bound mouse IgG antibodies were detected with a peroxidase-conjugated sheep anti-mouse antibody (GE Healthcare).

Passive transfer of IgE to basophils and histamine release experiments

Histamine release experiments were performed with peripheral blood polymorphonuclear leukocytes which were obtained by dextran sedimentation from a patient with chronic myeloid leukemia (CML) (21). Cells were preincubated for 3 h at 37°C with serum obtained from an allergic patient (Table 1: patient 2) before (total IgE: 12620 kU/l) or after IgE depletion (total IgE: 272 kU/l) or with histamine release buffer alone. After washing with histamine release buffer the cells were incubated with increasing concentrations (0.0001, 0.001, 0.01, 0.1 and 1 µg/ml) of recombinant Phl p 1 at 37°C for 30 min. Histamine released into the cell-free supernatant was measured by radioimmunoassay (Immunotech, Marseille, France) and is expressed as percentage of total histamine determined after cell lysis. Results are displayed as mean values of duplicate determinations.

Single-step isolation of IgE-bearing cells from peripheral blood using mAb12

Heparinized blood samples (12.5 ml) from allergic patients were washed with PBS, 0.1% (w/v) BSA, and subsequently incubated with 10 µg/ml of mAb12 or an isotype-matched control antibody without specificity for IgE for 30 min at room temperature. Cells were collected by centrifugation (800 g, 15 min) and washed two times with PBS, 0.1% (w/v) BSA. Anti-mouse IgG CELlection™ Dynabeads (Dyna, Hamburg, Germany) were incubated with the pretreated cells for 30 min at 4°C. The ratio of beads to target cells was at least 5 : 1. Target cells were separated by the Dynal Magnetic Particle Concentrator (Dyna, Hamburg, Germany) and washed two times with PBS, 0.1% (w/v) BSA. Cells were gently resuspended in RPMI 1640 medium, complemented with HEPES, L-glutamine and 1% (v/v) fetal calf serum (FCS) (Gibco, Paisley, UK). Beads

were removed from cells by DNase I digestion according to the manufacturer's instructions. The cell count was measured and the identity of the isolated cells was determined by Giemsa staining of cytospin preparations (Fig. 3A, Table 3).

Binding of FITC-labeled mAb12 to basophils and IgE-secreting plasma cells using flow cytometric analysis

Basophils were obtained from peripheral blood of a healthy donor by Ficoll-enrichment of mononuclear cells (MNC). The MNCs were washed in PBS at 4°C and were then incubated with mAb12 or an isotype-matched IgG₁ control antibody (Becton Dickinson Bioscience, San Jose, CA, USA), respectively, for 30 min. Then, cells were washed and stained with a FITC-labeled goat anti-mouse IgG₁ antibody (Caltag Laboratories, Burlingame, CA, USA). For detection of basophils, cells were stained with the PE-conjugated CD203c-specific mAb 97A6 (Immunotech) and with APC-labeled CD123-specific mAb AC145 (Miltenyi Biotech, Bergisch Gladbach, Germany). Cells were analyzed by multi-color flow cytometry on a FACS-Calibur (Becton Dickinson). Antibody-reactivity was expressed as mean fluorescence intensity.

In another set of experiments, mAb12 was FITC-labeled using the FITC-labeling kit from Calbiochem (Merck, Darmstadt, Germany) according to the manufacturer's instructions. An IgE producing human plasma-cell line (U266) (22) as well as a human B-cell line producing no IgE antibodies (Raji) were purchased from ATCC (Middlesex, UK). Cells were cultured in RPMI 1640 (Biochrom AG, Pasching, Austria) containing 10% FCS and 20 µg/ml gentamicin (Gibco). An aliquot of 500 000 cells was resuspended in 100 µl of human AB-serum (Biotest, Dreieich, Germany), diluted 1 : 5 in PBS, and incubated 20 min at 4°C. After centrifugation, the cells were incubated in 50 µl of 5 µg/ml FITC-labeled mAb12 in PBS for 1 h at 4°C, washed with 1 ml PBS and centrifuged. Then they were resuspended in 50 µl of a 25 µg/ml 7AAD (Sigma-Aldrich, St Louis, MO, USA)/PBS dilution and incubated for 15 min at 4°C. The cells were washed in 1 ml PBS, resuspended in 300 µl PBS and analyzed with FACScan (Becton Dickinson, Franklin Lakes, NJ, USA). Ten thousand cells were acquired and analyzed using CELLQUEST software (Becton Dickinson).

Results

Fast and high-affinity binding of mAb12 to human IgE; inhibition of IgE-binding to alpha-chain by mAb12

In a first set of Biacore experiments, the affinity of mAb12 to human IgE was determined. Twofold increasing concentrations (1–64 nM) of purified human IgE were injected into the flow cell containing immobilized mAb12 and binding as well as dissociation were recorded (Fig. 1A). Based on the experimental data, association- and dissociation-curves were calculated (black lines) and fitted to the recorded curves (colored lines). Association- (k_a) and dissociation-rate constants (k_d) were determined to be $k_a = 5.46 \times 10^5/\text{Ms}$ and $k_d = 8.8 \times 10^{-5}/\text{s}$, respectively. The affinity constant K_D was calculated from k_d and k_a to be 1.61×10^{-10} M.

Binding of mAb12 to alpha-chain bound IgE was shown by immobilization of FcepsilonRI to the sensor-surface and sequential injection of IgE and mAb12 or an isotype-control into the flow-cell (Fig. 1B). MAb12

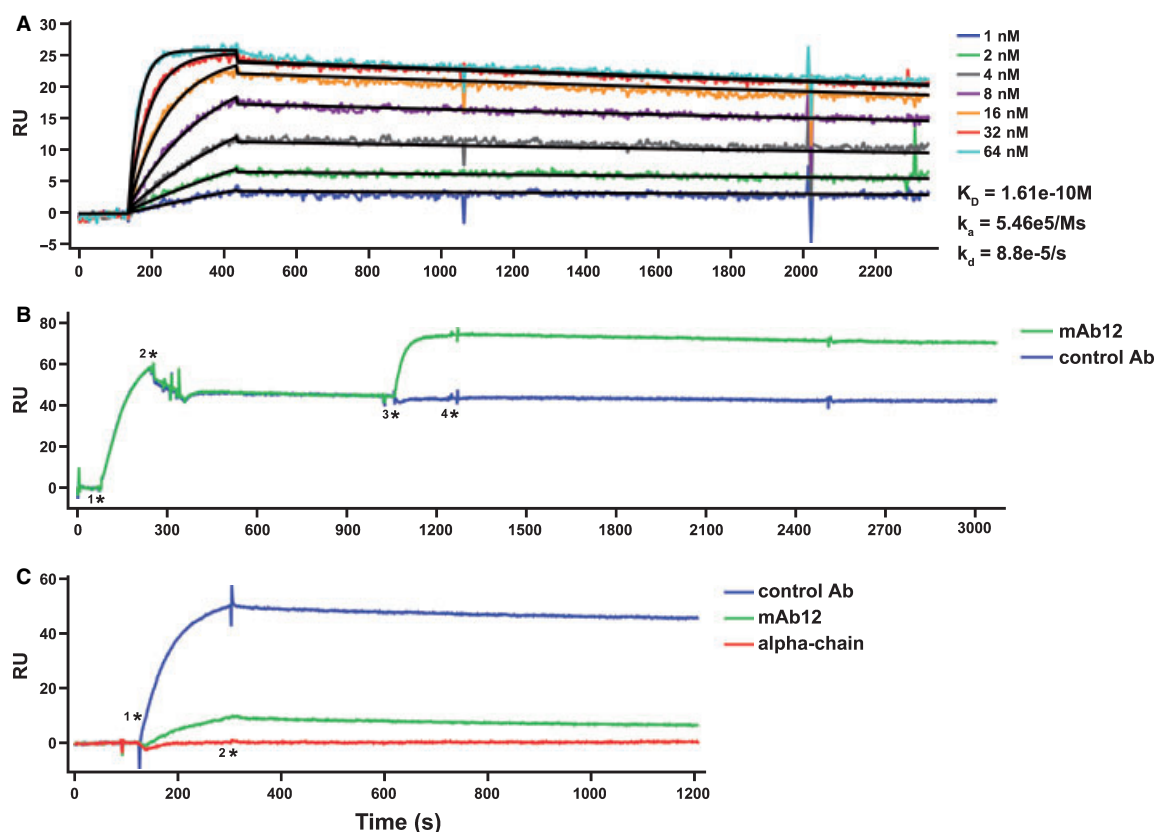


Figure 1. Affinity and kinetics of the interaction between mAb12 and human IgE as determined by surface plasmon resonance (Biacore) measurements. (A) IgE was injected at twofold increasing concentrations from 1 nM to 64 nM (curves bottom to top) into the flow cell containing immobilized mAb12. Calculated curves (black) were superimposed on recorded curves (coloured). (B) Association of mAb12 or an isotype control antibody with IgE which is bound to chip-coupled FcepsilonRI alpha-chain. Asterisks (left to right) indicate start (1) and stop (2) of the injection and binding of IgE to immobilized alpha-chain followed by a stabilization phase. Start (3) and stop (4) of injection of mAb12 (green) or the control antibody (blue), followed by monitoring of potential dissociation. (C) Inhibition of the interaction between IgE and FcepsilonRI alpha-chain by mAb12. IgE was preincubated with mAb12 (green curve), an isotype control (blue curve) or FcepsilonRI alpha-chain (red curve), respectively, and then exposed to chip-bound alpha-chain (1). The injection was stopped (2) and potential dissociation was monitored thereafter. Binding in A, B and C is expressed in resonance units on the y-axis over time (x-axis: s).

showed fast and strong binding to alpha-chain-bound IgE with almost no dissociation. No binding of the isotype control antibody was observed.

Preincubation of IgE with mAb12 at a molar ratio of 1 : 4 and injection into the flow-cell (Fig. 1C, green line) containing immobilized alpha-chain showed more than 80% reduction of IgE-binding to the alpha-chain, whereas preincubation with the isotype control showed no effect (blue line). Complete inhibition was obtained by preincubation of IgE with unbound alpha-chain (red line).

Efficient and selective depletion of IgE from serum in a single-step procedure with sepharose-bound mAb12

Twenty milliliter serum aliquots from five allergic patients (Table 1) were passed once through an mAb12 affinity column. Total IgE and allergen-specific IgE were quan-

tified before and after application to sepharose-bound mAb12 using UniCAP. The total IgE ranged from 179 kU/l (patient 5) to 12620 kU/l (patient 2) before application to sepharose-bound mAb12 (Table 1). In all patients a >90% reduction of total IgE-levels could be achieved after one cycle of preadsorption. We also determined IgE levels (kUA/l) specific for birch pollen, timothy grass pollen, mugwort, mites, cat and dog allergens in the same serum samples. A profound reduction of allergen-specific IgE below the detection limit of the UniCAP system was observed for almost each of the patients. Even in patient 2 who had allergen-specific IgE-levels of more than 100 kUA/l, specific IgE was reduced to <5 kUA/l (Table 2).

Furthermore, IgE levels specific for purified recombinant allergens were determined in the same sera by ELISA and again, a profound reduction of allergen-specific IgE levels was observed. For example, IgE levels

Table 2. Reduction in total and allergen-specific IgE levels after single-step preadsorption of serum with sepharose-bound mAb12

Patients	Total IgE (kU/l)			Specific IgE (kUA/l)		
	Before	After	% Reduction	Allergy	Before	After
1	1626	6	99.6	Birch	14.1	<0.35
				Timothy grass	46	<0.35
				Mites	39.8	<0.35
2	12 620	272	97.8	Birch	192	4.4
				Timothy grass	234	3.9
				Mites	218	3.8
3	280	15	94.6	Birch	8.5	<0.35
				Timothy grass	7.1	<0.35
				Mites	3	<0.35
				Cat	5.8	<0.35
4	427	41	90.4	Dog	4.3	<0.35
				Birch	1.2	<0.35
				Timothy grass	0.6	<0.35
				Mites	3.7	0.6
5	179	12	93.3	Birch	9.5	<0.35
				Timothy grass	64.5	2.1
				Mugwort	3.3	<0.35

of patient 2 specific for rBet v 1 were 1.92, for rPhl p 1 1.7, for rPhl p 2 0.19, for rPhl p 5 1.23 and for rDer p 2 1.57. After depletion, specific IgE for rBet v 1 was reduced to 0.09, for rPhl p 1 to 0.1, for rPhl p 2 to 0.06, for rPhl p 5 to 0.08 and for rDer p 2 to 0.14. The levels of allergen-specific IgE for patients 1, 3, 4, and 5 after IgE depletion were below the cut off level of 0.05. The concentrations of allergen-specific IgG₁, IgG₂, IgG₃ and IgG₄ remained unaffected by the IgE-depletion (data not shown).

Application of the sera to the column containing immobilized control antibody did not result in a reduction of IgE-levels (data not shown).

Reduction of basophil degranulation by depletion of IgE

To investigate whether depletion of allergen-specific IgE from serum reduces allergen-induced basophil degranulation, serum samples from patient 2, obtained before and after IgE-depletion, were incubated with peripheral blood polymorphonuclear leukocytes from a patient suffering from CML. The cells were then incubated with increasing concentrations of the major grass pollen allergen rPhl p 1 and the release of histamine was measured.

Preincubation of enriched basophils with serum from patient 2 obtained before IgE depletion lead to loading of basophils with IgE and to a dose-dependent release of histamine when rPhl p 1 was added (Fig. 2, filled circles). When basophils were exposed to serum after IgE depletion a 75% reduction of Phl p 1-induced histamine release was obtained and the cellular sensitivity declined more than 10-fold (Fig. 2, filled triangles). rPhl p 1 did not induce histamine release in basophils pretreated with buffer alone up to a concentration of 1 µg/ml (Fig. 2, filled squares).

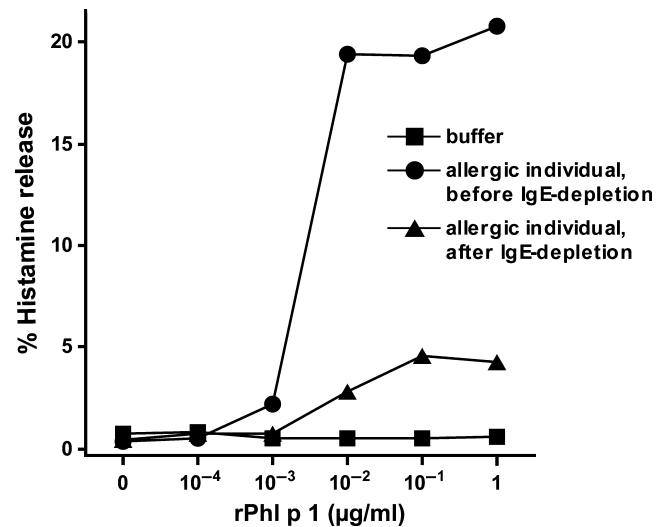


Figure 2. Removal of biologically active IgE from serum. Cultured basophils were exposed to serum from a pollen allergic patient (patient 2) before (circles) and after IgE-depletion (triangles) with mAb12 or to histamine release buffer (squares). Histamine release (y-axis: percentage of total histamine) was measured after addition of increasing concentrations of purified grass pollen allergen, rPhl p 1 (x-axis).

mAb12 for isolation of IgE-bearing cells from heparinized blood, detection of IgE-bearing basophils and IgE-secreting plasma cells

Heparinized whole blood samples from five atopic patients, two nonatopic individuals and from one non-allergic individual suffering from CML were incubated first with mAb12 or for control purposes with an isotype matched control antibody and then with Dynabeads containing anti-mouse IgG. Using mAb12, highly enriched basophils were isolated from whole blood of the allergic patients, nonallergic individuals and the CML patient. On average, 71.5% basophils, 11.6% lymphocytes, 10.4% monocytes, 3.9% eosinophils and 2.6% neutrophils, all cells which reportedly may contain cell-bound IgE, were isolated using mAb12 in a single step procedure (Table 3). Figure 3A shows purified basophils obtained via mAb12 affinity-purification. Binding of mAb12 to cell-bound IgE was verified using a polyclonal horse anti-mouse biotinylated antibody (data not shown).

Flow cytometric analysis was applied to investigate whether FITC-labeled mAb12 can react with basophils. PBMCs from a nonallergic donor were isolated and labeled with mAb12 in FACS experiments. The mAb12-reactive cells were identified with anti-CD203c and anti-CD123 antibodies as basophils (Fig. 3B). We also studied whether IgE-secreting plasma cells despite their low expression of cell-bound IgE can be detected with mAb12. For this purpose, the human cell line U266 and, for control purposes, Raji cells, a human Burkitt's lymphoma B-cell line, were exposed to mAb12. FITC-labeled mAb12 showed binding to U266 cells, whereas no

Table 3. Percentages of leukocytes in IgE-bearing cells which have been isolated from peripheral blood of five allergic (patients 1, 6, 7, 8, 9), and two nonallergic individuals (patients 10, 11), as well as from one patient suffering from chronic myeloid leukemia (CML), respectively, using mAb12

Patients	Percentages of cell types				
	Basophils	Eosinophils	Monocytes	Lymphocytes	Neutrophils
1	90	4	6	—	—
6	42	2	38	17	1
7	95	—	—	—	5
8	22	16	18	33	11
9	77	1	8	14	—
10	62	6	10	20	2
11	95	—	—	5	—
CML	89	2	3	4	2

binding to the human Raji cell line was observed (Fig. 3C).

Discussion

Immunoglobulin E is a universal target for the therapy of allergic diseases because of its fundamental role in the

underlying pathomechanisms. Omalizumab (23, 24), a humanized monoclonal anti-IgE antibody, which is repeatedly administered subcutaneously at intervals of 2–4 weeks, was the first drug for therapeutic targeting of IgE. Application of Omalizumab proved to induce a reversible, but significant reduction of IgE-levels and improvement of clinical signs providing clear evidence for the effectiveness of IgE-targeted therapy (17, 25). Interestingly, the expression of FcεpsilonRI on the surface of mast cells and basophils appeared to be downregulated under omalizumab treatment (12, 26–28). In addition, evidence was provided that reduction of IgE levels did not induce a compensatory rebound reaction and that both, secretory activity and number of B cells isolated from PBMCs of allergic patients treated with omalizumab were diminished compared with a placebo group (29).

However, potential disadvantages of treatment with omalizumab are that continuous and potentially life-long administration of high doses of the therapeutic antibody are necessary and may bear the risk of anaphylactic or serum-sickness-like reactions (30, 31). In fact, after 4 years of clinical experience with Xolair (FDA approval: June 2003) the overall risk of inducing a severe adverse reaction turned out to be 0.2% (32). Furthermore,

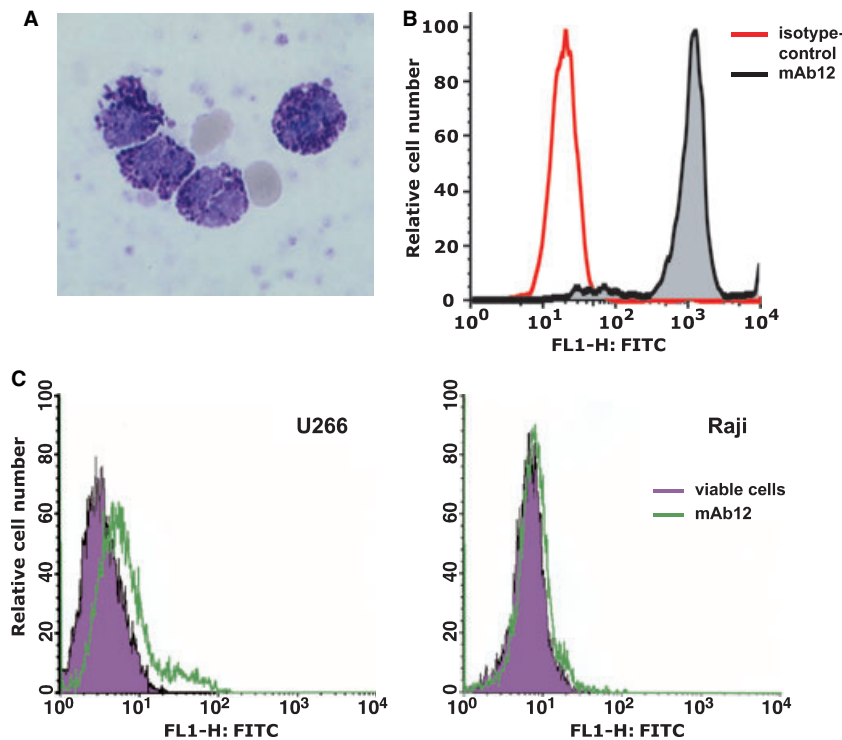


Figure 3. (A) Purified human basophils. Giemsa-stained cytopsin sample of basophils purified from blood of an atopic patient using mAb12 and Dynabead-coupled anti-mouse IgG. (B) FACS assays demonstrating specific targeting of human basophils with mAb12 (black) compared with an isotype-matched monoclonal antibody without reactivity with human IgE (red). x-axis: signal intensity, y-axis: relative cell number. (C) FITC-labeled mAb12 binds to the IgE-producing human cell line U266. Flow cytometric analysis showing specific binding of FITC-labeled mAb12 to the IgE producing human plasma cell line U266 (left box) and lack of binding to an IgE-negative human B-cell line (Raji) (right box). x-axis: signal intensity, y-axis: relative cell number; purple: viable cells; green: mAb12-positive viable cells.

omalizumab cannot be used to treat allergic patients with IgE levels exceeding 700 IU/ml and thus its use is limited to a subgroup of allergic patients only (33).

In this study, we characterized and performed the preclinical assessment of monoclonal anti-human IgE antibody mAb12 regarding its usefulness for the extracorporeal depletion of IgE and IgE-bearing cells from allergic patients as alternative treatment strategy. Actually, clinical studies performed with column-bound anti-IgE performed by Lebedin et al. (34) demonstrated that such a treatment is well tolerated and may reduce IgE levels.

According to our results, mAb12 appears to be a well suited reagent for the extracorporeal depletion of IgE and reduction of IgE-bearing cells. Sensor-chip based studies demonstrated that affinity, association- and dissociation-rate constants of the interaction between mAb12 and IgE are in the same range as the parameters characterizing the high-affinity interaction of IgE and the α -chain of the high affinity receptor for IgE. mAb12 thus binds IgE rapidly, with high affinity and forms a highly stable complex.

In accordance with the Biacore data, we found that sepharose-bound mAb12 is functional and 5 mg mAb12 allowed a more than 90% depletion of IgE from 20 ml serum containing IgE levels of more than 10 000 IU/ml after only one cycle. It should thus be possible to deplete IgE from 4000 ml plasma with 1 g of adsorbed mAb12 per patient and per cycle which is much less than what is used in existing products (35, 36). The amount of mAb12 needed for IgE depletion may be even scaled down because of the high affinity of mAb12. It should be no problem to produce the required quantities of mAb12 considering that patients currently receive up to 1 g of omalizumab per year of treatment. If it is possible to obtain also *in vivo* a more than 90% depletion of IgE, the IgE apheresis should be clinically more effective than omalizumab. After only one cycle of depletion, some specific IgE has remained but we plan to apply more treatment cycles, which should further reduce the IgE levels and affect newly synthesized IgE.

Earlier studies have demonstrated at least for seasonal allergens that allergen-specific IgE levels are boosted by allergen contact and decline in the absence of such an allergen contact (37, 38). The IgE depletion should thus be especially effective if given in the context of allergen exposure and a rebound of IgE production may eventually not take place in the absence of allergen exposure.

The depletion of IgE should also prevent reloading of effector cells, and similar to omalizumab treatment, should reduce the number of IgE receptors on effector cells and effector cell sensitivity to allergens.

The reduction in the potential leakage of an affinity column will certainly be an important production issue to prevent possible side-effects. In this context, it should be mentioned that the allergenic activity of the full mAb12 is already very low and may be completely abolished by using a Fab or a scFv derived from mAb12. It should be also borne in mind that the extracorporeal depletion of IgE will always be performed in an intensive care setting which should allow controlling potential side-effects by appropriate measures.

The mAb12-based column could be easily regenerated with 5 M $MgCl_2$, stored at 4°C, and as such remained stable and functional for several months.

In addition to the reduction of IgE, it seems possible to use mAb12 for the reduction of IgE-bearing cells, which may have beneficial effects for allergic patients as it may decrease the risk of systemic anaphylactic reactions because of extensive basophil degranulation. Additional therapeutic effects may be expected from the depletion of IgE-bearing antigen-presenting cells and eosinophils. In fact, we found that mAb12 could be used to isolate basophils, lymphocytes, antigen-presenting cells and eosinophils with magnetic beads bearing anti-mouse IgG antibodies, and we demonstrated by FACS assays the specific binding of mAb12 to basophils and IgE-secreting plasma cells. As IgE and IgE-bearing cells may also have important physiological functions, we do not aim at a complete elimination but rather at a reduction of IgE and IgE bearing cells.

Technologies for extracorporeal immunoadsorption are nowadays highly developed, bear a low risk of side-effects and are frequently applied in case of antibody-mediated autoimmune disorders for the removal of IgG antibodies which are present at 100 000 fold higher concentrations than IgE (35, 36).

In conclusion, our results identify mAb12 as a candidate molecule for the treatment of allergic patients by extracorporeal removal of IgE and/or IgE-bearing cells.

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