Inhibition of IgE synthesis by anti-IgE: Role in long-term inhibition of IgE synthesis by neonatally administered soluble IgE

(IgE suppression/autoantibodies to IgE)

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ABSTRACT Inoculation of syngeneic IgE into 2- to 12-day-old mice results in prolonged synthesis of anti-IgE antibodies without further challenge. These anti-IgE antibodies may be largely responsible for the long-term inhibition of synthesis of IgE that is known to result from a perinatal challenge with IgE. This conclusion is supported by the effect of passive inoculation of syngeneic polyclonal anti-IgE antibodies into young mice, which similarly results in selective inhibition of IgE synthesis. Further evidence is the close relationship between the age dependency of IgE-induced inhibition of subsequent IgE synthesis and the ability of IgE to induce anti-IgE antibodies. IgE synthesis was monitored at the level of secretion by B cells as well as serum IgE levels and IgE antibody responses.

We have reported that neonatal mice respond to a syngeneic IgE monoclonal antibody (mAb), administered in alum, complete Freund's adjuvant (CFA), or incomplete Freund's adjuvant, by producing anti-IgE antibodies (1). This responsiveness is lost by the age of 2–3 wk, which correlates closely with the appearance of detectable serum IgE. The loss of responsiveness was attributed to tolerization of T cells by endogenous IgE. That B cells are not tolerant was shown by the capacity of mice of any age to produce anti-IgE in response to a keyhole limpet hemocyanin (KLH)-IgE conjugate (2). T cells can also be tolerized on the day of birth by i.p. inoculation of IgE in saline (1).

Inoculation of an IgE monoclonal antibody into perinatal mice is known to result in prolonged inhibition of their capacity to produce IgE antibodies in response to antigen (3). The inhibition was attributed to isotype-specific suppressor T cells (4). We report here that such treatment causes a prolonged decrease in the level of serum IgE as well as antigen-specific IgE responses. In addition, we observed that perinatally IgE-treated mice produced significant serum titers of anti-IgE that persisted for the duration of the experiment (up to several months). This suggested the possibilities that the apparent inhibition of IgE responses and reduced serum IgE levels might actually be due to masking of epitopes of IgE by anti-IgE, to rapid clearance of IgE induced by anti-IgE, or to inhibition of the synthesis of IgE. That IgE synthesis is in fact inhibited was shown by measurements of the numbers of IgE-secreting cells (IgE-SC), which were greatly reduced in the anti-IgE-producing mice. Also, passive treatment of 4-wk-old mice with polyclonal syngeneic anti-IgE similarly resulted in greatly reduced levels of circulating IgE and of IgE-SC, which began to recover after disappearance of the anti-IgE; there was no significant effect on the total number of cells secreting immunoglobulin. The observations that mice treated perinatally with IgE mount a prolonged anti-IgE response and that IgE synthesis is suppressed by passively

administered anti-IgE suggest that anti-IgE antibodies are a major mediator of IgE-induced inhibition of IgE synthesis. Further support for this hypothesis is the close relationship of the age dependency of IgE-induced inhibition of IgE synthesis and IgE-induced anti-IgE antibody production.

Other investigators have reported that passive administration of xenogeneic anti-IgE results in decreased levels of serum IgE and reduced serum IgE responses to antigen (5–7). Marshall and Bell (8, 9) showed that low titers of anti-IgE are induced by inoculation of allogeneic IgE into adult BN rats and that this is accompanied by a reduction in serum IgE concentration and the serum IgE antibody response to ovalbumin. As indicated above, various alternative mechanisms might account for the reductions in IgE. Our results indicate that inhibition of synthesis or secretion of IgE by anti-IgE antibodies contributes to or is responsible for the observed effects.

MATERIALS AND METHODS

Mice. A/J and BALB/cJ mice were obtained from The Jackson Laboratory. (BALB/c $\times$ A/J) F_1 (CAF $_1$) mice were bred from female BALB/cJ and male A/J mice.

Antigens. Monoclonal IgE κ antibodies (1) SE17.1 (CRI $_{\lambda}^{-}$) and SE20.2 (CRI $_{\lambda}^{+}$) are of A/J derivation and are specific for *p*-azobenzenearsonate (Ars); each mAb was affinity-purified. KLH was obtained from Calbiochem–Behring. A conjugate of IgE (SE17.1) with KLH, 1:1 by weight, was prepared by using glutaraldehyde at pH 6.0 as described (2).

Polyclonal Antibodies to Syngeneic IgE. A/J mice were injected i.p. with 140 μ g of KLH-IgE in saline/CFA, 1:9 (vol/vol) (10). Ascites was tapped after the third injection. An IgG fraction was prepared from the pooled ascites. Anti-IgE was quantified by radioimmunoassay as described next.

Assays for Mouse Anti-IgE. Wells of polyvinyl chloride microtiter plates were exposed overnight to the IgE mAb SE20.2 (1 μ g in 100 μ l per well) and then saturated with 2.5% horse serum. Dilutions of samples to be assayed were then added and incubated for 5–6 hr at room temperature. Wells were washed and then developed with 100 ng of 125 I-labeled affinity-purified goat anti-mouse Fc (of IgG) that had been adsorbed with mouse IgE-Sepharose. As a standard, we used pooled hyperimmune anti-IgE antiserum whose antibody content had been determined by measuring its maximum capacity for IgE (2). Assays were done in duplicate.

Assay for Total IgE in Serum. Wells of polyvinyl chloride plates were coated with a 4:1 ratio of normal rabbit IgG to affinity-purified rabbit anti-mouse IgE (total, 5 μ g/ml) and then saturated with 2.5% horse serum (2). After exposure to 50 μ l of test sample per well for 6 hr at room temperature, wells were washed and exposed overnight to 12 ng of

¹²⁵I-labeled, affinity-purified rabbit anti-mouse IgE in 100 μ l. mAb SE20.2 (IgE_K) was used as the standard.

Assays for the Number of Secreting Cells (SC Assay). For assay of IgE-SC, we used the solid-phase immunoenzymatic technique of Sedgwick and Holt (11). Alkaline phosphatase (Boehringer Mannheim) was conjugated to affinity-purified antibodies with glutaraldehyde. As substrate we used 5-bromo-4-chloro-3-indolyl phosphate (5-BCIP; Sigma). Thirty-five-millimeter wells in polystyrene tissue culture plates were exposed overnight to affinity-purified rabbit anti-mouse IgE antibodies (200 μ g/ml) and then saturated with 3% (vol/vol) fetal calf serum (FCS). A cell suspension of spleen or mesenteric lymph nodes ($0.5\text{--}2 \times 10^6$ cells in 1 ml of RPMI 1640 medium containing 5% FCS) was added to each well and incubated at 37°C for 16 hr in a level CO₂ incubator. The plates were washed and then incubated with alkaline phosphatase-conjugated, affinity-purified rabbit anti-mouse IgE antibodies (≈ 2 μ g/ml) for 6 hr at room temperature. After another wash, a 5-BCIP-agarose solution was added at 44°C and allowed to solidify; 5-BCIP yields a blue spot when exposed to alkaline phosphatase. The spots were counted after a 4-hr or overnight incubation at room temperature. Very small spots due to residual cell material were readily distinguished microscopically. For enumeration of anti-IgE-SC, the wells were coated with mAb SE20.2 (200 μ g/ml) and then saturated with 3% FCS. After incubation with lymphoid cells as above, the plates were incubated with alkaline phosphatase-conjugated, affinity-purified goat anti-mouse Fc antibodies (≈ 2 μ g/ml), followed by exposure to 5-BCIP. For measurement of immunoglobulin-secreting cells, affinity-purified rabbit anti-mouse Fab (200 μ g/ml) was used to coat culture plates. After incubation with $0.2\text{--}2 \times 10^5$ lymphoid cells, the plates were exposed to alkaline phosphatase-conjugated rabbit anti-mouse Fab (≈ 2 μ g/ml), and blue spots were developed as above. In the IgE-SC assay, background levels for hybridomas secreting irrelevant proteins or for anti-IgE-SC nonimmune spleen or lymph node cells were virtually zero.

RESULTS

We have reported that neonatal mice respond to immunization with IgE in adjuvant by producing anti-IgE antibodies. Responsiveness is lost at the age of 2–3 wk, which corresponds with the appearance of IgE in serum. Data presented below indicate that anti-IgE antibodies may be in large part responsible for the prolonged inhibition of IgE responses caused by inoculation of IgE into perinatal mice (3).

Table 1 extends our previous observations to the use of IgE in saline, without adjuvant, as the antigen. A high percentage of mice inoculated at the ages of 3, 6, 8, or 10 days produced anti-IgE in response to 25, 100, or 500 μ g of IgE in saline; 5 μ g was inadequate. Mice challenged at the age of 15 or 29 days did not produce detectable serum anti-IgE. Also, mice

inoculated within 24 hr of birth (age 1 day) were totally unresponsive. We have previously shown that mice treated with IgE in saline at birth are tolerized to a subsequent challenge with IgE in CFA (1).

Table 2 shows the persistence of the anti-IgE response to 500 μ g of IgE, inoculated twice, and also presents data on serum IgE levels. The sera of nearly all mice that were inoculated at the age of 3 or 6 days and again 28 days later had detectable levels of serum anti-IgE antibodies 56 and 100 days after the first inoculation. Moreover, on day 100 after the first inoculation, all but 1 of 13 mice in those two groups had greatly reduced serum IgE, as compared with controls. Mice inoculated on the day of birth or on days 15 or 29 produced little or no anti-IgE and had normal or moderately reduced (day 15 group) serum IgE levels. Similar results were observed in mice inoculated twice with 25 μ g, rather than 500 μ g, of IgE in saline (data not shown).

Table 3 indicates that the responses of CAF₁ mice were similar to those of A/J. Most mice inoculated with 500 μ g of IgE in saline at the age of 8 days produced anti-IgE and showed greatly reduced serum IgE titers. Similar results were obtained with 2- to 4-day-old mice (data not shown); six of nine such mice produced anti-IgE antibodies and had undetectable levels of IgE at days 56, 100, or 134 after the first inoculation. Mice first immunized on the day of birth or at the age of 18 or 31 days failed to produce anti-IgE and maintained normal or increased serum IgE (Table 3). Thus, CAF₁ mice respond similarly to A/J. In all cases there was a strong inverse correlation between the anti-IgE response and serum IgE level. As with A/J mice, CAF₁ mice inoculated with IgE in saline on the day of birth or after they were 2–3 wk old were unresponsive.

The last four columns in Table 3 indicate, first, that the anti-IgE responses and diminished serum IgE levels persist at least through day 134 after the first inoculation—106 days after the last injection of IgE. In addition, those mice that produced anti-IgE also failed to respond to KLH-Ars immunization by producing detectable serum IgE anti-Ars antibodies. The total anti-Ars response (mainly IgG) was not inhibited.

Data on IgE anti-Ars antibody responses to challenge with antigen, of 2- to 4-day-old or 8-day-old mice, are consistent with those reported previously (3). Also, the previous results were extended by demonstrating greatly reduced levels of serum IgE. However, our observation that anti-IgE antibodies are produced suggested the possibility that the reduced serum IgE levels and reduced IgE antibody responses in the present and earlier experiments (3) may have been caused by anti-IgE antibodies. Such antibodies might act by inducing rapid clearance of IgE, by masking epitopes of IgE, or by inhibiting the synthesis of IgE antibodies. This question was approached by determining numbers of IgE-SC.

Enumeration of IgE-SC and Anti-IgE-SC. It was necessary first to ascertain whether the presence of anti-IgE-SC influenced the enumeration of IgE-SC, and vice versa. We used normal adult CAF₁ spleen cells as a source of IgE-SC. When

Table 1. Age and dose dependency of responses of A/J mice to syngeneic IgE without adjuvant

Age at first injection,* day(s)	Anti-IgE response to IgE mAb inoculated (twice), [†] μ g/ml (n)			
	500 μ g	100 μ g	25 μ g	5 μ g
1	All 0 (6)	—	All 0 (5)	—
3	6.6 $\pm$ 2.6 (4 LR/10)	10.8 $\pm$ 2.9 (0 LR/5)	12.4 $\pm$ 4.2 (3 LR/7)	0.4 $\pm$ 0.2 (4 LR/5)
6	17.2 $\pm$ 2.0 (1 LR/11)	18.7 $\pm$ 3.0 (1 LR/8)	15.5 $\pm$ 4.2 (2 LR/8)	<0.2 (5 LR/5)
8	10.3 $\pm$ 3.3 (1 LR/6)	—	9.1 $\pm$ 2.6 (0 LR/5)	—
10	15.6 $\pm$ 5.0 (0 LR/5)	7.4 $\pm$ 4.7 (2 LR/5)	4.1 $\pm$ 3.1 (3 LR/5)	0.3 $\pm$ 0.1 (5 LR/6)
15	All 0 (5)	—	All 0 (8)	—
29	All 0 (4)	—	All 0 (6)	—

*The immunogen was A/J mAb SE17.1 (IgE_K); mice were given a second inoculation 28 days later and bled after another 28 days.

[†]Data shown are mean values $\pm$ SEM. Numbers in parentheses are numbers of mice tested; LR = low responders (<1 μ g/ml).

Table 2. Persistence of the anti-IgE response in A/J mice after inoculations of IgE without adjuvant

Mice*		Response on days 56 and 100 after first inoculation†			
		Day 56		Day 100	
Age at first injection, day(s)	No.	IgE, ng/ml	Anti-IgE, µg/ml	IgE, ng/ml	Anti-IgE, µg/ml
<1	6	(120 ± 47)	All 0	(900 ± 310)	All 0
3	7	30	9.0	0	2.9
		200	0	90	0.1
		50	19.2	0	26.8
		30	1.0	10	0.4
		10	18.6	10	22.0
		30	0.4	790	0.4
		870	0.1	40	0.7
		(170 ± 120)	(6.9 ± 3.3)	(130 ± 110)	(7.6 ± 4.4)
6	6	60	0.2	0	0.3
		40	18.6	30	16.5
		10	19.8	0	36.4
		20	16.8	0	6.3
		20	20.0	0	21.9
		30	10.2	10	3.5
		(30 ± 7)	(14.2 ± 3.2)	(7 ± 5)	(13.5 ± 5.6)
15	5	(250 ± 90)	All 0	(320 ± 120)	(0.2 ± 0.1)
29	4	(130 ± 38)	All 0	(800 ± 190)	All 0
No inoculations	6‡	(210 ± 60)	All 0	(1160 ± 130)	All 0

*Each mouse received two 500-µg inoculations of syngeneic IgE (mAb SE17.1) on the day specified and 28 days later.

†Individual responses are shown for the second and third groups (mice inoculated first at ages 3 and 6 days). Numbers in parentheses are mean values ± SEM.

‡These mice were bled when 29, 57, and 101 days old.

1×10^6 such cells were mixed with an equal number of spleen cells from a CAF₁ mouse producing anti-IgE (immunized with KLH-IgE), the number of IgE-SC (52 cells) and anti-IgE-SC (149 cells) detected in the mixture agreed closely with the values for the individuals preparations (53 and 138). Similar results were obtained in two other experiments.

Tables 4–6 present data on numbers of IgE-SC and anti-IgE-SC in combined spleen and mesenteric lymph nodes as well as on serum titers of IgE and anti-IgE. The correct numbers of SC must be somewhat higher because of incomplete recovery of tissues.

The data in Table 4 show suppression of serum IgE titers and production of anti-IgE resulting from immunization of 8-day-old CAF₁ mice with IgE in CFA. Sera were tested 84 days after immunization. Similar results for this group were obtained 63 days after immunization (not shown). Again,

older mice (18 or 25 days old when first inoculated) did not produce anti-IgE, and their serum levels of IgE were only moderately reduced.

Mice were sacrificed on day 84, and the numbers of IgE-SC were determined. It is evident that IgE-SC were virtually eliminated in mice immunized when 8 days old. These mice also had significant numbers of anti-IgE-SC. In contrast, mice immunized with IgE at the age of 18 or 25 days did not exhibit anti-IgE-SC, and the numbers of IgE-SC were much higher than those of the 8-day group. However, there was some reduction in the numbers of IgE-SC, as compared with the control group ($P = 0.008$ and 0.031 for the 18-day and 25-day groups, respectively). The data indicate that the reductions in IgE serum titers are not simply due to masking by anti-IgE or to an enhanced rate of clearance of IgE but can be accounted for by a reduction in the number of IgE-SC.

Table 3. Age dependency of responses of CAF₁ mice to IgE without adjuvant

Mice*		Anti-IgE response on days 56, 100, and 134 after first inoculation						Anti-Ars response on day 134	
		Day 56		Day 100		Day 134			
Age at first injection, day(s)	No.	IgE, ng/ml	Anti-IgE, μ g/ml	IgE, ng/ml	Anti-IgE, μ g/ml	Total IgE, ng/ml	Anti-IgE, μ g/ml	IgE, ng/ml	Total, μ g/ml
0–1	6	(1530 $\pm$ 293)	0	(4770 $\pm$ 680)	(0.1 $\pm$ 0.05)	(10,640 $\pm$ 2,090)	(0.1 $\pm$ 0.05)	(1490 $\pm$ 690)	(400 $\pm$ 180)
8	7	0	8.8	100	35.0	0	15.8	0	470
		30	3.2	0	15.6	0	7.2	0	1870
		0	0.1	0	14.5	0	3.7	0	650
		0	3.0	0	1.8	0	0.5	30	100
		630	0.1	830	2.0	2,180	0	690	580
		2200	3.2	2330	0.3	9,200	0	730	70
		900	0	2340	0.1	14,080	0	2630	810
		(540 $\pm$ 310)	(2.8 $\pm$ 1.4)	(800 $\pm$ 410)	(9.9 $\pm$ 4.9)	(3,640 $\pm$ 2,150)	(3.9 $\pm$ 2.2)	(580 $\pm$ 360)	(650 $\pm$ 230)
18	4	(1140 $\pm$ 440)	0	(2960 $\pm$ 430)	0	(9,890 $\pm$ 1,750)	0	(940 $\pm$ 120)	(550 $\pm$ 180)
31	5	(5320 $\pm$ 990)	0	(4550 $\pm$ 690)	<0.2	(13,350 $\pm$ 400)	0	(1220 $\pm$ 460)	(430 $\pm$ 120)
No inoculations	5	(1170 $\pm$ 120)	0	(3260 $\pm$ 400)	0	(8,710 $\pm$ 880)	0	(1620 $\pm$ 810)	(340 $\pm$ 190)

*Each mouse received 500 µg of mAb SE17.1 (IgE_h) on the day specified and, again, 28 days later. They were challenged on day 100 after the first inoculation, after bleeding, and again 28 days later with 5 µg of KLH-Ars in alum i.p.; mice were bled again on day 134 after the first inoculation.

†Numbers in parentheses are mean values ± SEM. Other values are for individual mice.

Table 4. Age dependency of anti-IgE responses of CAF₁ mice to IgE in CFA

Mice*		Serum titers†		SC,‡ no.	
Age on inoculation, days	No.	IgE, ng/ml	Anti-IgE, µg/ml	IgE-SC	Anti-IgE-SC
8	4	13 ± 8 (0–40)	12.9 ± 5.9 (2.2–29.4)	3 ± 3 (0–10)	240 ± 100 (110–550)
18	4	2240 ± 120 (2000–2590)	0	310 ± 30 (230–370)	0
25	4	1000 ± 320 (450–1550)	<0.1	710 ± 320 (190–1600)	0
No inoculation§	3	3130 ± 550 (2190–4100)	0	1830 ± 240 (1590–2300)	0

*Each mouse received one inoculation of SE17.1 (25 µg in CFA). They were bled 84 days later and sacrificed on day 84 after the inoculation for determination of the number of SC.

†Serum titers of IgE and anti-IgE were comparable on days 63 and 84.

‡Total number of secreting cells in spleen and mesenteric lymph nodes.

§These mice were bled and then sacrificed at the age of 92 days.

Data in Table 5 confirm that 9-day-old CAF₁ mice, inoculated once with IgE without adjuvant, can produce anti-IgE and that this is accompanied by elimination of IgE-SC, as measured 130–150 days after the inoculation. However, in this case only about half of the mice (11 of 21) responded. (That not all CAF₁ mice of the appropriate age respond to IgE in saline was indicated also by the data in Table 3.) In contrast, 4 of 4 CAF₁ mice treated with IgE in CFA at the age of 8 days were responsive, as indicated by anti-IgE titers and reduced numbers of IgE-SC (Table 4). A large number of CAF₁ mice (>20) have been immunized with IgE in CFA at the ages of 8–10 days, and all have produced significant levels of anti-IgE; only 4 were tested for IgE-SC (Table 4). Therefore, the percentage of nonresponsive CAF₁ mice is greater when the immunogen is IgE in saline rather than CFA. The mice (Table 5) were also bled on days 63 and 108. Results were similar to those of days 130–150, except that anti-IgE titers of the responsive mice were somewhat higher (mean values, 2.7 and 3.4 µg/ml).

Effects of Passively Transferred Anti-IgE Antibodies on IgE Synthesis. The data presented indicate a strong correlation between the presence of anti-IgE antibodies and a reduced number of IgE-SC. Therefore, we determined effects of passive transfer of anti-IgE antibodies, using syngeneic polyclonal serum anti-IgE antibodies (IgG fraction). Each i.p. inoculation consisted of 4 mg of IgG containing ≈100 µg of anti-IgE. Mice were injected four times between the ages of 27 and 52 days (experiments 1 and 2 in Table 6) or between 65 and 71 days (experiment 3 in Table 6). Mice were tested at the age of 81, 84, or, in experiment 2 of Table 6, 129 days. Controls received normal mouse IgG or no treatment. The most pronounced effects were in experiment 1 in which mice were tested 32 days after the last inoculation of anti-IgE; the serum IgE concentrations were low, and a significant concentration (mean, 5.7 µg/ml) of anti-IgE persisted in the sera. The most pertinent data are those on the number of IgE-SC, which was reduced by 96% or 97% as compared with controls. A similarly treated group (experiment 2) that was tested at the age of 129 days (77 days after the last inoculation of anti-IgE) showed a marginal reduction in the number of IgE-SC ($P = 0.099$ as compared with normal IgG controls)

and in serum level of IgE ($P = 0.088$); the reductions were not nearly as pronounced as in experiment 1. Since anti-IgE was still present at day 84 but was undetectable at day 129, it appears that recovery of IgE synthesis begins after passively transferred anti-IgE is cleared.

The data of experiment 3 in Table 6 indicate that older mice, inoculated with anti-IgE between the ages of 65 and 71 days, are less susceptible to suppression of IgE synthesis by passively administered anti-IgE. Although serum IgE and IgE-SC were reduced as compared with controls, the effects were less pronounced than in experiment 1.

DISCUSSION

Our results confirm the prolonged inhibition of the IgE response to antigen resulting from inoculation of IgE into mice shortly after birth (3). In most mice this inhibition persisted for the duration of the experiment (up to 4 months after the initial challenge with IgE). The inhibition was demonstrated at the level of serum IgE as well as the IgE anti-Ars response to KLH-Ars in alum. Chen and Katz also mention decreased serum IgE levels (3). We extended these observations by demonstrating that inhibition of total serum IgE levels and IgE antibody responses was accompanied by the prolonged synthesis of anti-IgE antibodies. This in turn raised the possibilities that the reduced IgE levels might be caused by masking of epitopes on IgE, by rapid clearance of IgE-anti-IgE complexes, or by inhibition of IgE synthesis mediated by anti-IgE antibodies.

That IgE synthesis is in fact inhibited was demonstrated by measurements of numbers of IgE-SC in spleen and mesenteric lymph nodes. Control experiments showed that anti-IgE-SC in the same cell suspension did not affect the observed number of IgE-secreting cells, or *vice versa*.

That anti-IgE antibodies inhibit the IgE response was supported by experiments in which anti-IgE antibodies were passively transferred (four inoculations of 100 µg) into normal CAF₁ mice, starting at the age of 27 days (Table 6). When tested 32 days after the last inoculation, these mice had low numbers of IgE-SC (about 3–4% of controls) as well as greatly reduced serum IgE levels. Some anti-IgE antibodies

Table 5. Anti-IgE responses of 9-day-old CAF₁ mice to a single injection of IgE without adjuvant

IgE injected, µg	Mice, no.	Serum titer*		
		IgE, ng/ml	Anti-IgE, µg/ml	IgE-SC, no.
250	11	40 ± 6 (10–70)	1.0 ± 0.3 (0–3.1)†	All 0
250	10	3240 ± 810 (460–7660)	<0.1 (0–0.4)‡	1240 ± 280 (190–3340)
None	6	2100 ± 420 (750–3000)	All 0	1000 ± 210 (590–1970)

Each 9-day-old mouse received a single i.p. inoculation of 250 µg of IgE in saline or received saline alone. Mice were bled between the ages of 130 and 150 days and then were sacrificed for determinations of the numbers of IgE-SC and anti-IgE-SC.

*Numbers shown are mean values ± SEM. The range of values is given in parentheses.

†Two of the 11 mice had titers <0.2 µg/ml.

‡Eight of 10 mice had titers <0.2 µg/ml.

Table 6. Suppressive effect of passively administered syngeneic anti-IgE antibodies on IgE production in CAF₁ mice

Antibodies injected	Serum titer		SC,* no.	
	IgE, ng/ml	Anti-IgE, μ g/ml	IgE-SC	Ig-SC $\times 10^{-3}$
Experiment 1				
Anti-IgE	15 $\pm$ 12 (0–50)	5.7 $\pm$ 0.3 (5.1–6.4)	44 $\pm$ 26 (0–100)	210 $\pm$ 18 (100–250)
NIgG	1710 $\pm$ 720 (930–3850)	0	1000 $\pm$ 276 (720–1800)	150 $\pm$ 23 (110–210)
None	1585 $\pm$ 390 (680–2350)	0	1180 $\pm$ 302 (540–1960)	170 $\pm$ 18 (120–210)
Experiment 2				
Anti-IgE	930 $\pm$ 380 (360–1640)	0	410 $\pm$ 180 (180–760)	190 $\pm$ 52 (140–300)
NIgG	4050 $\pm$ 1190 (1680–5420)	0	1630 $\pm$ 540 (930–2700)	140 $\pm$ 9 (130–160)
None	3770 $\pm$ 110 (2450–5960)	0	1840 $\pm$ 370 (1400–2570)	150 $\pm$ 20 (120–190)
Experiment 3				
Anti-IgE	180 $\pm$ 39 (80–270)	7.1 $\pm$ 0.4 (6.1–7.7)	800 $\pm$ 173 (440–1250)	280 $\pm$ 40 (210–380)
NIgG	3920 $\pm$ 513 (2700–5180)	0	2940 $\pm$ 530 (1550–3790)	200 $\pm$ 23 (140–250)
None	2180 $\pm$ 443 (1400–3440)	0	2230 $\pm$ 323 (1530–2940)	230 $\pm$ 11 (210–250)

Each mouse received four 4-mg i.p. inoculations of polyclonal, syngeneic (A/J) anti-IgE IgG, containing $\approx 100 \mu$ g of specific antibody in 0.5 ml of saline; controls received 4 mg of A/J normal IgG (NIgG). Each group consisted of four mice in experiments 1 and 3 and three mice in experiment 2. Mice in experiments 1 and 2 were inoculated between the ages of 27 and 52 days. Mice in experiment 1 were assayed at 84 days of age (32 days after the last inoculation); in experiment 2, mice were assayed at 129 days of age. In experiment 3, mice were inoculated between the ages of 65 and 71 days and were assayed at 81 days of age. Results shown are mean values $\pm$ SEM. Numbers in parentheses are the range of values.

*Total number of SC in spleen and mesenteric lymph nodes.

(mean, 5.7 μ g/ml) remained in their sera. Another, similarly treated group, tested 77 days after the last inoculation of anti-IgE, also showed significant suppression of serum IgE and IgE-SC, but some recovery was evident. Because these mice no longer had detectable serum anti-IgE, the data suggest that recovery of IgE synthesis begins after the disappearance of anti-IgE antibodies.

Further evidence that anti-IgE is responsible for the inhibition of the IgE response comes from data on the age dependency of the anti-IgE response. We have reported (1) and confirm here that mice are responsive to IgE (produce anti-IgE) when first inoculated between the ages of ≈ 2 days and 2–3 wk. Tolerance to IgE, beginning at the age of 2–3 wk, coincides with the initial appearance of endogenous IgE in serum. Mice inoculated with IgE in saline on the day of birth are tolerized and will not respond to IgE in CFA between the ages of 6 and 12 days (1). The results reported here on serum IgE levels and IgE-SC in mice treated with IgE show a similar age dependency. Challenge with IgE on the day of birth or after 2 weeks of age was ineffective (or marginally effective at 15 or 18 days) in reducing subsequent serum IgE levels or numbers of IgE-SC (Tables 2–4). Thus, age dependencies of the two effects (IgE inhibition and anti-IgE synthesis) are completely parallel.

Because IgE levels are greatly reduced for a long period of time, it seems likely that inhibition of synthesis, and not simply inhibition of its secretion, takes place. If rates of synthesis were normal, rapid turnover of IgE in nonsecreting cells would be the alternative mechanism. Of course, it is possible that both synthesis and secretion are inhibited.

In contrast to Chen and Katz (3), we repeatedly found that initial challenge with IgE in saline on the day of birth does not result in inhibition of the subsequent IgE response. Since both laboratories used CAF₁ mice in some experiments, the reason for this discrepancy is not clear. They also showed that adoptive transfer of T cells from IgE-suppressed KLH-immune adult mice into irradiated recipients suppressed a subsequent KLH-specific IgE antibody response. This suggests the possibility that anti-IgE antibodies and suppressive T cells participate in the inhibition of IgE synthesis.

The duration of anti-IgE production is of interest, particularly in view of the short half-life in serum (5–8 hr; ref. 12) of IgE used as immunogen. The anti-IgE response continued

for the duration of the experiment (over 4 months in one case) and may well prove to persist for a large fraction of the life-span of a mouse. A possible explanation is that IgE produced in small amounts by the IgE-treated mice provides a continuous supply of immunogen.

Suppression of immunoglobulin synthesis by anti-isotype antibodies has been extensively studied in the case of anti- μ chain antibodies. Treatment of neonatal mice with xenogeneic anti- μ chain causes suppression of synthesis of all immunoglobulin isotypes and is accompanied by a large reduction in the number of splenic B cells and germinal centers (13, 14). In contrast, a noncytotoxic mAb, directed against allotypic determinants on the μ chain, apparently tolerized target B cells, which could expand and produce mainly IgG3 antibodies upon immunization (15).

Our data indicate that passive transfer of anti-IgE antibodies can regulate IgE at the level of synthesis or secretion by B cells and that perinatally administered IgE can induce regulatory anti-IgE antibodies.

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