

Rat Monoclonal Anti-Murine IgE Antibody Removes IgE Molecules Already Bound to Mast Cells or Basophilic Leukemia Cells, Resulting in the Inhibition of Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis

Hiroaki Miyajima^a Naohiro Watanabe^b Zoltan Ovary^c Ko Okumura^d
Takao Hirano^e

^aDivision of Pathobiology, Juntendo University School of Medicine, and ^bDepartment of Tropical Medicine, Jikei University School of Medicine, Tokyo, Japan; ^cDepartment of Pathology, New York University Medical School and Kaplan Comprehensive Cancer Center, New York, N.Y., USA; ^dDepartment of Immunology and ^eDivision of Hematology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan

Key Words

IgE · Anti-IgE · Anaphylaxis · Mast cell · FcεRI

Abstract

IgE plays a central role in allergic reactions. Some anti-IgE antibodies (HMK-12, 6HD5) inhibit the binding of IgE to the FcεRI of mast cells/basophilic leukemia cells (PT-18, RBL/2H3), but less inhibition is seen with the anti-allotypic JKS-6 and the anti-idiotypic Eb-1. Anti-IgE HMK-12 can detach bound IgE molecules from the FcεRI. When mast cells or basophils were incubated with monoclonal anti-DNP-IgE SPE-7, washed and treated with anti-IgE HMK-12, anti-IgE/IgE complexes were found in the supernatant. Similar results were obtained with the Fab fragment of HMK-12. Mice injected with anti-DNP-IgE SPE-7 and later with DNP-BSA had the typical systemic anaphylactic shock. However, if they were injected with the anti-IgE antibody (HMK-12) before the challenge, they did not get an anaphylactic shock. In the sera of mice injected with monoclonal IgE SPE-7 and anti-IgE antibody (HMK-12), IgE/anti-IgE complexes were detected. No passive

cutaneous anaphylaxis occurred if the rats were injected with anti-IgE antibodies before the challenge. In summary, anti-IgE antibodies can remove IgE antibodies from the FcεRI; anti-IgE/IgE complexes can be detected in vitro and in vivo, and anti-IgE antibodies can inhibit IgE-mediated systemic or local anaphylactic reactions.

Copyright © 2002 S. Karger AG, Basel

Introduction

The reagenic antibody (IgE) plays a central role in allergic reactions [1]. Many lines of evidence have led to the concept that the interaction between IgE and mast cells/basophils is the primary effector pathway in allergic responses [2].

Mast-cell activation occurs by cross-linking cell-bound IgE with multivalent allergens, resulting in the release of mediators such as histamines, which directly produce allergic symptoms in allergic disease.

As a result of genetic or environmental factors, atopic patients synthesize allergen-specific IgE that attaches to

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2002 S. Karger AG, Basel
1018–2438/02/1281–0024\$18.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Takao Hirano
Division of Hematology, Department of Internal Medicine
Juntendo University School of Medicine
2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421 (Japan)
Tel. +81 3 3813 3111 ext. 3386, Fax +81 3 3813 0421, E-Mail thirano@med.juntendo.ac.jp

circulating basophils and tissue mast cells via high-affinity receptors (FcεRI). The binding of multivalent allergens by surface-bound-specific IgE cross-links FcεRI and triggers cellular events resulting in the release of mediators, such as histamines and serotonin [2–4].

These facts explain the newer treatments used in human studies on chronic allergic diseases such as rhinitis and asthma [5–7].

The recombinant humanized monoclonal antibody (rhumAb-E25) was developed by immunizing mice with human IgE. A monoclonal antibody was selected that recognizes IgE at the same site as the high-affinity receptor for IgE (FcεRI) [8]. This antibody forms complexes with free (unbound) IgE but not with IgG or IgA. It blocks the binding of IgE to cell-membrane receptors, thereby inhibiting the release of receptors, but it does not bind to cell-bound IgE [9]. For clinical use, the amino acid residues of the variable immunoglobulin regions of mouse origin that were implicated in the binding of human IgG1 resulted in an immunoglobulin protein that is more than 95% human. Early clinical trials of rhumAB-E25 infusion in allergic asthmatic patients demonstrated the suppression of both early and late responses to inhaled allergens.

In their studies [5–8], *in vivo* treatment with rhumAb-E25 led to rapid and dramatic falls in free serum IgE levels and FcεRI receptors on circulating basophils. Reductions in allergen-specific basophilic histamine release were also seen. Therefore, rhumAb-E25 may be a useful tool for clinical investigations.

It is also known that in experimental systems using mice, the anti-IgE antibody may play an important role *in vitro* and *in vivo* [10, 11].

Baniyash et al. [12] had shown that an anti-IgE antibody, as well as the Fab fragment of this antibody, inhibited the binding of IgE to rat basophilic leukemia cells (RBL/2H3) and also the antigen-mediated IgE-dependent serotonin release from RBL/2H3. We established several rat monoclonal antibodies specific for murine IgE [13]. Using these anti-IgE antibodies, we showed that antigen-specific IgE antibodies could be detected in sera or culture supernatants and surface IgE expression on B cells as well [14, 15]. Our studies also demonstrated that the anti-IgE antibody inhibited passive cutaneous anaphylaxis (PCA) and elicited reverse PCA (RPCA) [13, 16, 17].

We attempted to examine here the inhibition of the IgE-mediated anaphylactic reactions on mast cells and basophils *in vitro* and especially that of a systemic anaphylactic shock or the PCA reaction *in vivo* with our rat anti-murine IgE antibodies.

Materials and Methods

Animals

Female BALB/c mice and female Sprague-Dawley rats were obtained from the Charles River Laboratory, Japan.

Cells

The mouse mast cells (PT-18) and the rat basophilic leukemia cells (RBL/2H3) were generous gifts of Dr. C. Ra (Department of Immunology, Juntendo University, Tokyo, Japan). The PT-18 cells were maintained in tissue culture in MEM + 3 mM *L*-glutamine and 1 mM sodium pyruvate with penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% heat-inactivated FCS. The RBL/2H3 cells were maintained in tissue culture in MEM plus 10% heat-inactivated FCS, 3 mM *L*-glutamine with penicillin (100 U/ml), and streptomycin (100 µg/ml).

Monoclonal Antibodies

SPE-7 (anti-DNP murine IgE antibody) [18] was purchased from Seikagaku (Tokyo, Japan). Rat mAb 6HD5 and HMK-12 (specific for the murine IgE) [13] and JKS-6 (specific for the IgE^b allotype) [17] have been described previously. The idiotype-specific Eb-1 has also been established in our laboratory [unpubl. data].

Antigen and Staining

DNP₁₄-BSA: 14 molecules of DNP (70 kD molecular weight) of bovine serum albumin were prepared in our laboratory [13]. Evans blue dye was obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Flow-Cytometric Analysis

Inhibition of Binding of IgE to FcεRI on Mast Cells (PT-18) and Basophilic Leukemia Cells (RBL/2H3) by Anti-IgE Antibodies. FITC-labeled SPE-7 was preincubated at room temperature for 30 min with an equal volume of two concentrations (10 and 1 µg/ml) of each of the following antibodies: 6HD5, HMK-12, JKS-6 and Eb-1. After 30 min at room temperature, 0.1 ml of each of the above concentrations were added to 1 × 10⁶/0.1 ml of 2H3/RBL of PT-18 cells, then kept at 4°C for 30 min. The cells were then washed twice in PBS and centrifuged for 5 min at 1,200 rpm, resuspended in 0.3 ml of PBS, and analyzed by FACS Vantage (Becton-Dickinson, Calif., USA).

Removal of IgE Bound to FcεRI on Mast Cells (PT-18) and Basophilic Leukemia Cells (RBL/2H3). FITC-labeled SPE-7 was incubated for 1 h at 4°C with 0.1 ml of 1 × 10⁷/ml RBL/2H3 and a second series with PT-18 cells. After washing, the cells were resuspended in 0.2 ml of MEM culture medium and 10 µl of 10 mg/ml of anti-IgE antibodies were added to each tube. The anti-IgE antibodies were 6HD5, HMK-12, JKS-6, Eb-1, and rat IgG (control). The cells were incubated in 5% CO₂ at 37°C for 30, 60, or 120 min. After incubation, the cells were centrifuged and then resuspended in the initial volume for FACS analysis. The culture supernatants were decanted and kept at 4°C for the IgE complex assay by ELISA. FACS data were calculated by the following equation:

Inhibition (%) or removal (%) =

$$100 - \left(\frac{\text{sample MFI} - \text{auto-MFI}}{\text{sIgE positive cell MFI} - \text{auto-MFI}} \times 100 \right)$$

where MFI = mean fluorescence intensity and sIgE = surface IgE.

IgE Complex Assay

The IgE complex was detected using the ELISA system [19] with a slight modification. The anti-DNP-IgE/rat-anti-mouse IgE antibody complex in culture supernatants and serum was detected by a sandwich ELISA. The plates were coated with DNP-BSA at room temperature for 1 h and then blocked with 1% BSA-PBS. Samples were placed in the well, and the plates were incubated for 1 h at room temperature. The bound anti-DNP-IgE/rat anti-mouse IgE antibody complex was detected by an HRP-coupled anti-rat IgG (Zymed, San Francisco, Calif., USA). The plates were developed by the addition of a peroxidase substrate and read in an ELISA plate reader at 450 nm.

PCA Reactions

PCA reactions were performed as described [20]. Several sites of the freshly shaved skin of 3 rats for each group were injected intradermally with 0.1 ml of anti-DNP-IgE SPE-7 (10 ng/0.1 ml) and, 24 h later, 0.1-ml dilutions of 6HD5 or HMK-12 were injected into the same sites. Two hours after the second series of injections, the rats were injected intravenously with 0.2 ml of a mixture of equal quantities of 0.5% Evans blue dye in saline and 1 mg/ml DNP₁₄-BSA. The reactions (the diameters of the blue spots) were measured 30 min later.

Systemic Anaphylaxis

Systemic anaphylaxis was performed as described by Ando et al. [21]. Three of the BALB/c mice for each group were injected intravenously with 20 µg of anti-DNP-IgE SPE-7 in 0.1 ml of saline and, after 24 h, they were injected intravenously with 0.1 ml of 1 mg of the anti-IgE antibody (HMK-12). The control was rat IgG. After 2 h, they were challenged with an intravenous injection of 0.2 ml of 1 mg/ml of DNP₁₄-BSA in 0.5% Evans blue dye.

Quantitative Extraction of Dye Extravasation to the Skin (Ear)

The systemic anaphylaxis reaction was quantified by measuring the dye (Evans blue) concentration in the extracts of the skin (ear) of BALB/c mice, as described previously [22]. Thirty minutes after the intravenous injection of the dye, the blue area of the skin (ears) was cut into small fragments (10 from each ear). The dye was then extracted for 24 h in 2 ml of a mixture of 1.4 ml of acetone and 0.6 ml of a 0.5% aqueous solution of sodium sulfate, and the preparation was then centrifuged for 10 min at 1,000 rpm. The supernatant was measured for OD at 620 nm. The percentage recovery of the dye was calculated by comparing the absorbance of the supernatants at 620 nm with that of the test and control mice. Normal skin (dye only) was similarly extracted as a negative control.

Purification and Fragmentation of the Anti-IgE mAb

Fab fragments were prepared with immobilized papain (Pierce, Rockford, Ill., USA), and the fragments of papain cleavage were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gel [23].

Table 1. Percent inhibition of PT-18

Antibody	Concentration mg	Inhibition %
HMK-12	10	96.9
	1	58.6
6HD5	10	30.9
	1	28.0
JKS-6	10	62.5
	1	51.2
Eb-1	10	24.2
	1	30.1

Results

Inhibition Assay of IgE Binding to FcεRI on Mast Cells (PT-18) and Basophilic Leukemia Cells (RBL/2H3) by Anti-Isotypic IgE Antibodies (HMK-12, 6HD5), Anti-Allotypic (JKS-6), and Anti-Idiotypic (Eb-1) IgE Antibodies

To check whether the anti-IgE mAbs are directed against sites within the Fc region, which bind to the FcεRI, we analyzed the ability of different mAbs to interfere with the binding of IgE to RBL/2H3 or PT-18.

FITC-SPE-7 was incubated with HMK-12, 6HD5, Eb-1 and JKS-6 to see the eventual inhibition of binding of FITC-SPE-7 to mast cells (PT-18) and basophilic leukemia cells (RBL/2H3). FITC-SPE-7-bound PT-18 or RBL/2H3 cells (surface IgE positive) were analyzed by FACS.

As shown in figure 1, HMK-12 inhibited the binding of IgE to PT-18 and RBL/2H3 in a dose-dependent pattern. Table 1 shows the percent inhibition of IgE binding to PT-18. While a strong inhibitory effect was observed with HMK-12 (inhibition: 96.9%), less inhibition was seen with other anti-IgE antibodies, such as 6HD5, JKS-6, and Eb-1 (inhibition was 30.9, 62.5, and 24.1%, respectively) by FACS analysis.

Anti-IgE Antibody (HMK-12) Removes IgE Binding to FcεRI on Mast Cells (PT-18) and Basophilic Leukemia Cells (RBL/2H3)

FITC-SPE-7 was incubated with PT-18 and RBL/2H3 for 1 h and then washed. Cold antibodies (HMK-12, 6HD5, JKS-6, Eb-1) were added to the cultures. Two hours later, the surface expression of IgE-positive cells was analyzed and compared to the controls.

As shown in figure 2, FITC-SPE-7-bound cells (sIgE-positive cells) decreased in RBL/2H3 or in PT-18 treated with HMK-12 as the incubation times were lengthened.

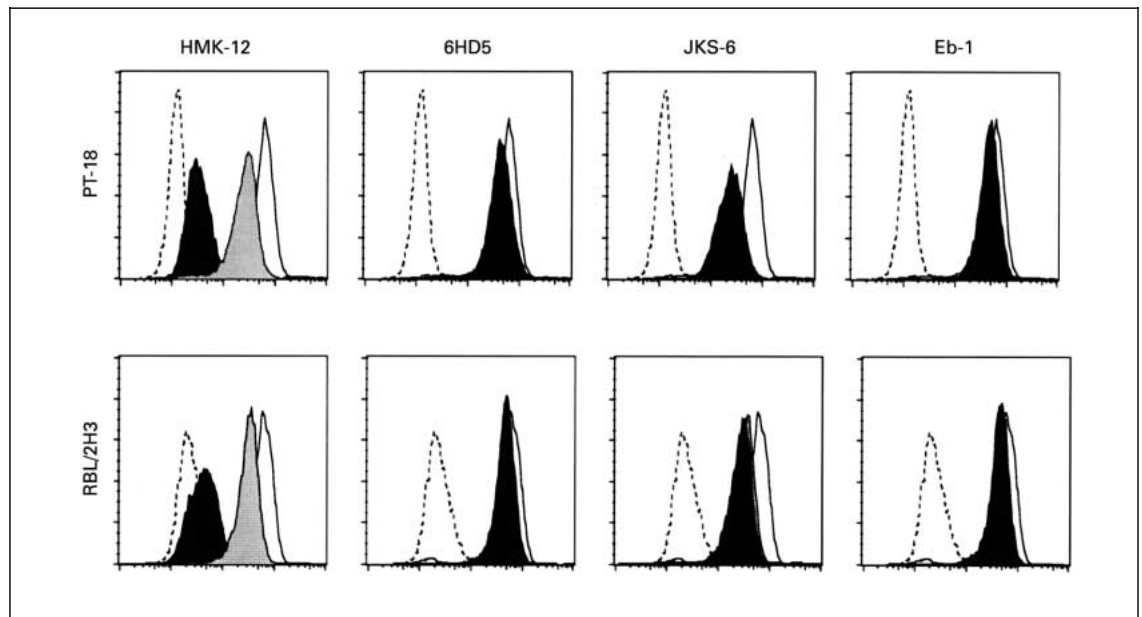


Fig. 1. Inhibition assay of IgE binding to Fc ϵ RI on mast cells (PT-18) or basophilic leukemia cells (RBL/2H3) with various anti-IgE antibodies measured by FACS. FITC-SPE-7 was incubated with HMK-12, 6HD5, Eb-1 and JKS-6. Antibody absorbed with 10 (black shading) or 1 μ g (gray shading) was added to mast cells (PT-18) or basophilic leukemia cells (RBL/2H3). Unshaded areas with broken outline: autofluorescence; unshaded areas with solid outline: nontreated (SPE-7 only). FITC-SPE-7-bound (sIgE-positive) PT-18 and RBL/2H3 cells were analyzed by FACS.

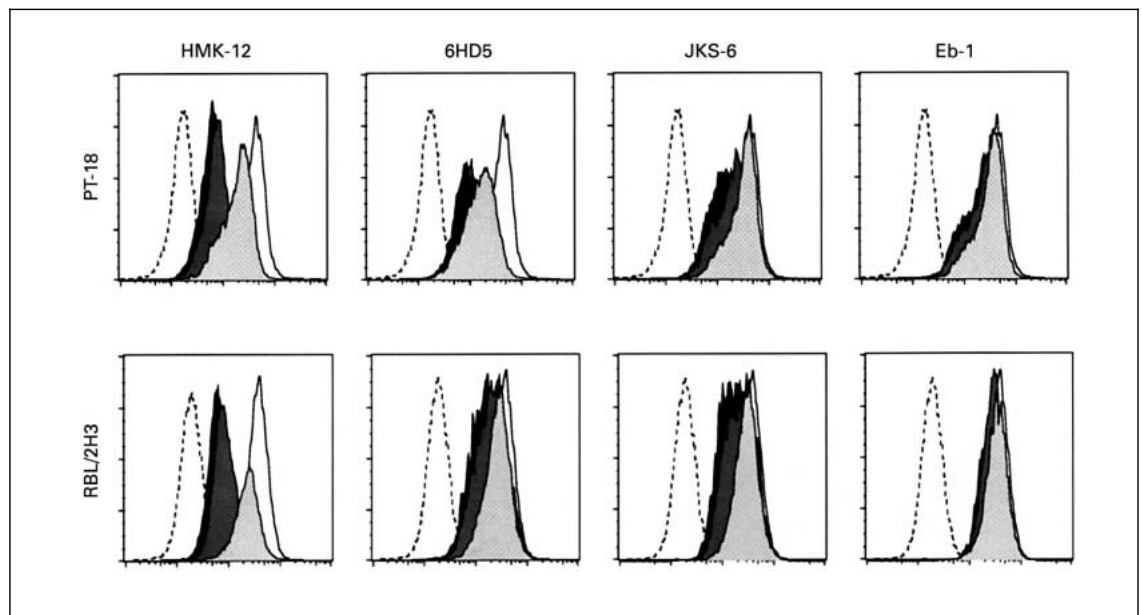


Fig. 2. The anti-IgE antibody (HMK-12) removes IgE bound to Fc ϵ RI on mast cells (PT-18) or basophilic leukemia cells (RBL/2H3). FITC-SPE-7 was incubated with PT-18 and RBL/2H3 for 1 h. Then cold antibodies (HMK-12, 6HD5, JKS-6, Eb-1) were added to the culture. We then analyzed IgE on both cells. Incubation time with cultured cells (PT-18 and RBL/2H3) and anti-IgE antibodies were 30 min (light-gray shading), 1 h (dark-gray shading) and 2 h (black shading). Unshaded areas with solid outline: control rat IgG; unshaded areas with broken outline: autofluorescence.

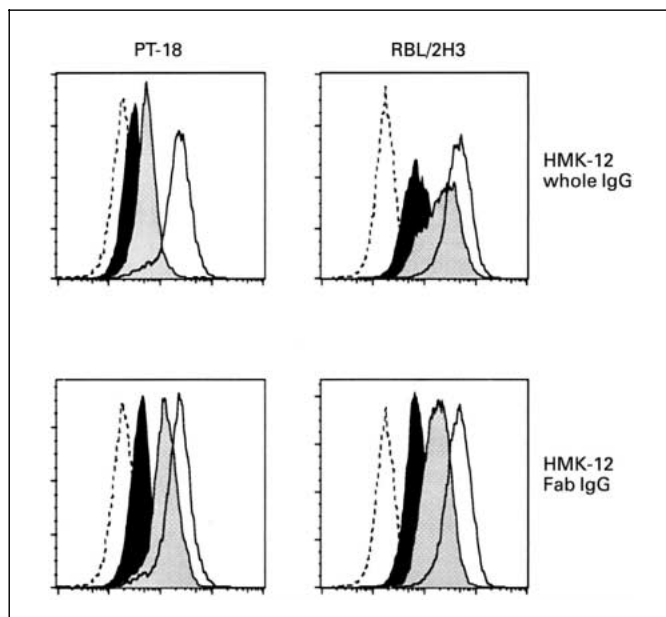


Fig. 3. Fab fragment of HMK-12 removes IgE bound to Fc ϵ RI of PT-18 and RBL/2H3. FITC-SPE-7 was incubated with PT-18 and RBL/2H3. Two hours later, cold antibodies (intact HMK-12, or Fab fragments) were added to the culture. The antibody concentrations were 10 (black shading) and 1 μ g (gray shading). Unshaded areas with broken outline: autofluorescence; unshaded areas with solid outline: nontreated (SPE-7 only).

The percent removal of IgE binding (table 2) confirmed that HMK-12 (removal: 88.1%) had stronger removal effects than other anti-IgE antibodies (6HD5: 75%; JKS-6: 78%, and Eb-1: 53.1%). The same results were observed when Fab fragments instead of intact IgG of HMK-12 were used for the experiments shown in figure 3.

Figure 3 demonstrated that SPE-7-bound positive cells were dose-dependently decreased in RBL/2H3 treated with Fab fragments and intact IgG of HMK-12.

IgE/Anti-IgE-Immune Complexes Detected in Culture Supernatants of Mast Cells or Basophilic Leukemia Cells Already Binding IgE with Anti-IgE Antibodies

When mast cells or basophilic leukemia cells already binding with IgE were cultured with HMK-12, immune complexes could be detected in the supernatants. When 6HD5 was used instead of HMK-12, the amount of immune complexes detected was low. No immune complexes were detected with JKS-6 or Eb-1 (fig. 4).

Table 2. Percent inhibition data presented in figure 2

Antibody	Time min	Removal, %	
		RBL/2H3	PT-18
HMK-12	120	86.8	88.1
	60	80.8	83.6
	30	40.8	52.1
6HD5	120	53.0	75.3
	60	48.6	73.9
	30	28.9	60.6
JKS-6	120	50.0	67.8
	60	42.2	53.9
	30	19.2	15.8
Eb-1	120	24.8	53.1
	60	20.2	44.4
	30	9.3	18.4

Anti-IgE Antibodies Prevent Systemic Anaphylaxis

Systemic anaphylaxis was performed as described. For each group, 3 BALB/c mice were injected intravenously with 1 mg of HMK-12 (fig. 5d), 6HD5 (fig. 5c), rat IgG (fig. 5b) and PBS only (fig. 5a) 2 h before injection of the antigen (DNP-BSA). The mice of the 6 HD5 and HMK-12 groups did not show any anaphylactic symptoms, and dye extravasation on their ears could not be seen. However, the mice of the PBS and rat IgG groups showed systemic anaphylactic symptoms, and dye extravasation could be observed (fig. 5).

The extracts of the skin lesions were quantified by measuring the dye concentration (fig. 5e). The percentage of dye extravasation was very high in the mice treated with PBS (fig. 5a) and rat IgG (fig. 5b), but it was low in the mice treated with 6HD5 (fig. 5c) and HMK-12 (fig. 5d). These data confirmed the results of figure 5a–d, suggesting that HMK-12 and 6HD5 had a strong inhibitory effect on systemic anaphylactic reactions.

Anti-IgE Antibodies Inhibit PCA

Serial dilutions of SPE-7 were injected intradermally into 3 rats of each group. Twenty-four hours later, the rats were challenged by intravenous injection of 1 mg of DNP₁₄-BSA and Evans blue dye. These rats served as controls.

In a second series, the rats were injected intravenously, just like the controls, except that 24 h later 0.1-ml dilutions of the different monoclonal anti-IgE antibodies were injected into the same sites. Two hours later, the animals were challenged by intravenous injection of DNP₁₄-BSA

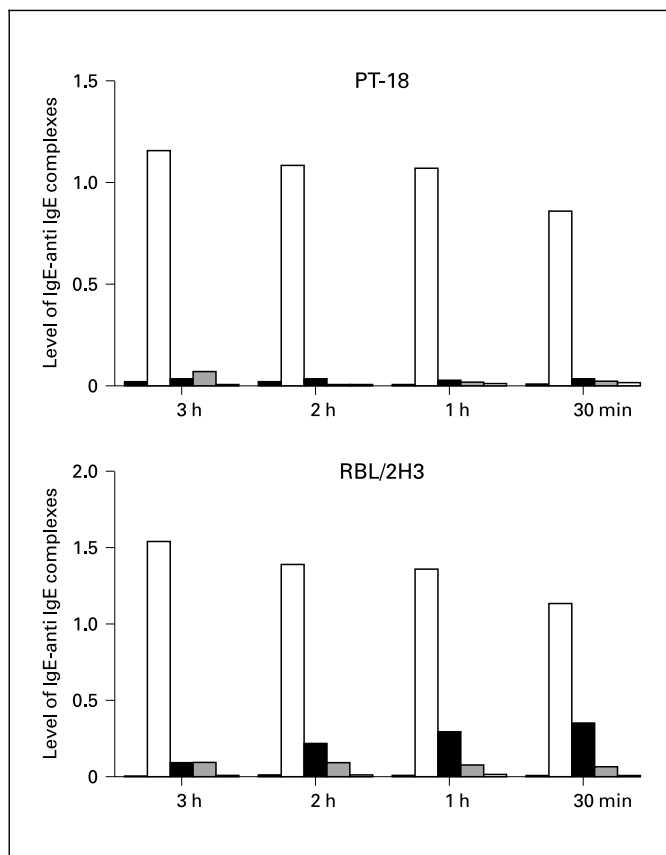


Fig. 4. Anti-DNP-IgE/anti-IgE immune complexes in culture supernatants. Anti-DNP-IgE/anti-IgE immune complexes detected in culture supernatants of IgE-fixing mast cells treated with several anti-IgE antibodies: HMK-12 (white columns), 6HD5 (black columns), JKS-6 (dark-gray columns) and Eb-1 (light-gray columns). OD: 490 nm.

and Evans blue dye (table 3). As shown in table 3, sites injected with HMK-12 or Fab of HMK-12 did not induce PCA reactions, but sites injected with JKS-6 and rat IgG did.

IgE/Anti-IgE-Immune Complexes Detected in Sera of Mice Treated with the Anti-IgE Antibody (HMK-12)

BALB/c mice were injected intravenously with SPE-7 and were treated 1 day thereafter with the anti-IgE antibody (HMK-12 or rat IgG). These mice were bled from the tail vein 1–3 days after the anti-IgE antibody injection.

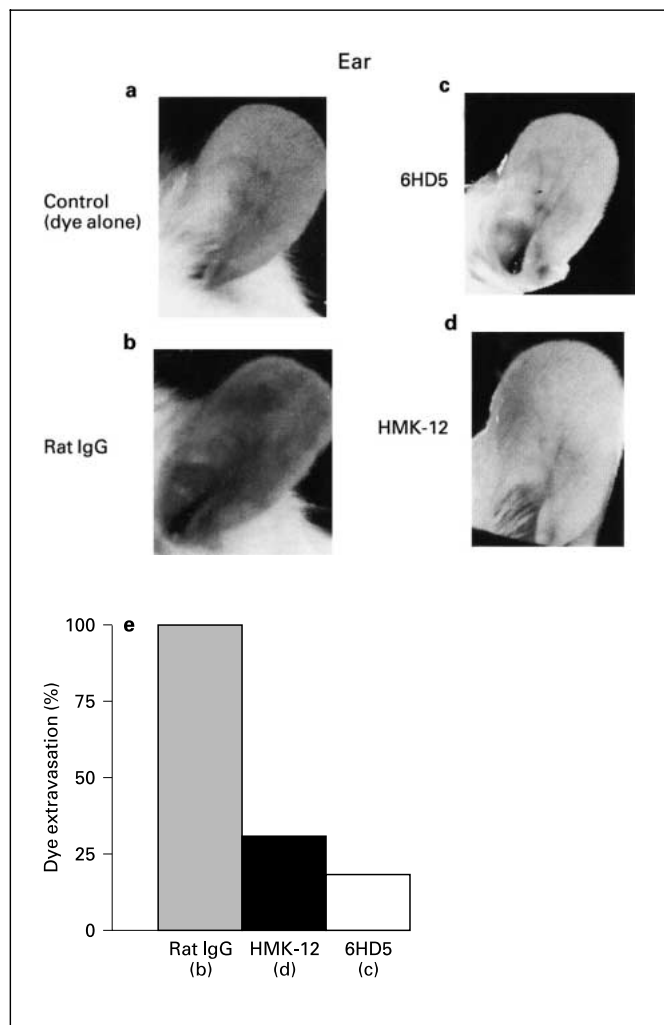


Fig. 5. a–d Anti-IgE antibodies prevent IgE-induced systemic anaphylaxis. Dye extravasations in anti-DNP-IgE-sensitized mice during IgE-induced systemic anaphylaxis. BALB/c mice were injected intravenously with 20 μ g of anti-DNP IgE. 24 h later, they were injected intravenously with 1 mg of anti-IgE antibodies. a Control. b Rat IgG. c 6HD5. d HMK-12. 2 h later, they were injected intravenously with 1 mg of DNP-BSA in 0.5% Evans blue. As a control, mice were injected intravenously with only 1 mg of DNP-BSA in 0.5% Evans blue. e Extracts of the skin lesions were quantified by measuring the dye (Evans blue) concentration. Gray column: rat IgG; black column: HMK-12; white column: 6HD5.

tions. IgE/anti-IgE immune complexes were detected in the sera of mice injected intravenously with HMK-12 from day 1 through day 3, but the amounts of immune complexes were low in the sera of those injected intravenously with rat IgG (control; fig. 6).

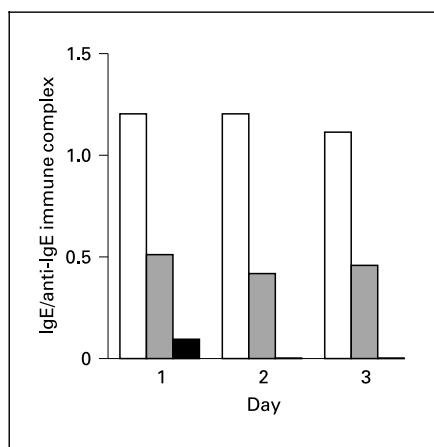


Fig. 6. Anti-DNP-IgE/anti-IgE immune complexes were detected in the sera of mice injected intravenously with the anti-IgE antibody (HMK-12). Mice were injected intravenously with SPE-7 and 1 day later with the anti-IgE antibody [HMK-12 (white columns), 6HD5 (gray columns), rat IgG (black columns)], also intravenously. The sera were obtained by bleeding from tail veins 1–3 days after anti-IgE injections. OD measured at 450 nm.

Table 3. Anti-IgE antibodies inhibit PCA

	Antibodies, µg				
	100	10	1	0.1	0
6HD5	—	—	—	++	+++
HMK-12	—	—	+++	+++	+++
HMK-12 Fab	—	±	+++	+++	+++
JKS-6	+++	+++	+++	+++	+++
Rat IgG	+++	+++	+++	+++	+++

Serial dilutions of SPE-7 were injected intradermally into rats. 24 h later, several dilutions of anti-IgE antibodies (HMK-12, Fab of HMK-12, 6HD5, JKS-6, or rat IgG) were injected into the same sites. Another 2 h later, DNP-BSA + Evans blue were injected intravenously for the challenge. +++ = Blue spot of ≥ 15 mm in diameter; ++ = ≥ 10 mm in diameter; + = < 10 mm in diameter; ± = < 5 mm in diameter; — = no visible blue spot.

Discussion

Mast cells are usually distributed in vascularized tissues and serosal cavities, where they can function as effector cells in IgE-dependent immune responses. Indeed, IgE-dependent immune responses of mast cells mediator secretions are thought to contribute significantly to the pathogenesis of allergic diseases.

Baniyash et al. [12] described monoclonal anti-IgE antibodies in 1986. They showed that anti-IgE antibodies inhibit the binding of IgE to mast cells/basophilic leukemia cells and suppress serotonin release in vitro, and PCA reactions in vivo. We have also established several monoclonal antibodies specific for the murine IgE (the isotype, allotype and idiotype) [13, 17]. In our studies, isotype- or allotype-specific IgE antibodies could be detected in the sera or culture supernatants and surface IgE expression on B cells, too [14, 15].

We found that HMK-12 inhibited the IgE binding to FcεRI, because it competed with the binding site of IgE to FcεRI on mast cells and basophils by FACS analysis (fig. 1).

HMK-12 also removed IgE molecules from mast cells, even if an anti-IgE antibody was added after IgE had been fixed to mast cells (fig. 2, 3). Why is HMK-12 able to detach IgE from FcεRI? It is speculated that the affinity of HMK-12 for IgE is stronger than that of FcεRI. However, further studies are required to solve this problem. We also found that IgE/anti-IgE immune complexes were detected in supernatants of IgE-binding mast cells cultured with anti-IgE antibodies (fig. 4). The Fab fragments of the anti-IgE (HMK-12) antibody could have the same ability as the intact antibodies to inhibit allergic reactions using mast/basophilic leukemia cells in vitro. Our experiments showed that an anti-IgE antibody could get rid of IgE molecules from mast cells and produce IgE/anti-IgE immune complexes. When we used B cells instead of mast/basophilic leukemia cells, we could not detach the bound IgE [unpubl. data].

This was the first observation that anti-IgE antibodies could detach IgE molecules from mast cells. Therefore, we carried out the in vivo studies on systemic anaphylaxis and PCA with the use of these antibodies.

In the case of systemic anaphylaxis, mice injected with the anti-IgE antibody (HMK-12) did not show symptoms of a systemic anaphylactic shock, and dye extravasation could not be seen on the ear in this group, but mice treated with other antibodies (JKS-6/Eb-1) showed shaking, dyspnea, and incontinence (data not shown), and dye extravasation could also be observed (fig. 5a–d). As shown in figure 5e, the eluates of the skin confirmed that the anti-IgE antibody (HMK-12) prevents systemic anaphylaxis.

IgE/anti-IgE immune complexes were also detected in the sera of mice treated with HMK-12 (fig. 6). PCA reactions with anti-IgE antibody were inhibited by HMK-12 (table 3). We showed that anti-IgE antibodies inhibit or suppress allergic reactions in vivo without any side effects.

Why did anti-IgE antibodies inhibit/suppress allergic reactions in mast cells or basophils? The reason is that the anti-IgE antibody (HMK-12) can remove IgE molecules from FcεRI on mast cells and can produce IgE/anti-IgE immune complexes. Many studies demonstrated the physiological significance of the FcεRI interaction with FcγRs in vitro and in vivo, and a novel regulatory pathway for the type I hypersensitivity response was represented [24–28].

The ability of IgE to bind with low affinity to Fc receptors for IgG (FcγRIIB and FcγRIII) is complex. Takai et al. [24] showed that FcγRIIB knockout mice display an enhanced IgE-mediated anaphylactic response, whereas FcγRIII knockout mice display a decrease in the IgE-mediated response, suggesting that in relation to FcεRI the IgE-mediated response was downregulated by FcγRIIB but upregulated by FcγRIII.

It would be interesting to study the importance of the FcγRIIB/FcγRIII interaction with FcεRI in our future work.

Saini et al. [29] and MacGlashan et al. [30] showed that anti-IgE antibodies could decrease free IgE levels and finally downregulated FcεRI on mast cells/basophilic cells. Therefore, FcεRI densities may be regulated by plasma free IgE levels. Our studies show that anti-IgE antibodies remove IgE molecules from mast/basophil cells and generate immune complexes.

Recently, Milgrom et al. [31] showed in clinical studies that a humanized monoclonal antibody against IgE has potential as a treatment for patients with severe allergic asthma. We have reported here in vivo studies of systemic anaphylaxis and PCA reactions using anti-IgE antibodies. Anti-IgE antibodies inhibit systemic anaphylaxis and PCA reactions. These studies show that monoclonal anti-IgE antibodies could be important in regulating IgE-mediated anaphylactic reactions in animal systems and also in treating human patients with type I allergic diseases.

References

- 1 Ishizaka T, Ishizaka K: Activation of mast cells for mediator release through IgE receptors. *Prog Allergy* 1984;34:188–235.
- 2 Serafin WE, Austen KF: Mediators of immediate hypersensitivity reactions. *N Engl J Med* 1987;317:30–34.
- 3 Metzger H: The receptor with high affinity for IgE. *Immunol Rev* 1992;125:37–48.
- 4 Ovary Z: Activité des substances à faible poids moléculaire dans les réactions antigène-anticorps in vivo et in vitro. *CR Séances Acad Sci* 1961;253:582–583.
- 5 Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM: Humanization of an antibody directed against IgE. *J Immunol* 1993;151:2623–2632.
- 6 Jardieu PM, Fick RB Jr: IgE inhibition as a therapy for allergic disease. *Int Arch Allergy Immunol* 1999;118:112–115.
- 7 Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB Jr, Cockcroft DW: Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response (see comments). *Am J Respir Crit Care Med* 1997;155:1835–1840.
- 8 Presta L, Shields R, O'Connell L, Lahr S, Porter J, Gorman C, Jardieu P: The binding site on human immunoglobulin E for its high affinity receptor. *J Biol Chem* 1994;269:26368–26373.
- 9 Shields RL, Werther WR, Zioncheck K, O'Connell L, Klassen T, Fendly B, Presta LG, Jardieu PM: Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int Arch Allergy Immunol* 1995;107:412–413.
- 10 Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P: Active anaphylaxis in IgE-deficient mice. *Nature* 1994;370:367–370.
- 11 Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP: Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* 1993;75:969–976.
- 12 Baniyash M, Eshhar Z, Rivnay B: Relationships between epitopes on IgE recognized by defined monoclonal antibodies and by the Fc epsilon receptor on basophils. *J Immunol* 1986;136:588–593.
- 13 Hirano T, Miyajima H, Kitagawa H, Watanabe N, Azuma M, Taniguchi O, Hashimoto H, Hirose S, Yagita H, Furusawa S, et al: Studies on murine IgE with monoclonal antibodies. I. Characterization of rat monoclonal anti-IgE antibodies and the use of these antibodies for determinations of serum IgE levels and for anaphylactic reactions. *Int Arch Allergy Appl Immunol* 1988;85:47–54.
- 14 Azuma M, Hirano T, Miyajima H, Watanabe N, Yagita H, Enomoto S, Furusawa S, Ovary Z, Kinashi T, Honjo T, et al: Regulation of murine IgE production in SJA/9 and nude mice. Potentiation of IgE production by recombinant interleukin 4. *J Immunol* 1987;139:2538–2544.
- 15 Miyajima H, Hirano T, Hirose S, Karasuyama H, Okumura K, Ovary Z: Suppression by IL-2 of IgE production by B cells stimulated by IL-4. *J Immunol* 1991;146:457–462.
- 16 Usui M, Hirano T, Miyajima H, Ando S, Kurimoto M, Yamaji C, Matuhashi T, Ovary Z, Okumura K: Construction of a monoclonal antibody against Igh-7a. *Immunogenetics* 1993;37:301–304.
- 17 Yamada M, Hirano T, Miyajima H, Hirose S, Ovary Z, Okumura K: Construction of a monoclonal antibody against Igh-7b. *Immunogenetics* 1994;40:296–299.
- 18 Eshhar Z, Ofarim M, Waks T: Generation of hybridomas secreting murine reagenic antibodies of anti-DNP specificity. *J Immunol* 1980;124:775–780.
- 19 Zuberi RI, Apgar JR, Chen SS, Liu FT: Role for IgE in airway secretions: IgE immune complexes are more potent inducers than antigen alone of airway inflammation in a murine model. *J Immunol* 2000;164:2667–2673.
- 20 Ovary Z, Caiazza SS, Kojima S: PCA reactions with mouse antibodies in mice and rats. *Int Arch Allergy Appl Immunol* 1975;48:16–21.
- 21 Ando A, Martin TR, Galli SJ: Effects of chronic treatment with the c-kit ligand, stem cell factor, on immunoglobulin E-dependent anaphylaxis in mice. Genetically mast cell-deficient SI/Sld mice acquire anaphylactic responsiveness, but the congenic normal mice do not exhibit augmented responses. *J Clin Invest* 1993;92:1639–1649.

- 22 Harada M, Takeuchi M, Fukao T, Katagiri K: A simple method for the quantitative extraction of dye extravasated into the skin. *J Pharm Pharmacol* 1971;23:218–219.
- 23 Rousseaux J, Rousseaux-Prevost R, Bazin H: Optimal conditions for the preparation of Fab and F(ab')₂ fragments from monoclonal IgG of different rat IgG subclasses. *J Immunol Methods* 1983;64:141–146.
- 24 Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV: Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* 1996;379:346–349.
- 25 Takizawa F, Adamczewski M, Kinet JP: Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as Fc gamma RII and Fc gamma RIII. *J Exp Med* 1992;176:469–475.
- 26 Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, Ravetch JV, Takai T: Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med* 1999;189:1573–1579.
- 27 Malbec O, Fong DC, Turner M, Tybulewicz VL, Cambier JC, Fridman WH, Dacron M: Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. *J Immunol* 1998;160:1647–1658.
- 28 Philips JR, Brouwer W, Edwards M, Mahler S, Ruhno J, Collins AM: The effectiveness of different rat IgG subclasses as IgE-blocking antibodies in the rat basophil leukaemia cell model. *Immunol Cell Biol* 1999;77:121–126.
- 29 Saini SS, MacGlashan DW Jr, Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, Bochner BS: Down-regulation of human basophil IgE and Fc epsilon RI alpha surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. *J Immunol* 1999;162:5624–5630.
- 30 MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM: Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438–1445.
- 31 Milgrom H, Fick RB Jr, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ: Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Engl J Med* 1999;341:1966–1973.