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Immunology

Role of antibody and T cells in the long-term inhibition of IgE synthesis

(IgE regulation/autoantibodies/self-tolerance)

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ABSTRACT **We have shown that the long-term inhibition of IgE synthesis associated with perinatal inoculation of syngeneic IgE is accompanied by the synthesis of autoantibodies to IgE. Synthesis of IgE can also be inhibited by passive transfer of syngeneic anti-IgE antibodies. In the present investigation we made use of adoptive transfer experiments to assess the relative roles of antibodies and T cells in the inhibitory process. It was found that spleen cells from IgE-suppressed mice (synthesizing anti-IgE antibodies) could adoptively transfer the state of inhibition to syngeneic adult mice. The inhibition occurred only under conditions in which the recipient mice synthesized anti-IgE antibodies. Separated B cells, CD4$^+$ T cells, CD8$^+$ T cells, or a mixture of B and CD8$^+$ T cells were ineffective. However, strong inhibition of IgE synthesis (as indicated by serum levels and numbers of IgE-secreting cells in the spleen) was observed after transfer of a mixture of B cells and CD4$^+$ (helper) T cells. The results indicate that in this experimental model anti-IgE antibodies are the suppressive agent and that T cells do not play a role other than that of providing help to B cells for anti-IgE synthesis.**

In adult mice tolerance to endogenous IgE resides in T cells. A vigorous anti-IgE response is obtained when syngeneic monoclonal IgE is inoculated as a conjugate to keyhole limpet hemocyanin (KLH), indicating the presence of IgE-specific B cells (1). Unconjugated IgE is not immunogenic in adult mice, which suggests that the KLH is needed to provide the required T-cell help (1). Unconjugated syngeneic IgE can, however, induce anti-IgE antibody formation if inoculated in amounts of ≥ 2.5 μg after the day of birth but before the age of 10 days; the IgE is immunogenic when inoculated either in complete Freund's adjuvant (CFA) or in saline (2, 3). The loss of ability to respond to unconjugated IgE corresponds closely with the age at the first appearance of IgE-secreting cells in lymphoid tissue; they are first detectable in the thymus between the ages of 7 and 11 days and soon afterwards in lymph nodes and spleen (4). It thus appears that T cells are tolerized by IgE and that the delayed onset of tolerance is attributable to the absence of IgE in the neonatal mouse. Tolerance to IgE can be artificially induced by inoculation of IgE (2, 5), or B cells expressing surface IgE (6), on the day of birth or by repeated inoculations of very small amounts of IgE between days 3 and 7 after birth (6).

The induction of anti-IgE antibodies before day 10 was shown to be related to the observation of Chen and Katz (7) that inoculation of syngeneic IgE into perinatal mice resulted in long-term inhibition of the ability of the mice to synthesize IgE antibodies in response to challenge with antigen. [We subsequently found that such immunization also resulted in a prolonged decrease in serum IgE levels (3).] The short time period after birth during which syngeneic IgE can induce inhibition of subsequent IgE synthesis was found to correspond closely with the period when anti-IgE antibodies can be induced by immunization with unconjugated IgE (2, 4). The inference we drew, that anti-IgE antibodies are responsible at least in part for the inhibition of IgE synthesis, was supported by the observation that syngeneic anti-IgE, passively administered to 4- to 8-week-old mice, can profoundly inhibit total and specific IgE synthesis in response to a subsequent challenge with antigen (3). We confirmed each of these observations by measurements of numbers of IgE-secreting cells (total and antigen-specific) as well as serum IgE concentrations. The measurements of secreting cells demonstrate central inhibition of IgE synthesis and rule out clearance or masking of serum IgE antibodies as the explanation for the inhibitory effects of anti-IgE antibodies. Inhibition of serum levels of IgE in animals treated with xenogeneic or allogeneic anti-IgE was reported by others (8–12); measurements of numbers of secreting cells were not described.

A demonstration of a role of anti-IgE antibodies in the inhibition of IgE synthesis does not preclude the additional participation of suppressive T cells induced by IgE, as proposed by others (7, 13). The present research addresses the question of the possible role of T cells, as well as anti-IgE antibodies, in the inhibition of IgE synthesis in IgE-treated mice.

MATERIALS AND METHODS

Mice. A/J mice were obtained from The Jackson Laboratory.

Monoclonal Antibodies (mAbs). IgE(κ) anti-*p*-azobenzenearsonate mAbs SE20.2 and SE17.1 are of A/J origin (14); TIB142 [IgE(κ); American Type Culture Collection] is of BALB/c derivation and is specific for the trinitrophenyl (TNP) hapten (15). mAbs were affinity purified as described (16). The anti-IgE-secreting hybridoma AE11 [IgG1(κ)] was described previously (17). Hybridomas GK1.5 (18) [anti-CD4; rat IgG2b(κ)] and 2.43 (19) [anti-CD8, rat IgG2b(κ)] were obtained from the American Type Culture Collection. These mAbs were purified from a culture supernatant by ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-cellulose.

Radioimmunoassays. Assays for total mouse IgE and mouse anti-IgE in serum were carried out by using polyvinyl chloride plates coated with rabbit anti-IgE or mouse IgE, respectively (1). The assay for total anti-TNP antibodies was carried out on polyvinyl chloride plates whose wells were coated with bovine serum albumin (BSA)-TNP (500 μg/ml). ^{125}I-labeled affinity-purified rabbit anti-mouse Fab (100 ng in 100 μl per well) was the developing reagent (16). The standard for total anti-TNP was a DEAE-cellulose-purified fraction of

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Abbreviations: BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CGG, chicken gamma globulin; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; TNP, trinitrophenyl.

ascitic fluid from KLH-TNP-immune A/J mice whose anti-TNP content was determined by a quantitative precipitin test. Assays for IgE anti-TNP antibodies were done in a similar manner but used ^{125}I-labeled affinity-purified rabbit anti-mouse IgE for development. mAb TIB142 [anti-TNP; IgE(κ)] was used as the standard. When relatively high amounts of non-IgE anti-TNP antibodies were detected, the serum samples were precipitated with ammonium sulfate at 39% of saturation, before the assay for IgE anti-TNP, in order to remove most of the IgG anti-TNP antibodies (16).

Assays for Numbers of Secreting Cells. Numbers of cells secreting IgE or anti-IgE immunoglobulins were determined by our modified method (3) of Sedgwick and Holt (20). Anti-TNP IgE-secreting cells were enumerated on culture plates precoated with BSA-TNP (500 μg/ml). Alkaline phosphatase-conjugated affinity-purified rabbit anti-mouse IgE ($\approx$2 μg/ml) was used as the developing reagent. Anti-TNP IgG-secreting cells were detected in a similar manner by using alkaline phosphatase-conjugated affinity-purified goat anti-mouse Fc ($\approx$2 μg/ml) as the detecting reagent.

Cell Preparations. Single-cell suspensions of spleen were prepared in cold Dulbecco's modified Eagle's medium and erythrocytes were lysed with 0.83% ammonium chloride.

An enriched B-cell fraction was obtained by treating 2.5 $\times$ 10^8 pooled spleen cells with anti-Thy-1.2 plus complement. The recovery of enriched B cells was $\approx$50% of the original population and >90% were viable. A second treatment with anti-Thy-1.2 plus complement resulted in <3% killing.

To enrich T cells, 2.5 $\times$ 10^9 pooled spleen cells were depleted of B cells by two cycles of panning (21). Petri dishes (100 mm) were coated with affinity-purified goat anti-mouse Fab (300 μg/ml). Cells (10^8 in 5 ml per dish) were plated and allowed to stand for 90 min at 4°C. Nonadherent cells were resuspended and recovered by gentle agitation; $\approx 10^9$ cells were recovered as a T-cell-enriched fraction.

To purify CD4$^+$ cells, T-enriched cells were positively selected on Petri dishes precoated with mAb GK1.5 (anti-CD4, used at 100 μg/ml for coating). The adherent cells were recovered in phosphate-buffered saline by pipetting at room temperature and were then treated with mAb 2.43 (anti-CD8, 10 μg/ml) plus rabbit complement. About 8% of the original spleen cells were recovered of which $\approx$80% were viable; >93% could be killed by anti-CD4 (10 μg/ml) plus complement, and <3% by a second treatment with anti-CD8 plus complement.

To purify CD8$^+$ cells, 5 $\times$ 10^8 cells nonadherent to anti-CD4-coated dishes were applied to dishes coated with mAb 2.43 (anti-CD8, 100 μg/ml for coating). After removal of nonadherent cells, the adherent cells were recovered by pipetting and then treated with anti-CD4 (10 μg/ml) plus complement. The recovery of CD8$^+$-enriched T cells was $\approx$4% of the original cell population; $\approx$70% were viable and >95% were killed by anti-CD8 plus complement, and <1% were killed by a second cycle of anti-CD4 plus complement.

Conjugation of IgE to KLH or Chicken Gamma Globulin (CGG). This procedure was carried out as described (1), by using a 1:1 weight ratio of the two proteins with glutaraldehyde as the coupling reagent.

RESULTS

The data in Table 1 confirm and extend earlier results (2, 3) by demonstrating a decrease in IgE synthesis in mice immunized against syngeneic IgE or conjugates of IgE with KLH or CGG. Unconjugated IgE given in CFA on day 25 did not induce the formation of anti-IgE antibodies and had no major effect on serum IgE concentration or the number of IgE-secreting cells in lymphoid tissues, both measured on day 99. In contrast, when IgE was administered at the age of 8 days, in CFA or in saline, anti-IgE antibodies were induced and IgE synthesis was almost completely inhibited by the criteria of serum levels of IgE or numbers of IgE-secreting cells.

KLH-IgE administered in CFA on day 9 or on days 28 and 49 after birth induced anti-IgE formation and caused profound inhibition of IgE synthesis by both criteria. Similar effects were observed when CGG-IgE was inoculated in incomplete Freund's adjuvant on days 49 and 63. Thus, CGG-IgE and KLH-IgE induced anti-IgE formation and suppressed subsequent IgE synthesis in adult mice whereas unconjugated IgE was effective only when administered within a few days after birth, as previously observed (3). The latter results confirm the delayed onset of T-cell tolerance to IgE in perinatal mice.

Tables 2 and 4 provide information on the role of lymphocyte subsets in the inhibition of IgE synthesis. Table 2 shows that the transfer of 2 $\times$ 10^7 spleen cells from mice immunized perinatally with IgE caused a large decrease in the IgE serum level and the number of IgE-secreting cells in spleens of recipient mice, both measured 16 weeks after the cell transfer. In addition, anti-IgE antibodies (mean value, 5.1 μg/ml) were detected in the recipient mice. In contrast, spleen cells from mice immunized with CGG-IgE had little, if any, suppressive effect on IgE synthesis in the recipients by either criterion (P = 0.15 for IgE serum concentration). Anti-IgE levels in the same recipients were barely detectable. (The reason for the superior inhibitory capacity of cells from mice immunized with unconjugated IgE is considered in the *Discussion*.)

Also shown in Table 2 are data obtained after transfer of serum (0.3 ml $\times$ 2), rather than cells, from mice immunized

Table 1. Suppressed IgE synthesis in mice producing anti-IgE antibodies elicited by various regimens of immunization

Immunogen	Mice: No.	Mice: Age, days: Inoculation*	Mice: Age, days: Assays	Serum concentration: IgE, ng/ml	Serum concentration: Anti-IgE, μg/ml	No. of secreting cells†: IgE	No. of secreting cells†: Anti-IgE
Nonimmune control	5	—	99	1880 ± 390	<0.2	1460 ± 170	<15
IgE in CFA (25 μg)	4	8	92	10 ± 8	13 ± 6	<15	240 ± 100
IgE in saline (50 μg)	5	8	99	20 ± 10	5 ± 2	<15	140 ± 60
IgE in CFA (25 μg)	4	25	99	1000 ± 310	<0.2	710 ± 320	<15
KLH-IgE (50 μg)	5	9	93	<10	10 ± 4	40 ± 30	720 ± 210
KLH (25 μg)	4	9	93	2060 ± 180	<0.2	1080 ± 90	<15
KLH-IgE (100 μg)	5	28, 49	91	250 ± 140	9 ± 1	140 ± 40	80 ± 60
KLH (25 μg)	4	28, 49	91	3500 ± 690	<0.2	3200 ± 620	<15
CGG-IgE (50 μg)	3	49, 63	91	10 ± 3	19 ± 5	160 ± 30	200 ± 55
CGG (25 μg)	4	49, 63	91	560 ± 90	<0.2	1110 ± 10	<15

*Inoculation was intraperitoneal. KLH and KLH-IgE were given in CFA; CGG and CGG-IgE were given in incomplete Freund's adjuvant.
†Mice were sacrificed on the day of bleeding. The data are the total number (mean ± SEM) of secreting cells in the spleen and mesenteric lymph nodes.

Table 2. Transfer of suppression of IgE synthesis by cells from mice immunized with IgE or CGG-IgE

Immunization of donors	Spleen cells or serum transferred	Analysis of recipients 16 weeks after cell or serum transfer				
		Serum concentration		Secreting cells, no. per spleen		
		IgE, ng/ml	Anti-IgE, μg/ml	IgE	Total Ig ($\times 10^{-3}$)	Anti-IgE
None	No transfer	750 ± 330	<0.2	770 ± 300	230 ± 26	<15
IgE*	2×10^7 cells	20 ± 1	5.1 ± 0.3	<15	330 ± 30	280 ± 50
CGG-IgE†	2×10^7 cells	320 ± 70	0.2 ± 0.1	650 ± 210	350 ± 39	<15
None	2×10^7 cells	620 ± 250	<0.2	640 ± 210	300 ± 30	<15
IgE	0.3 ml of serum,‡ twice	45 ± 10	1.5 ± 0.3	50 ± 5	220 ± 30	<15
None	0.3 ml of serum,‡ twice	540 ± 20	<0.2	1560 ± 320	190 ± 17	<15

*Mice were immunized intraperitoneally with 100 μg of IgE (mAb SE17.1) in saline at the ages of 6, 9, and 42 days. Spleen cells from six donor mice were pooled ≈11 weeks later and 2×10^7 cells were inoculated intravenously into each recipient (four recipients per group).
†Adult mice (7 weeks old) were inoculated intraperitoneally with 100 μg of CGG-IgE in incomplete Freund's adjuvant and given a similar booster injection 3 weeks later. Spleen cells from donor mice were pooled 7 weeks later and 2×10^7 cells were inoculated intravenously into each recipient. All recipients were 7 weeks old at the time of transfer of cells or the first inoculation of serum.
‡Concentration of anti-IgE, ≈60 μg/ml; serum was inoculated into recipients 16 and 8 weeks before the assays.

with IgE. The transfer resulted in a very large decrease in IgE serum concentration and numbers of IgE-secreting cells in the recipient mice; nonimmune serum, used as the control, was not suppressive.

Table 3 further illustrates the effects of adoptive transfers of cells. However, the recipients in these experiments were immunized with KLH-TNP in alum (72 days before and 7 days after cell transfer). Assays carried out on recipients 6 days after the second inoculation of KLH-TNP included total serum IgE, total and IgE-specific anti-TNP serums titers, and numbers of secreting cells of four different specificities. The principal result in Table 3 is that CGG-IgE-immune cells can adoptively transfer a state of IgE suppression, but only if the recipient mice are further immunized with CGG-IgE. Compared with controls, such mice (second group in Table 3) showed a large decrease in total serum IgE and IgE anti-TNP concentrations; there was no significant effect on the total level of serum anti-TNP antibodies (mainly non-IgE). Suppression was also evident at the level of secreting cells (total IgE- and, particularly, anti-TNP IgE-secreting cells). At the same time the recipients produced anti-IgE antibodies.

Significant suppression of IgE synthesis (Table 3) did not occur in mice that received CGG-IgE-immune cells but which were not further challenged with CGG-IgE or were challenged with CGG rather than CGG-IgE. Also, mice that received cells from CGG-immune donors were not suppressed with respect to IgE synthesis, even when these recipients were challenged with CGG-IgE. This may be related to the short interval (13 days) between the challenge with CGG-IgE and the assay, which did not permit significant buildup of synthesis of anti-IgE antibodies unless the donor mice had already been primed with CGG-IgE (second group, Table 3).

Table 4 provides information as to the subset of lymphocytes responsible for the adoptive transfer of inhibition of IgE synthesis. Donor mice were immunized three times intraperitoneally with 100-μg portions of syngeneic IgE (SE17.1) in saline, 6, 9, and 43 days after birth. Mice selected for use as donors had serum titers of anti-IgE > 20 μg/ml on day 57. Recipient mice were primed with 0.5 μg of KLH-TNP in alum 13 weeks before the cell transfer and were similarly challenged with KLH-TNP 7 days after the transfer. Assays of serum antibodies and numbers of secreting cells were carried out 6 days later. The transfer of 1.2×10^7 unfractionated spleen cells resulted in a decrease of about 70–75% in total serum IgE or anti-TNP IgE in the recipient mice. The inhibitory effect was somewhat greater on the number of cells secreting IgE or IgE anti-TNP. No inhibition of total anti-TNP (mainly non-IgE) antibodies or IgG anti-TNP-secreting cells was observed.

The transfer of 1.2×10^7 enriched B cells had a marginal effect on the serum concentration of IgE in recipients and a nonsignificant effect on the numbers of cells secreting IgE or IgE anti-TNP in the recipient mice. Similarly, CD4-enriched or CD8-enriched T cells were noninhibitory.

The strongest overall inhibition of IgE synthesis was observed when a mixture of $CD4^+$ T cells and B cells was transferred. This resulted in a large decrease in total serum IgE and IgE anti-TNP antibodies in the recipients. Also, the numbers of cells secreting IgE or IgE anti-TNP were greatly

Table 3. Effects of IgE synthesis in recipient mice of 2×10^7 spleen cells transferred from IgE-immune donors

Immunization of donors	Immunization of recipients	Serum titer				Secreting cells, no. per spleen			
			Anti-TNP				Anti-TNP		
		Total IgE, ng/ml	IgE, ng/ml	Total, μg/ml	Anti-IgE, μg/ml	Total IgE	IgE	Total ($\times 10^{-3}$)	Anti-IgE
CGG-IgE	None	5000 ± 560	940 ± 120	1050 ± 40	<0.2	17,900 ± 3740	6430 ± 270	260 ± 23	<15
CGG-IgE	CGG-IgE	510 ± 260	120 ± 50	930 ± 50	2.2 ± 0.4	2,670 ± 1030	280 ± 140	290 ± 23	280 ± 100
CGG-IgE	CGG	NT	NT	NT	NT	20,480 ± 2100	7470 ± 2100	390 ± 82	<15
CGG	None	NT	NT	NT	NT	20,000 ± 430	8730 ± 430	194 ± 35	<15
CGG	CGG-IgE	NT	NT	NT	NT	18,600 ± 110	6500 ± 110	236 ± 46	<15
No transfer	None	4360 ± 1210	950 ± 210	790 ± 100	<0.2	18,080 ± 1600	7700 ± 300	240 ± 52	<15
No transfer	CGG-IgE	7450 ± 290	1150 ± 160	1000 ± 50	<0.2	20,350 ± 660	5950 ± 500	300 ± 69	<15

Adult donor mice were immunized intraperitoneally twice, with a 4-week interval, with CGG-IgE (100 μg) or CGG (50 μg) in incomplete Freund's adjuvant; they were sacrificed for cell transfer ≈15 weeks after the second inoculation. Spleen cells from mice immunized with a given antigen were pooled and 2×10^7 cells were administered to each recipient. Each recipient received KLH-TNP (0.5 μg in alum, intraperitoneally) 72 days before and 7 days after cell transfer. They were also inoculated intraperitoneally with 100 μg of the antigen specified in the second column, immediately after the cell transfer. Assays were carried out 13 days after cell transfer. NT, not tested.

Table 4. Adoptive transfer of the IgE-suppressed state by lymphocytes from mice immunized perinatally with IgE in saline

Spleen cells transferred	No. of recipients	Serum titer				Secreting cells, no. per spleen			
			Anti-TNP				Anti-TNP		
		Total IgE, ng/ml	IgE, ng/ml	Total, μg/ml	Anti-IgE, μg/ml	Total IgE	IgE	IgG ($\times 10^{-3}$)	Anti-IgE
None	4	7320 ± 2180	4230 ± 1070	360 ± 100	<0.2	17,400 ± 2290	6300 ± 1700	350 ± 83	<15
Unfractionated (1.2×10^7)	4	2250 ± 420	1120 ± 130	600 ± 100	1.5 ± 0.5	2,430 ± 950	710 ± 120	370 ± 65	600 ± 140
B cells (1.2×10^7)	4	3080 ± 1330	2010 ± 480	480 ± 86	0.4 ± 0.1	13,850 ± 1960	4050 ± 600	420 ± 93	63 ± 30
$CD4^+$ T cells (1.2×10^7)	4	8890 ± 430	4200 ± 1000	680 ± 110	<0.2	24,700 ± 2050	7700 ± 500	440 ± 80	<15
$CD8^+$ T cells (1.2×10^7)	4	5530 ± 1230	4250 ± 1560	520 ± 33	<0.2	18,700 ± 3400	7300 ± 1400	410 ± 70	<15
$CD4^+$ T cells (0.6×10^7) plus B cells (0.6×10^7)	4	980 ± 480	540 ± 250	390 ± 100	0.6 ± 0.2	3,500 ± 1670	850 ± 600	400 ± 86	680 ± 200
$CD8^+$ T cells (0.6×10^7) plus B cells (0.6×10^7)	4	4920 ± 920	NT	NT	NT	11,830 ± 2310	4980 ± 1350	290 ± 76	25 ± 14

Donor mice were immunized with 100 μg of IgE in saline on days 6, 9, and 43 after birth. Approximately 3 months later the donors were sacrificed and their spleen cells were pooled. Recipient mice (≈20 weeks old) were primed with KLH-TNP (0.5 μg in alum, intraperitoneally) 13 weeks before the cell transfer and challenged with KLH-TNP 7 days after the transfer. They were bled and sacrificed 6 days later. NT, not tested.

reduced. The data on numbers of secreting cells are comparable to those obtained after transfer of unfractionated spleen cells (Table 4).

In contrast to $CD4^+$ cells plus B cells, the mixture of $CD8^+$ T cells plus B cells had a small or negligible inhibitory effect on IgE synthesis.

DISCUSSION

The work reported here made use of adoptive transfer experiments to explore the role of anti-IgE antibodies and T cells in the inhibition of IgE responses. Earlier experiments, described in the Introduction, had already implicated anti-IgE antibodies in the inhibition of IgE synthesis induced by perinatal administration of IgE and, further, demonstrated that the synthesis of IgE could be inhibited by passive administration of syngeneic anti-IgE antibodies.

Data presented here show that 2×10^7 spleen cells from mice immunized with IgE in saline (on days 6, 9, and 42 after birth) can adoptively transfer inhibition of IgE synthesis to adult mice of the same strain. This was shown by measurements of serum concentrations of IgE and numbers of IgE-secreting cells in the recipient mice. In contrast, cells from mice immunized with CGG-IgE (and producing anti-IgE antibodies) did not transfer the state of inhibition unless the recipients were also challenged with CGG-IgE (Table 3). In addition, mice that received IgE-primed cells produced anti-IgE antibodies, whereas mice that received CGG-IgE-primed cells did not, unless they were challenged with CGG-IgE. A possible explanation for the apparent absence of a requirement for a secondary challenge after transfer of cells from mice immunized with unconjugated IgE is that the IgE antigen is in fact needed to stimulate anti-IgE synthesis in recipients but is supplied by the recipient mice. Unconjugated CGG could not replace CGG-IgE either for priming of donors or for stimulation of the recipients of CGG-IgE-primed cells.

Experiments to explore the role of B and T cells made use of splenic cells from donors immunized with unconjugated IgE (on days 6, 9, and 43 after birth). In these experiments the recipient mice were primed with KLH-TNP 72 days before the adoptive transfer and challenged with KLH-TNP 7 days after the transfer; assays were carried out 6 days later. The transfer of 1.2×10^7 unfractionated IgE-immune spleen cells inhibited total IgE synthesis and the synthesis of IgE anti-TNP antibodies in the recipients. The inhibition was most pronounced at the level of numbers of IgE-secreting cells (total or anti-TNP). Transfer of B-enriched cells did not cause inhibition of IgE production, nor did the transfer of $CD4^+$ or $CD8^+$ T cells. Also, a combination of B cells and $CD8^+$ T cells was not inhibitory. However, strong inhibition of IgE synthesis (total or anti-TNP) was caused by the cotransfer of B cells and $CD4^+$ T cells. This cotransfer also resulted in the synthesis of anti-IgE antibodies in the recipient mice. The amounts of anti-IgE produced, as well as the degrees of inhibition of IgE synthesis, were comparable in mice that received unfractionated spleen cells or a combination of B and $CD4^+$ T cells.

The results strongly suggest that inhibition of IgE synthesis in the recipient mice was caused by anti-IgE antibodies. Inhibition was observed only when the transferred cells (B cells plus $CD4^+$ T cells) would be expected to generate anti-IgE antibodies, and in each case anti-IgE synthesis was detected in those mice in which IgE production was inhibited. It should be noted that all of our results suggest that the continuous presence of anti-IgE antibodies is necessary for inhibition of IgE synthesis (3).

A role for T cells alone in the suppression of IgE is suggested by the data of Chen *et al.* (13). Their experimental system differed from ours; they measured suppression by adoptive transfer of a mixture of T cells from IgE-suppressed mice and syngeneic spleen cells from KLH-primed mice. They immunized the recipients with KLH and showed that the synthesis of IgE anti-KLH antibodies was inhibited by the presence of T cells from IgE-suppressed donors. More recently, Chen (22) reported that the suppressive T cells were $CD4^-CD8^+$. It is uncertain whether the specificity of their suppressor cells was directed to isotype (IgE) or to antigen (KLH), since both the donors and recipients were immunized with KLH; it has been reported that antigen-specific suppression can be restricted to the IgE isotype (23–27). Also, Jardieu *et al.* (28) have isolated T-cell clones that produce soluble factors which inhibit antigen-specific IgE production. Although T cells appear to play a role in the suppression of IgE in other systems, anti-IgE antibodies are strongly inhibitory and predominant in the experimental model that we employed.

There are at present no data directly relevant to the mechanism through which anti-IgE antibodies inhibit IgE synthesis in an isotype-specific manner. Prolonged inhibition of immunoglobulin synthesis after administration of antibodies specific for the μ chain of IgM was demonstrated >20 years ago (29). Anti-μ antibodies are inhibitory when administered to neonatal but not to older animals. In contrast, anti-IgE is effective in suppressing IgE synthesis in mice that are up to 8 weeks old. A factor that may account, at least in

part, for the difference is the relatively low concentration of IgE as compared with IgM in adult mice; the serum concentrations of the two isotypes differ by a factor of 200–1000. The high concentration of circulating IgM may help to prevent an inhibitory effect of anti-IgM in adult animals. Another example of inhibition by anti-isotype antibody is the inhibition of differentiation in lipopolysaccharide-stimulated cultured murine spleen cells *in vitro* (30). Antibodies specific for IgG3 prevented lipopolysaccharide-induced differentiation into IgG3-secreting plasma cells; other isotypes were not affected. IgE appears unique in that inhibition can be induced *in vivo* in adult animals.

One possible mechanism for the inhibition of immunoglobulin synthesis by anti-immunoglobulin is the induction of apoptosis in B cells. This has been demonstrated after treatment of WEHI-231 or CH31 (both immature, surface IgM-positive B-cell lines) with anti-immunoglobulin antibody (31, 32). The apoptosis is prevented in the presence of T helper cells, whose effect appears to be mediated by the interaction of CD40L molecules on T cells with CD40 on the surface of the WEHI-231 cells (33), suggesting that crosslinking of the surface immunoglobulin receptor by antigen or anti-immunoglobulin is the initiating event. Apoptosis of B cells induced by antigen has also been reported (33). In mice carrying a transgene encoding an autoantibody, the self-reactive B cells were eliminated on contact with the self-antigen in either the bone marrow or the periphery, indicating that both immature and mature B cells can be eliminated as a consequence of crosslinking of surface immunoglobulin (34–38). An alternative mechanism for tolerance induction is the inactivation, rather than elimination, of self-reactive B cells (38–41).

On the basis of the above data, it appears likely that anti-IgE mediates its inhibitory effect either by elimination or by inactivation (induction of anergy) in IgE-bearing B cells. Direct experiments to establish the mechanism would be facilitated by the availability of a suitable cell line bearing surface IgE. The ε switch variant of the I.29 line (42), which does express surface IgE, has proven resistant to effects of anti-IgE in our laboratory.

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