Induction of high titers of anti-IgE by immunization of inbred mice with syngeneic IgE

(immune tolerance/autoantibodies)

Seiji Haba and Alfred Nisonoff

Rosenstiel Research Center, Department of Biology, Brandeis University, Waltham, MA 02254

ABSTRACT We have generated high titers (up to 1 mg/ml) of antibodies to isotypic determinants of IgE by immunization of A/J mice with syngeneic monoclonal IgE conjugated to keyhole limpet hemocyanin. As much as 3 mg of anti-idiotypic antibodies per ml was induced at the same time. In contrast to conventional rheumatoid factors, the anti-isotypic antibodies are of moderately high affinity (10^7–10^8 M^{-1}). Assays of the anti-IgE antisera indicated the presence of IgE, both free and in the form of immune complexes; the latter values are minimum estimates owing to masking of isotypic determinants. Regulatory effects of these high titers of anti-IgE can now be investigated. Such studies will be facilitated by the availability of monoclonal, syngeneic anti-IgE antibodies.

The appearance of anti-globulin autoantibodies, most frequently IgM anti-IgG, is observed after immunization with certain bacteria (1, 2), heterologous proteins (3, 4), or immune complexes (5, 6). It has been suggested that polymers of IgG, such as occur in immune complexes, may cause the IgG to act as a thymus-independent antigen (3). Anti-immunoglobulin autoantibodies can also be produced by stimulation with bacterial lipopolysaccharide (LPS) (7–9). This may simply be a consequence of polyclonal activation of IgM antibodies by LPS. The autoantibody response is increased by conjugating the LPS to mouse IgG prior to inoculation (10). Autoantibodies specific for immunoglobulins are referred to as rheumatoid factors (RF) because of their occurrence in the serum of patients with rheumatoid arthritis (11, 12). RF are generally of low affinity ($\approx 10^4$–10^5 M^{-1}; ref. 13). RF specific for IgE has been detected at low levels (<1 μg/ml) in patients with allergic diseases, particularly allergic asthma (14). IgG anti-IgE antibodies were also detected in the sera of patients with hyper-IgE syndrome, allergic respiratory disease, or atopic dermatitis (15). In the latter report, data on anti-IgE concentrations were expressed as OD units in an ELISA; the highest value was ≈10 times the average level of anti-IgE antibodies in normal individuals. Antibodies of low titer, directed to allotypic determinants of IgE, have been prepared by immunization of BALB/c mice with immune complexes of IgE anti-dinitrophenyl monoclonal antibodies (mAb) of C57BL/6 origin; the converse anti-allotype was also produced by immunization of C57BL/6 mice (16).

A very high frequency (up to 10–15%) of mouse B cells stimulated by LPS produce IgM anti-IgG (17). This high frequency has recently been attributed to the ability of the framework regions of the light chains of RF to interact directly with IgG, independently of the heavy chain of the RF (18). This suggests the possibility that many RF may have dual specificity mediated, respectively, by the light chain alone and by the more conventional combining site.

In the present paper, we demonstrate the feasibility of producing high titers (approaching 1 mg/ml) of antibodies to isotypic determinants of syngeneic IgE in A/J mice. In contrast to most RF, these antibodies are of moderately high affinity (0.8–9 × 10^7 M^{-1}) and their concentration is far greater than that of normal levels (<2 μg/ml) of IgE. The observations are discussed in terms of tolerance to autologous proteins. The regulatory influences of such high titers of autoantibodies can now be investigated.

MATERIALS AND METHODS

A/J, BALB/c, and (BALB/c × A/J)F$_1$ (CAF$_1$) mice were obtained from The Jackson Laboratory. Mice were at least 6 wk old at the start of the experiment.

Monoclonal IgE was obtained from hybridomas. mAb SE20.2 (19) and SE21.1 (both IgEκ, anti-p-azobenzene arsonate) are of A/J derivation and were prepared in our laboratory. The nonsecreting hybridoma cell line SP2/0 was used as fusion partner for spleen cells. The BALB/c hybridoma, TIB-142 (IgEκ anti-trinitrophenyl), was obtained from the American Type Culture Collection (donated by M. Wabl, Max Planck Institute, Tubingen, F.R.G.). The mAb was affinity-purified (20) on a column of dinitrophenyl–bovine serum albumin conjugated to Sepharose 4B; elution was carried out with 0.15 M dinitrophenol. Other mAb used, each of which is p-azobenzene arsonate-specific, are R16.7 (IgG1κ; ref. 21), SM1.5 (IgMκ; ref. 22), and SA131 (IgAκ; this paper). Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem. KLH was conjugated to IgE (KLH–IgE) with glutaraldehyde (23) using a 1:1 ratio (wt/wt) of the proteins, each at a final concentration of 5 mg/ml, and 2.5 mmol of glutaraldehyde per g of protein.

Assay for Total IgE. Polyvinylchloride (PVC) microtiter plates were exposed overnight to a mixture containing a 3:1 ratio (wt/wt) of normal rabbit IgG to affinity-purified rabbit anti-mouse IgE at a total concentration of 5 μg/ml. After washing, the wells were saturated by exposure to 2.5% horse serum. Samples to be assayed for IgE were then added to the wells; after 5–6 hr at room temperature, the plates were washed and exposed to ^{125}I-labeled affinity-purified rabbit anti-mouse IgE (12 ng in 0.1 ml). After overnight incubation, wells were washed and assayed. Protein SE20.2 (IgEκ) was used as the standard for the assay. The useful range of concentrations of IgE was 20 pg to 3 ng in 0.1 ml. All assays were done in duplicate (20).

Assays for Anti-IgE in Mouse Antisera (Prepared Against mAb SE21.1). Two assays were used. In one, PVC plates were coated with the IgE protein, SE20.2 (2 μg in 0.1 ml), and saturated with 2.5% horse serum. Dilutions of antisera to be assayed were then added and incubated for 5–6 hr at room temperature. After washing, wells were developed with 50 ng

Abbreviations: KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; mAb, monoclonal antibody; PVC, polyvinylchloride; RF, rheumatoid factor.

of ^{125}I-labeled affinity-purified goat antibodies to the Fc fragment of mouse IgG. (Control experiments showed complete absence of reactivity of the latter reagent with IgE.) As a standard for this assay, we used an anti-IgE antiserum whose antibody content was estimated by measuring its maximum binding capacity for IgE, as described below. Nonimmune A/J serum was used as the negative control in each set of experiments.

In the second assay, we measured the capacity of serum to bind various amounts of ^{125}I-labeled purified IgE (SE20.2). Each test mixture also contained, as carrier, 5 μl of normal A/J serum that had been adsorbed by passage through a rabbit anti-IgE-Sepharose column. Complexes were precipitated with an excess of rabbit antibodies to the Fc fragment of mouse IgG (adsorbed with IgE). When normal mouse serum was tested in this assay, <1% of the ^{125}I label was precipitated at each concentration of labeled IgE tested.

RESULTS

Table 1 shows titers of anti-IgE and (apparent) IgE in the sera of A/J mice that received KLH–IgE emulsified in complete Freund's adjuvant (CFA) (group 1). Also shown are data for mice that received KLH (group 2), monomeric IgE (group 3), or glutaraldehyde-polymerized IgE (group 4). Mice of group 5 were nonimmunized controls. All of the mice of the 5 groups were close to 6 wk old when the first inoculation was given. Each inoculation comprised 200 μg (group 1) or 100 μg (groups 2–4) of protein emulsified in CFA. Mice were inoculated on days 0 and 14 and bled on day 35. Some of the mice in groups 1–4 were inoculated again on day 35 and bled again on day 49. IgE and anti-IgE concentrations were measured in sera by radioimmunoassays using PVC microtiter plates. For the day-35 sera, the mean value for IgE concentration in the 20 untreated mice (Table 1, group 5) was

362 ng/ml; the group of mice that received the KLH–IgE conjugate (group 1) had a somewhat lower average IgE titer (142 ng/ml). The values for the treated group must be considered as apparent IgE concentrations because of the simultaneous presence of anti-IgE (discussed below). Mice of group 2, which received unconjugated KLH in CFA had concentrations of serum IgE (mean, 1570 ng/ml) that were much higher than the mean value for the control group 5 or for group 1, which received KLH–IgE. There was a small decrease in the serum IgE content of group 3, that received monomeric IgE, as compared to the untreated mice of group 5 (mean IgE concentrations, 179 vs. 362 ng/ml). Immunization with glutaraldehyde-polymerized IgE (group 4) had no significant effect on apparent IgE concentration; the mean value on day 35 was 459 ng/ml.

The most striking results are the high titers of anti-IgE antibodies that were elicited in sera of mice of group 1, which received KLH–IgE; the average value of anti-IgE on day 35 is 60 μg/ml. Undetectable levels of anti-IgE (<0.2 μg/ml) were present in the sera of all mice of groups 2 or 5, whereas detectable but low levels of anti-IgE (0.2–2.8 μg/ml) were observed in the sera of most of the mice of groups 3 and 4, which received monomeric or polymeric IgE, respectively.

Results obtained for those mice of group 1 that were inoculated again on day 35 and bled on day 49 are shown in the last column of Table 1. The range of anti-IgE titers was 69–970 μg/ml with a mean value of 366 μg/ml. The range of apparent IgE concentrations was 8–650 ng/ml; for most of the mice, the apparent IgE titers were <100 ng/ml. The individual serum that contained the highest concentration of IgE in this group expressed the lowest titer of anti-IgE; in general, there was an inverse correlation between the two values.

Affinities of A/J Anti-IgE Antibodies. These values were measured by using ^{125}I-labeled IgE (SE20.2), A/J anti-SE21.1

Table 1. Production of anti-IgE against syngeneic IgE in A/J mice

			Day 35				Day 49			
Group	Immunogen	μg per inoculation	Serum IgE, ng/ml		Anti-IgE, μg/ml		Serum IgE, ng/ml		Anti-IgE, μg/ml	
1	KLH–IgE	200	0–50	(10)	28–40	(6)	8–50	(11)	69–200	(5)
			50–100	(17)	40–60	(20)	50–100	(5)	200–500	(10)
			100–200	(11)	60–80	(12)	100–150	(2)	500–800	(3)
			200–400	(8)	88–99	(9)	650	(1)	970	(1)
			810	(1)						
			Mean 142		60		78		366	
2	KLH	100	400–1000	(5)	<0.2	(19)	1100–2000	(4)	<0.2	(9)
			1000–2000	(7)			2000–3000	(2)		
			2000–3000	(5)			3000–3990	(3)		
			3000–3550	(2)						
			Mean 1570				2562			
3	Monomeric IgE	100	70–150	(4)	<0.2	(3)				
			150–300	(4)	0.2–0.5	(3)	80–300	(5)	0.6–1	(2)
			360	(1)	0.5–1.0	(2)	300–600	(3)	1–2	(5)
					2.8	(1)	4000	(1)	2–10	(2)
			Mean 179		0.6		688		2.6	
4	Polymeric IgE	100	240–300	(2)	0.2–0.5	(4)	160–300	(3)	0.2–1	(4)
			300–600	(4)	0.5–1.0	(1)	300–500	(4)	1–2	(3)
			600–840	(2)	1.0–2.0	(3)	880	(1)	2.8	(1)
			Mean 459		0.7		436		1.3	
5	None		80–200	(7)	<0.2	(20)	64–300	(8)	<0.2	(20)
			200–500	(8)			300–600	(5)		
			500–800	(2)			600–1000	(4)		
			800–970	(3)			1000–1900	(3)		
			Mean 362				718			

IgE and anti-IgE were determined by radioimmunoassays in the wells of PVC plates. Inoculations were given in CFA on days 0 and 14 and mice were bled on day 35. Some but not all of the mice were given a third inoculation on day 35 (after bleeding) and bled again on day 49. Numbers of mice are in parentheses.

(diluted antiserum), and an antiglobulin reagent to precipitate immune complexes (see *Materials and Methods*). Binding curves were obtained with pools of antiserum or, for the day-49 bleedings, with sera of individual mice. Average binding affinities (K_a) (shown in Table 2) ranged from 0.8 to 9×10^7 M^{-1}. The individual mice selected on the basis of relatively high titers of anti-IgE (day 49) exhibited a somewhat higher average K_a than the unselected mice of the day-49 pool. Among pooled antisera, the highest value of K_a (4.3×10^7 M^{-1}) was obtained after the latest bleeding (day 56). All binding curves (Scatchard plots) were nonlinear, indicating heterogeneity with respect to K_a.

Double Diffusion in Agarose Gel (Ouchterlony Tests). All of the anti-IgE antisera of high titer tested were found to contain strongly precipitating antibodies. Two examples are shown in Fig. 1. Each antiserum contained antibodies specific for the IgE isotype, as evidenced by precipitation with monoclonal IgE antibodies other than that (SE21.1) used as immunogen and the failure to give precipitation lines against mouse IgM or IgG. In addition, each antiserum contained precipitating anti-idiotypic antibodies, as shown by the spur of the line formed by the immunogen, when placed in a well adjacent to that containing another IgE mAb (Fig. 1 *Middle*). Two different IgE mAb (TIB-142 and SE20.2) gave lines of identity with one another when placed in adjacent wells.

Further Evidence for Specificity of Anti-IgE (Anti-SE21.1). This experiment was done by coating PVC plates with SE20.2 and exposing to diluted anti-IgE (SE21.1) antiserum, followed by ^{125}I-labeled affinity-purified goat anti-mouse Fc of IgG. The amount of anti-SE21.1 added to each well was ≈5 ng in 0.05 ml. We tested antisera from 5 individual mice (day-49 bleeding) and four pools of antiserum from two groups of 19 or 20 mice collected on days 28, 42, 49, and 56. (These are the same pools used to collect the data in Table 2.) In the presence of 5 μg of unlabeled TIB-142 (IgEκ), the amount of label bound varied from 2% to 10% of the control value. In the presence of 5 μg of unlabeled serum IgG, the amount of uptake of radiolabel varied from 109% to 147% of the control; for six of those nine samples the values were 100%–111% of control. In the presence of 5 μg of unlabeled IgM (mAb SM1.5), the amount of radiolabel bound in the nine samples varied from 93% to 110% of the control value. The values obtained in the presence of 5 μg of unlabeled IgA (mAb SA131) ranged from 90% to 109% of the control. Thus, only IgE was an effective inhibitor in this assay.

Concentration of Anti-Idiotypic Antibodies in Anti-SE21.1 Antiserum. The double-diffusion (Ouchterlony) patterns discussed above indicated the presence of anti-idiotypic as well as anti-isotypic anti-IgE antibodies in anti-KLH–IgE antisera. A quantitative estimate of the relative amounts was made with the same two antisera (mice A18 and A19) that were used for the double-diffusion analyses. This was done by adding

Table 2. Apparent binding affinities of anti-IgE antisera*

Mice	No. of inoculations[†]	Day of bleeding	K_a, $\times 10^7$ M^{-1}
5 individual mice[‡]	3	49	2.6, 2.9, 8.0, 9.0, 9.0
Pool A (20 mice)	2	28	0.8
Pool A (20 mice)	2	42	1.0
Pool B (19 mice)	3	49	1.4
Pool B (19 mice)	3	56	4.3

*Determined by using various concentrations of ^{125}I-labeled SE20.2 and anti-IgE (SE21.1) antiserum diluted to give a final anti-IgE concentration of ≈5 μg/ml. Complexes were precipitated with rabbit anti-mouse Fc of IgG (see *Materials and Methods*).
[†]Inoculations were of 200 μg of KLH–IgE conjugate.
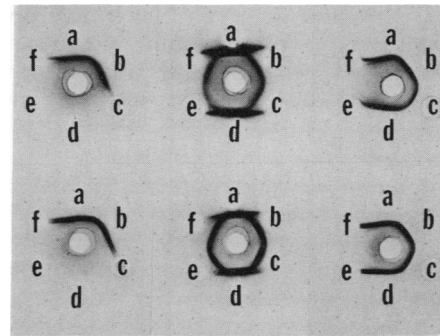[‡]These 5 mice were selected from those constituting pool B on the basis of high anti-IgE titers.

FIG. 1. Analysis by double diffusion in agarose of anti-IgE (SE21.1) antisera (day-49 bleedings) from mouse A18 (*Upper*) and mouse A19 (*Lower*). (*Upper Left*) a and b, IgE mAb SE20.2, 2 and 1 mg/ml; c, IgM mAb, SM1.5, 1 mg/ml; d–f, normal mouse IgG, 0.25, 0.5, and 1 mg/ml. (*Lower Left*) Same as *Upper Left*, but with a different antiserum (from mouse A19) in the center well. (*Upper Middle*) a and d, IgE mAb SE21.1 (the immunogen), 2 and 1 mg/ml; b and c, SE20.2 (IgE mAb), 2 and 1 mg/ml; e and f, IgE mAb TIB-142, 1 and 2 mg/ml. (*Lower Middle*) Same as *Upper Middle*, but with antiserum from mouse A19 in the center well. (*Upper Right*) a and d, SE20.2, 2 and 1 mg/ml; b and c, TIB-142, 2 and 1 mg/ml; e and f, normal mouse IgG, 0.5 and 1 mg/ml. (*Lower Right*) Same as *Upper Right*, but with antiserum A19 in the center well.

increasing amounts of ^{125}I-labeled SE21.1 or SE20.2 to 50-μl quantities of a 1:500 dilution of antiserum and determining the amount of radioactivity bound (see *Materials and Methods*). The total concentrations of anti-IgE antibodies were estimated by extrapolation of plots of $1/b$ vs. $1/f$ to $1/f = 0$; i.e., to an infinite value of f. (The symbols b and f refer to bound and free ligand concentrations.) The values thus obtained for the two antisera were 3750 and 4400 μg/ml for the binding of SE21.1 (the immunogen) as compared to 960 and 1170 μg/ml for binding of the nonimmunogen SE20.2. Further evidence for the presence of anti-idiotype was the only partial inhibition (≈15%) of binding of 10 ng of ^{125}I-labeled SE21.1 to anti-SE21.1 antiserum by 3 μg of unlabeled SE20.2. The same amount (3 μg) of unlabeled SE21.1 caused >95% inhibition. In contrast, 3 μg of unlabeled SE20.2, SE21.1, or TIB-142 each inhibited the binding of 10 ng of ^{125}I-labeled SE20.2 to anti-SE21.1 by >90%.

Presence of Immune Complexes in Anti-IgE Antisera. Portions of anti-IgE antisera (0.3 ml) were passed through a 90-cm³ column of Sephacryl S-300 and titers of IgE and anti-IgE were determined (using PVC plates) for individual fractions. Two of eight experiments that gave typical results are represented in Figs. 2 and 3, which show the data for a "late" and an "early" antiserum, respectively. For each fraction, the anti-IgE titer was measured by using labeled goat anti-Fc of IgG as the developing reagent. (This assay does not detect IgM anti-IgE.) In both experiments (Figs. 2 and 3), the anti-IgE activity was eluted as a single peak at an elution volume close to that obtained with pure IgG (in a separate run through the same column).

For the late antiserum (anti-IgE, 190 μg/ml) nearly all of the IgE activity was eluted before the elution volume of monomeric IgE, indicating that the IgE was present in immune complexes (Fig. 2). (From these experiments one cannot deduce the true concentration of IgE because of probable masking of determinants by anti-IgE.) A small amount of ^{125}I-labeled IgE (SE20.2), added to the antiserum before gel filtration, showed an elution pattern of radioactivity similar to that obtained by radioimmunoassays for the bulk of the IgE (Figs. 2 and 3).

Fig. 3 shows the elution pattern for an early antiserum (anti-IgE titer, 58 μg/ml). Again, essentially all the anti-IgE activity was eluted at a volume corresponding closely to
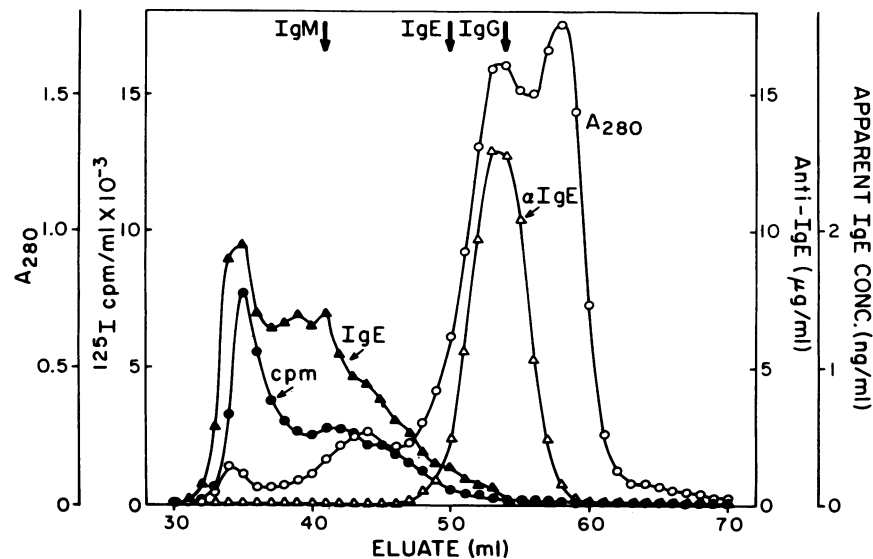
FIG. 2.   Gel filtration patterns of 0.3 ml of A/J anti-IgE antiserum pooled from the sera of 25 mice immunized with KLH–IgE (SE21.1). The mice were inoculated on days 0 and 14 and bled on day 42. ^{125}I-labeled IgE (mAb SE20.2) (4 ng) was added as a tracer. Anti-IgE and apparent total IgE concentrations were determined by radioimmunoassays on PVC plates. The location of the arrows is based on separate runs through the same column of purified mAbs.

monomeric IgG, but two peaks of IgE activity were obtained by the radioimmunoassay for total IgE. One peak corresponded with that of monomeric IgE; the second was of higher molecular weight (presumably immune complexes). Two peaks of radioactivity were similarly obtained for ^{125}I-labeled IgE, artificially added as a tracer to the antiserum (Fig. 3).

Assay for IgM Anti-IgE Antibodies. The assay used to detect IgG anti-IgE (on PVC plates) was modified by using affinity-purified ^{125}I-labeled rabbit anti-IgM as the developing reagent. The two pools of anti-IgE antiserum used for the gel filtration experiments described above were tested. The signals (cpm) thus obtained per μl of serum were $\approx$1/160th and 1/180th as large as those obtained with labeled goat anti-rabbit Fc with the same antisera and on the same PVC plate (coated with the IgE mAb SE20.2). To compare the efficacy of the two labeled developing reagents (anti-Fc or anti-IgM) an assay was set up using bovine serum albumin-*p*-azobenzene arsonate-coated wells and equivalent weights (10

or 20 ng) of IgM anti-*p*-azobenzene arsonate and IgG anti-*p*-azobenzene arsonate monoclonal antibodies. In this case, the ratio of the two signals (cpm) was 3.9:1 (IgG/IgM) when 10 ng of each isotype was present, and 3.2:1 with 20 ng of each isotype (IgG or IgM). These data suggest the presence of a relatively low ($\approx$1/40th as great as IgG) but measurable concentration of IgM anti-IgE antibodies in the mice immunized with KLH–IgE.

DISCUSSION

We have shown that it is possible to generate high titers (approaching 1 mg/ml) of antibodies against syngeneic IgE in inbred mice. Such concentrations greatly exceed the concentration of IgE normally present in A/J mice ($<$2 μg/ml). The immunogen used was a conjugate of KLH and IgE polymerized with glutaraldehyde and inoculated as an emulsion in CFA. Very low titers of anti-IgE were induced by monomeric or glutaraldehyde-polymerized IgE. The IgE preparations used were mAb of A/J derivation specific for
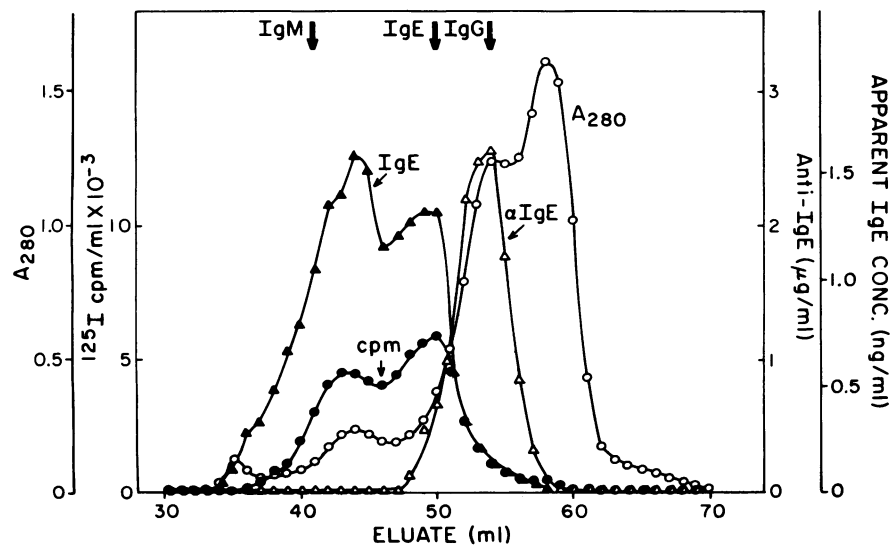

FIG. 3.   Same as Fig. 2 except that the serum was pooled from earlier bleedings (day 28) of the same 25 mice.

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the p-azobenzene arsonate hapten. mAb SE21.1 was used as immunogen in most of these experiments. However, the IgEκ mAb SE17.1 (A/J anti-p-azobenzene arsonate) was also effective in inducing anti-IgE. High titers of anti-IgE were also induced in BALB/c mice against the BALB/c mAb TIB-142 (IgEκ, anti-trinitrophenyl; data not shown). Assays for anti-isotype activity were carried out in each case by using an IgE mAb other than the immunogen as ligand. Assays carried out with the immunogen (SE21.1) indicated that high titers of anti-idiotype, generally exceeding those of anti-isotype antibodies, were also induced.

Three different assays confirmed the presence of anti-IgE: a radioimmunoassay carried out in the wells of PVC microtiter plates coated with IgE; direct binding of radiolabeled IgE in solution followed by precipitation of complexes with an antiglobulin reagent; and double diffusion in agarose gel (Ouchterlony analysis). Isotype specificity was shown by the double-diffusion experiments and by the failure of unlabeled mouse IgM, IgG, or IgA to inhibit the binding of labeled IgE to the anti-IgE antibodies; IgE mAb were strongly inhibitory. (An IgE mAb other than the immunogen was used as ligand in these assays.) Radioimmunoassays indicated that nearly all of the anti-IgE antibodies are of the IgG class. Small amounts of IgM anti-IgE were detected, but the amounts were ≈40 times lower than those of IgG anti-IgE.

The requirement for KLH as a component of the immunogen suggests that strong T-cell help may be essential for the induction of syngeneic anti-IgE. Some support for this comes from the fact that the anti-IgE antibodies induced were principally IgG and that monomeric or polymeric IgE were relatively poor immunogens.

In contrast to conventional rheumatoid factors, which are generally of low affinity ($\approx 10^4$–10^5 M^{-1}; ref. 13) the anti-IgE antibodies produced were of moderately high affinity (10^7–10^8 M^{-1}). Another distinction is in the isotype of the antiglobulin; RF are, in general, predominantly IgM, whereas our anti-IgE antibodies were, as indicated, principally IgG.

Because of the presence of immune complexes we could not accurately assess IgE concentrations in our immune sera, although low titers were measurable by the radioimmunoassay that makes use of PVC plates. These assays indicated that nearly all of the IgE detected was present as immune complexes in pooled late antisera, whereas a substantial proportion ($\approx 1/3$rd) was present as unbound IgE in earlier antisera. This difference may reflect differences in anti-IgE titers of the early and late antisera since the binding affinities did not vary markedly. Gel filtration experiments indicated that virtually all of the anti-IgE was present as monomeric IgG rather than as immune complexes; this would be expected since the anti-IgE concentrations greatly exceeded concentrations of IgE that are normally present in mice.

The data thus suggest that IgE continues to be synthesized in the presence of large amounts of anti-IgE, but the actual levels are highly uncertain because of probable masking of determinants by anti-IgE and possible changes in the half-life of circulating IgE attributable to the presence of anti-IgE. The presence of some free IgE in early antisera is not inconsistent with our affinity measurements; in the early antisera, the concentration of anti-IgE antibody combining sites averaged $\approx 8 \times 10^7$ M^{-1}. The product of this value times the average affinity constant of early antisera is ≈ 10, a value consistent with the presence of some free IgE in serum. (The concentration of bound IgE may considerably exceed that measured by our assay). The possibility of changes in the rate of synthesis of IgE is a question of central interest with regard to possible regulatory influences of very high anti-IgE concentrations.

The results also have implications with respect to the question of tolerance to autologous immunoglobulins. All normal A/J mice that we have tested have measurable concentrations of serum IgE (averaging ≈150 ng/ml in 6-wk-old mice and increasing to ≈1 μg/ml 7 wk later), yet it proved easy to generate high titers of anti-IgE with the appropriate immunogen (KLH–IgE). This together with the failure of polymerized IgE to induce anti-IgE suggests that tolerance resides in the T-cell compartment; clearly, B cells with anti-IgE receptors are present and available.

The ability to generate high titers of anti-IgE may prove useful in studies of regulation of this isotype. Using the immunization protocol described here, we have produced several syngeneic monoclonal anti-IgE mAb. It should be of interest to determine the effects of such antibodies, administered neonatally, on the subsequent ability of a mouse to produce IgE antibodies, in view of the profound effects of early administration of heterologous anti-μ-chain antibodies (24, 25). It should also be of considerable interest to attempt to generate high titers of anti-IgD.

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