

INHIBITION OF ALLERGIC REACTIONS WITH MONOCLONAL ANTIBODY TO THE HIGH AFFINITY IgE RECEPTOR

SEIICHI KITANI, DANIEL KRAFT, CYNTHIA FISCHLER, STEPHEN E. MERGENHAGEN, AND REUBEN P. SIRAGANIAN¹

From the Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892

A mAb that reacts with the high affinity IgE-R on the rat basophilic leukemia cells (RBL-2H3) was used to inhibit allergic reactions. *In vitro*, the intact mAb BA3 and its Fab fragment inhibited radiolabeled IgE binding to the RBL-2H3 cells. The mAb binds to the IgE-R with a higher affinity than does IgE. Whereas the intact mAb released histamine from the RBL-2H3 cells, the Fab was inactive. The addition of the Fab fragments to RBL-2H3 inhibited the IgE-mediated histamine release reaction. The Fab fragments also inhibited *in vivo* passive cutaneous reactions in rats when injected intradermally either before or after IgE. The injection of the mAb Fab *i.v.* before the injection of the IgE into the skin sites also inhibited reactions, although it was less effective. The results demonstrate that anti-R antibodies can be used as a model for inhibiting immediate hypersensitivity reactions.

Mediators of immediate hypersensitivity are released from basophils and mast cells after the cross-linking of the high affinity IgE-R on these cells (1-3). There are conflicting reports on the capacity of nonspecific IgE to inhibit allergic reactions (4-6). This could be due to several reasons. Only a few IgE molecules on the surface of the basophils are sufficient for activating the cell for secretion and the competing non-Ag-specific IgE has the same affinity of binding to the receptor as the Ag-specific IgE.

Cells from the rat basophilic leukemia cell line RBL-2H3 have high affinity IgE-R and release histamine after the cross-linking of their IgE-R (7). Here we report the isolation of an anti-R mAb that binds with a higher affinity than does IgE to its own receptor. This mAb inhibits allergic reactions.

MATERIALS AND METHODS

Materials. All materials were obtained as described previously (8). The monoclonal anti-TNP IgE was purified from mouse ascites (9).

Preparation of anti-Fc_εR² mAb. The method previously described was used in these studies (8, 10). Briefly, BALB/c mice were immu-

nized *i.m.* with 10⁷ RBL-2H3 cells emulsified in CFA. The injections were repeated every 10 to 14 days for six times and then at monthly intervals. The spleen cells from one mouse were used for adoptive transfer into irradiated mice as described previously. Hybridoma cell lines were selected that reacted with the high affinity IgE-R; these were expanded, cloned, and then eventually injected into pristane-primed mice to form ascites. The mAb BA3 was purified from ascites by ammonium sulfate precipitation followed by ion exchange chromatography.

The mAb BA3 was digested with papain (2 to 5% by weight) for 2 to 4 h at 37°C in 0.2 M sodium acetate buffer (pH 5.6) with 4.0 mM cysteine and 4.0 mM EDTA. The Fab were then separated by DE-52 ion exchange chromatography followed by gel filtration. The Fab preparations did not contain intact or partially digested molecules as determined by SDS-PAGE.

Histamine release. The RBL-2H3 cells were maintained as described previously (7). The assay for histamine release from the RBL-2H3 cells in monolayer culture was performed as described previously (11, 12).

Binding assays. The different ¹²⁵I-labeled Ig were prepared and used in binding assays as described previously (8). The ¹²⁵I-IgE was the purified monoclonal anti-DNP IgE (13). The association and dissociation rate for ¹²⁵I-mAb BA3 Fab was determined (14, 15).

PCA reactions. The PCA reaction was done in Sprague-Dawley rats (16).

Immunoprecipitation with the mAb. The RBL-2H3 cells were cell surface labeled with ¹²⁵I, and the immunoprecipitation with different antibodies was done as reported previously (8). The samples were analyzed by SDS-PAGE.

RESULTS

The mAb BA3 was of the IgG₁ isotype and had similar characteristics to the previously described anti-Fc_εR mAb (8). By 14% SDS-PAGE electrophoresis the mAb BA3, IgE, and the previously described anti-receptor antibodies immunoprecipitated a broad 50 to 60-kDa band from surface-labeled cells (Fig. 1). The number of radiolabeled mAb BA3 molecules bound to the RBL-2H3 cells was similar to the Fc_εR number ($1.88 \pm 0.76 \times 10^5$ intact mAb BA3 and $2.11 \pm 0.75 \times 10^5$ Fab BA3 molecules/cell bound; mean $\pm$ SD). Intact mAb BA3 and the Fab fragment efficiently inhibited the binding of ¹²⁵I-IgE to the RBL-2H3 cells (Fig. 2). The concentrations (mean $\pm$ SD) for 50% inhibition of binding (IC₅₀) were as follows: mAb BA3, 0.028 ± 0.010 μ g/ml; Fab mAb BA3, 0.038 ± 0.011 μ g/ml; and IgE, 6.03 ± 1.2 μ g/ml. Therefore, both the intact mAb and the Fab fragment are 40- to 150-fold more efficient than IgE in blocking the binding of IgE to the Fc_εR. The mAb inhibited the binding of IgE at nanomolar concentrations. The high affinity of this mAb for binding to the RBL-2H3 was also found in binding studies. With the ¹²⁵I-labeled Fab fragment, the association rate constant at 4°C, k_1 , was 4.96×10^6 M⁻¹ sec⁻¹; this value is higher than the k_1 for IgE binding to RBL-2H3 cells (7, 14, 15, 17). The dissociation rate constant at 4°C, k_{-1} , was 1.87×10^{-5} sec⁻¹, a value similar to that of

Received for publication July 10, 1987.

Accepted for publication January 22, 1988.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Please address all correspondence and requests for reprints to Reuben P. Siraganian, Clinical Immunology Section, Laboratory of Microbiology and Immunology, NIDR, NIH, Bethesda, MD 20892.

² Abbreviations used in this paper: Fc_εR, high affinity IgE-R on basophils/mast cells; PCA, passive cutaneous anaphylaxis.

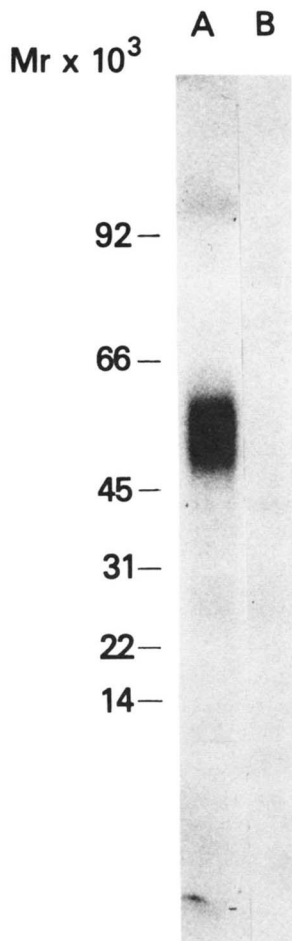


Figure 1. Proteins immunoprecipitated from ^{125}I surface-labeled RBL-2H3 cells by mAb BA3. The cells were surface labeled as described and the solubilized cells incubated with the antibodies attached to Sepharose 4B. After washing, the immunoprecipitated proteins were eluted and analyzed by 14% SDS-PAGE under nonreducing conditions, followed by autoradiography. A, mAb BA3; B, mouse IgG.

IgE. Therefore, the binding constant, K_a , is $2.65 \times 10^{11} \text{ M}^{-1}$ for this mAb. As has been observed with IgE, the K_a was 10-fold lower when estimated from Scatchard analysis of binding data. In other experiments, the addition of Fab mAb BA3 did not affect the rate of the dissociation of ^{125}I -IgE from cells. Therefore, the anti-receptor antibody did not induce a structural change in the receptor that resulted in an increased IgE dissociation.

Cross-linking of IgE or of the Fc ϵ R results in histamine release. Therefore, as expected, the intact mAb BA3 released histamine from the RBL-2H3 cells; there was 30% release with $0.004 \mu\text{g/ml}$. In contrast, the Fab fragment failed to release histamine even at concentrations of $10 \mu\text{g/ml}$. The addition of rabbit anti-mouse Ig to the Fab bound to the RBL-2H3 cells caused histamine release. The Fab mAb BA3 inhibited histamine release from the IgE-sensitized RBL-2H3 cells (Fig. 3). This inhibition was most prominent when the anti-R antibody was added before the Ag-specific IgE. When the IgE was added before the Fab there still was inhibition of histamine release, although more of the Fab mAb BA3 had to be added for an equivalent decrease. The inhibition of histamine release can be due to a decrease in cell surface IgE due to a competition between the IgE and the Fab for binding to the Fc ϵ R. In addition to the decrease in surface IgE the anti-R Fab results in the desensitization of basophils (S.

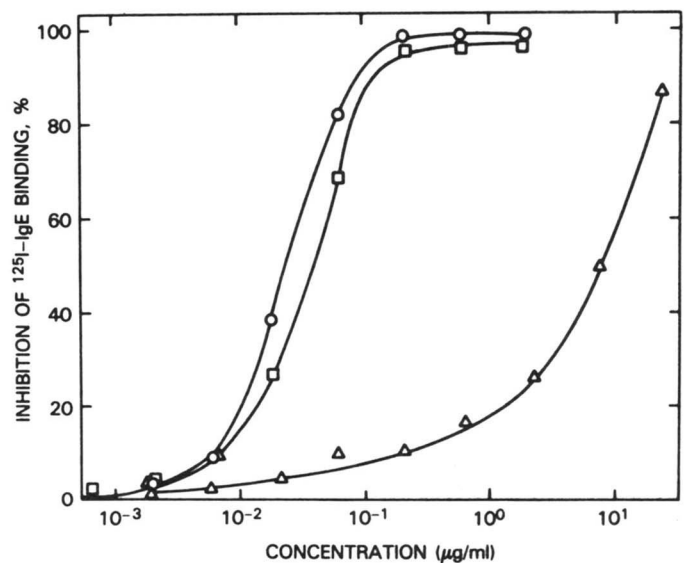


Figure 2. Inhibition of ^{125}I -IgE binding to rat basophilic leukemia cells. $\circ$, mAb BA3; $\square$, Fab fragment of mAb BA3; $\triangle$, IgE. The RBL-2H3 cells at $10^6/0.5 \text{ ml}$ were incubated for 60 min in an ice bath with the different concentrations of the indicated antibodies. ^{125}I -IgE at a concentration for 50% saturation was then added for a second incubation of 60 min. Triplicate aliquots were removed and centrifuged through oil, and the tips of the tubes containing the cell pellets were cut off and the radioactivity determined (8). Nonspecific binding was determined by the addition of a 100-fold excess of unlabeled IgE. The experimental determinations were in triplicate; the results are from five separate experiments.

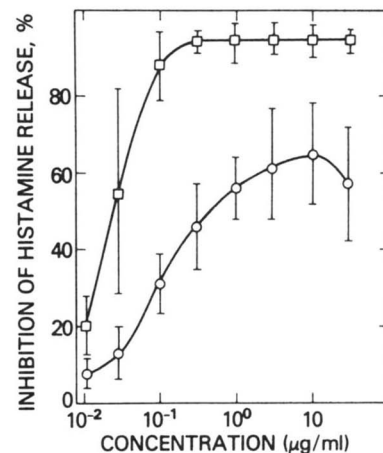


Figure 3. Inhibition of histamine release from the RBL-2H3 cells with the anti-Fc ϵ R mAb. $\square$, Different concentrations of the Fab mAb BA3 added first to the cells for 2 h at 37°C , followed by anti-TNP IgE for another 2 h. $\circ$, IgE added first, followed 2 h later by the Fab mAb BA3 and a further 2-h incubation. The monolayers were then washed with a Pipes-buffered solution and activated for histamine release for 45 min with the Ag DNP-human serum albumin at $0.025 \mu\text{g/ml}$. Control release from cells that were incubated with only IgE antibody was $54.6 \pm 12.8\%$ (mean $\pm$ SD). The experimental determinations were in duplicate; the results are from four experiments.

Kitani, and R. P. Siraganian, manuscript in preparation).

This mAb inhibited the PCA reaction in the skin (Tables I and II). When the Fab mAb BA3 was injected 2 h before $0.1 \mu\text{g}$ of IgE into the skin, 50% inhibition was observed with $0.10 \pm 0.02 \mu\text{g/site}$ Fab (Table I). This amounts to a four-fold molar excess of Fab mAb vs IgE at each site. There was inhibition of skin reactions even with a longer interval between the intradermal injection of the anti-R Fab and IgE. Rats were injected intradermally with the Fab BA3 1 to 3 days before receiving $0.1 \mu\text{g}$ of IgE per site and challenged i.v. 16 h later. Under these conditions and with $1.0 \mu\text{g}$ of Fab BA3 per site,

TABLE I
 Inhibition of PCA reactions by intradermal injection of anti-Fc_R antibody^a

Expt.	IgE (μg/site)	% Inhibition with Fab mAb BA3 ^b				IC ₅₀ (μg/site) ^c
		0.1 μg/site	0.3 μg/site	1 μg/site	3 μg/site	
1	0.1	60.3 ± 19.6	78.7 ± 15.8	93.2 ± 4.9	99.3 ± 0.4	0.10 ± 0.02
	0.3	39.0 ± 14.6	65.2 ± 15.1	76.0 ± 9.9	85.0 ± 5.7	0.18 ± 0.07
2	0.1	33.9 ± 16.9	43.6 ± 24.9	46.8 ± 21.5	58.6 ± 6.7	1.44 ± 0.91

^a Sprague-Dawley rats were injected intradermally on shaved backs with 100 μl of the different antibodies. Experiment 1, Fab mAb BA3 injected 2 h before the IgE; experiment 2, IgE injected 2 h before the Fab mAb BA3. Skin sites were injected with the different amounts of Fab mAb BA3; control sites received either the same amount of a nonspecific Fab prepared from an IgG, mAb against bacterial Ag (26) or PBS. The same sites were injected with anti-TNP IgE (9). After 16 h, the rats were injected i.v. with a solution of 1.2 mg of DNP-human serum albumin in 2% Evans blue. After 20 min, the rats were killed and the area of skin bluing at each site was measured.

^b Area of skin bluing in mm² (mean ± SD) in the control was as follows: with 0.1 μg of IgE per site, 383.4 ± 91.2, and with 0.3 μg of IgE per site, 492.0 ± 65.0. Values were calculated by measuring the area of skin bluing compared with the control and are presented as mean ± SD of three experiments.

^c IC₅₀, concentration of mAb that inhibited PCA reaction by 50%, presented as mean ± SD.

 TABLE II
 Inhibition of PCA reaction by i.v. injection of mAb BA3 Fab^a

IgE (μg/site)	% Inhibition with Fab mAb BA3 ^b		
	1 μg/site	3 μg/site	6 μg/site
0.01	98.5 ± 2.4	96.0 ± 3.5	84.5 ± 7.5
0.02	92.0 ± 10.6	83.3 ± 14.9	68.8 ± 25.5
0.03	77.5 ± 16.2	50.5 ± 20.1	49.3 ± 5.1
0.10	56.5 ± 22.2	56.3 ± 19.6	38.8 ± 27.9

^a Different rats received 30 μg i.v. of Fab mAb BA3 or, as control, Fab prepared from an IgG₁ hybridoma. At 1, 3, or 6 h, skin sites were injected intradermally with 100 μl of different concentrations of anti-TNP IgE. After 16 h, the rats were challenged and evaluated as described in the legend to Table I.

^b Area of skin bluing in mm² (mean ± SD) in the controls was as follows: with 0.01 μg of IgE per site, 118.6 ± 72.7; with 0.02 μg of IgE per site, 167.8 ± 28.0; with 0.03 μg of IgE per site, 223.7 ± 57.8; and with 0.10 μg of IgE per site, 403.3 ± 130.0. Results are the mean ± SD of four experiments.

^c Interval (h) between i.v. mAb and intradermal IgE.

there was 27 to 65% inhibition of the PCA reaction. Therefore, the $t_{1/2}$ for 50% inhibition with 1.0 μg of the Fab per site was 55 h, and with 3.0 μg of the Fab BA3, the $t_{1/2}$ was 90 h. In other experiments, it was observed that ¹²⁵I-IgE and ¹²⁵I-Fab BA3 disappeared from rat skin at similar rates. The injection of the Fab after the IgE also inhibited the PCA reaction, although, as was observed in vitro, the anti-R antibodies were 14-fold less efficient. The anti-R Fab injected i.v. inhibited the PCA reaction when the IgE was injected into the skin (Table II). This inhibition was seen when there was up to 6 h between the i.v. administration of the Fab and the injection of the IgE into the skin.

DISCUSSION

The present experiments demonstrate that the PCA reaction can be blocked by a mAb that reacts with the high affinity IgE-R. In the in vitro studies, this mAb has a significantly higher affinity for the R than does IgE itself. In radiolabeled binding studies, the Fab fragment of this mAb was at least 50-fold more effective than IgE in inhibiting binding to the receptor. In the PCA reaction, a 4-fold molar excess of the Fab vs IgE injected per site was required for 50% inhibition.

The binding of IgE to basophils or mast cells can be inhibited by myeloma IgE (18) and IgE induced by parasites (4) and by the Fc fragment prepared either by digestion (19–21) or by molecular cloning of the gene for IgE (22–25). There are conflicting reports on the capacity of high levels of nonspecific IgE to inhibit allergic reactions (4–6). In those studies, the competition for cell binding

was with non-Ag-specific IgE that had the same affinity as the Ag-specific IgE. Therefore, inhibition required a large excess of the nonspecific IgE. In contrast, the present study was with a mAb that has a higher affinity than IgE for the R and thus can compete more effectively for binding to the cells. In the present experiments, the anti-R antibody was still capable of inhibiting allergic reactions even when the cells had previously been exposed to IgE. Therefore, this is a model that is closer to the clinical situation in which the allergic patient already has IgE on the basophils and mast cells. Our study demonstrates that an agent binding with high affinity to the Fc_R can inhibit allergic reactions. The design of drugs that mimic such anti-receptor mAb may be very useful immunopharmacologic agents.

REFERENCES

- Ishizaka, T., and K. Ishizaka. 1984. Activation of mast cells for mediator release through IgE receptors. *Prog. Allergy* 34:188.
- Metzger, H., G. Alcaraz, R. Hohman, J. P. Kinet, V. Pribluda, and R. Quarto. 1986. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* 4:419.
- Siraganian, R. P. 1985. Biochemical events in basophil/mast cell activation and mediator secretion. In *Allergy*. A. P. Kaplan, ed. Churchill Livingstone, New York, p. 31–51.
- Godfrey, R. C., and C. F. Graddidge. 1976. Allergic sensitization of human lung fragments prevented by saturation of IgE binding sites. *Nature* 259:484.
- Coleman, J. W., and R. C. Godfrey. 1981. Inhibition of human lung mast cell sensitization by IgE in allergic sera. *Int. Arch. Allergy Appl. Immunol.* 66:464.
- Jarrett, E., S. Mackenzie, and H. Bennich. 1980. Parasite-induced 'nonspecific' IgE does not protect against allergic reactions. *Nature* 283:302.
- Barsumian, E. L., C. Isersky, M. G. Petrino, and R. P. Siraganian. 1981. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur. J. Immunol.* 11:317.
- Basciano, M. L., E. H. Berenstein, L. Kmak, and R. P. Siraganian. 1986. Monoclonal antibodies that inhibit IgE binding. *J. Biol. Chem.* 261:11823.
- Rudolph, A. K., P. D. Burrows, and M. R. Wabl. 1981. Thirteen hybridomas secreting hapten-specific immunoglobulin E from mice with Iga or Igb heavy chain haplotype. *Eur. J. Immunol.* 11:527.
- Siraganian, R. P., P. C. Fox, and E. H. Berenstein. 1983. Methods of enhancing the frequency of antigen-specific hybridomas. *Methods Enzymol.* 92:17.
- Morita, Y., and R. P. Siraganian. 1981. Inhibition of IgE-mediated histamine release from rat basophilic leukemia cells and rat mast cells by inhibitors of transmethylation. *J. Immunol.* 127:1339.
- Siraganian, R. P. 1974. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* 57:383.
- Stracke, M. L., L. K. Basciano, C. Fischler, E. H. Berenstein, and R. P. Siraganian. 1987. Characterization of monoclonal antibodies produced by immunization with partially purified IgE receptor complexes. *Mol. Immunol.* 24:347.
- Sterk, A. R., and T. Ishizaka. 1982. Binding properties of IgE receptors on normal mouse mast cells. *J. Immunol.* 128:838.

15. **Kulczycki A., Jr., and H. Metzger.** 1974. The interaction of IgE with rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. *J. Exp. Med.* 140:1676.
16. **Ovary, Z.** 1976. Local anaphylaxis. I. Passive cutaneous anaphylaxis. *Methods Immunol. Immunochem.* 5:16.
17. **Wank, S. A., C. DeLisi, and H. Metzger.** 1983. Analysis of the rate-limiting step in a ligand-cell receptor interaction: the immunoglobulin E system. *Biochemistry* 22:954.
18. **Spiegelberg, H. L., K. M. Canning, M. Scheetz, G. Koppel, and J. M. Chiller.** 1986. Effect of myeloma IgE injections on passive and active cutaneous anaphylaxis in rats. *J. Immunol.* 136:131.
19. **Stanworth, D. R., J. H. Humphrey, H. Bennich, and S. G. Johansson.** 1968. Inhibition of Prausnitz-Küstner reaction by proteolytic-cleavage fragments of a human myeloma protein of immunoglobulin class E. *Lancet* 2:17.
20. **Ishizaka, K., T. Ishizaka, and E. H. Lee.** 1970. Biologic function of the Fc fragments of E myeloma protein. *Immunochemistry* 7:687.
21. **Bennich, H., and S. G. Johansson.** 1971. Structure and function of human immunoglobulin E. *Adv. Immunol.* 13:1.
22. **Liu, F. T., K. A. Albrandt, C. G. Bry, and T. Ishizaka.** 1984. Expression of a biologically active fragment of human IgE ϵ -chain in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 81:5369.
23. **Kenten, J., B. Helm, T. Ishizaka, P. Cattini, and H. Gould.** 1984. Properties of a human immunoglobulin epsilon-chain fragment synthesized in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 81:2955.
24. **Ishizaka, T., B. Helm, J. Hakimi, J. Niebyl, K. Ishizaka, and H. Gould.** 1986. Biological properties of a recombinant human immunoglobulin ϵ -chain fragment. *Proc. Natl. Acad. Sci. USA* 83:8323.
25. **Geha, R. S., B. Helm, and H. Gould.** 1985. Inhibition of the Prausnitz-Küstner reaction by an immunoglobulin ϵ -chain fragment synthesized in *E. coli*. *Nature* 315:577.
26. **Cisar, J. O., E. L. Barsumian, S. H. Curl, A. E. Vatter, A. L. Sandberg, and R. P. Siraganian.** 1981. Detection and localization of a lectin on *Actinomyces viscosus* T14V by monoclonal antibodies. *J. Immunol.* 127:1318.