IMMUNOLOGICAL RESPONSIVENESS OF NEONATAL A/J MICE TO ISOTYPIC DETERMINANTS OF SYNGENEIC IgE

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We recently demonstrated the induction, in adult inbred mice, of high titers of syngeneic anti-IgE with isotypic specificity and moderately high affinity ($K_a \simeq 10^7$ M^{-1}) (1). The antibodies could be elicited by immunization with syngeneic monoclonal IgE conjugated to keyhole limpet hemocyanin (KLH)[1], but not by unconjugated IgE. The ability of the mice to respond indicates the presence of B cells with specificity for isotypic determinants of IgE. The requirement for KLH suggests that tolerance is maintained in adult mice at the level of T cells and that this tolerance can be broken by the use of an immunogenic carrier molecule. These observations facilitated the preparation of syngeneic monoclonal anti-IgE antibodies (2). We have prepared syngeneic anti-IgD by a similar procedure (3).

In studying levels of serum IgE in neonatal A/J mice, we noted that IgE was undetectable (<30 ng/ml) by our assay in newborn mice and that IgE could first be detected at the approximate age of 2–3 wk. This suggested the possibility that neonatal mice might be responsive to unconjugated syngeneic IgE and that tolerance might be acquired as IgE appeared in the serum. This proved to be the case; our investigation of tolerance as a function of age and preliminary studies of the mechanism are reported here.

Materials and Methods

Mice. 5-wk-old A/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME).

Antigens. Monoclonal IgEκ antibodies were obtained from hybridomas. SE20.2 (4), SE17.1 and SE21.1 (1) are of A/J derivation and are specific for *p*-azobenzenearsonate (Ars). mAb SE20.2, but not SE21.1 or SE17.1, expresses a major intrastrain crossreactive idiotype designated CRI_A. Each mAb was affinity purified on a column of Ars-bovine gamma globulin coupled to Sepharose 4B; elution was carried out with 0.5 M arsanilate, pH 8.0. TIB-142 (IgEκ) is of BALB/c origin and is specific for the TNP hapten. It was obtained from the American Type Culture Collection, Rockville, MD (donated by M. Wabl), and affinity purified as described (5). KLH was obtained from Calbiochem-Behring Corp. (La Jolla, CA). A conjugate of IgE (SE21.1) with KLH (KLH-IgE) was prepared by using glutaraldehyde, as described elsewhere (1). A 1:1 wt ratio of the proteins, each at final concentration of 5 mg/ml, was used for conjugation.

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[1] *Abbreviations used in this paper:* Ars, *p*-azobenzenearsonate; KLH, keyhole limpet hemocyanin; PVC, polyvinylchloride.

Assays for Total IgE in Serum. Polyvinylchloride (PVC) microtiter plates were exposed overnight to a mixture containing a 4:1 ratio of normal rabbit IgG to affinity-purified rabbit anti-mouse IgE, at a total concentration of 5 μg/ml (5, 6). After washing, the wells were saturated with 2.5% horse serum. Serum dilutions to be assayed for IgE were then added to the wells; after 6 h at room temperature, the plates were washed and developed with ^{125}I-labeled affinity-purified rabbit anti-mouse IgE (12 ng in 0.1 ml/well). After overnight incubation, wells were washed and counted. Protein SE20.2 (IgEκ) was used as the standard for the assay. All assays were done in duplicate.

Assays for Anti-IgE in Mouse Antisera. PVC plates were coated with mAb SE20.2 (1 μg in 0.1 ml/well), then saturated with 2.5% horse serum (1). After exposing to 50 μl of the test sample per well for 6 h at room temperature, the wells were washed and exposed overnight at room temperature to 100 ng in 0.1 ml of ^{125}I-labeled affinity-purified goat anti-mouse Fc (of IgG) that had been adsorbed with mouse IgE-Sepharose. This reagent did not react with mouse IgE but reacted with mouse IgG of each subclass. As a standard for the assay, we used pooled hyperimmune anti-KLH-IgE (SE21.1) antiserum whose anti-IgE content was determined by measuring its maximum IgE-binding capacity, as described elsewhere (1). Because immunizations were carried out with mAb SE21.1, assays on SE20.2-coated wells detected antibodies with isotypic, but not idiotypic specificity.

In some cases, sera were tested for the presence of IgM antibodies specific for IgE. The developing reagent used was ^{125}I-labeled affinity purified rabbit anti-mouse IgM.

Tests for Isotypic Specificity of Antibodies Elicited by IgE mAb SE21.1. Isotypic specificity of antibodies was determined for the mice of group 2, Table I, the 7-d-old mice of Table II, and all mice represented in Fig. 1 and Table III that had significant anti-IgE titers. Inhibition assays were used to test for isotypic specificity. Antisera to be added to SE20.2-coated wells were first mixed with 10 μg of unlabeled monoclonal IgE (TIB-142), IgM (SM1.5; reference 7), or normal A/J IgG. After washing, wells were developed with ^{125}I-labeled anti-Fc (of IgG). In each case, the IgE caused >90% inhibition of binding, whereas IgM or IgG caused <10% inhibition.

Test for Idiotypic Specificity of Antibodies Elicited by mAb SE21.1. For these assays, wells were coated with the immunogen (SE21.1) and exposed to anti-SE21.1 antiserum in the presence or absence of a large excess (20 μg) of an unlabeled unrelated mouse IgEκ mAb (TIB-142 or SE20.2). Wells were developed with affinity-purified ^{125}I-labeled rabbit anti-mouse Fc (of IgG).

Binding Affinities of Antiisotypic Anti-IgE Antibodies. Average affinities were determined by a liquid phase assay using anti-IgE (SE21.1) serum with ^{125}I-labeled SE20.2 as the ligand, as described in reference 1. Immune complexes were precipitated with excess rabbit anti-mouse Fc (of IgG).

Preparations of Cells for Adoptive Transfers. Single cell suspensions of spleen were prepared in RPMI 1640 medium. Red cells were lysed with 0.155M NH_4Cl solution containing 0.01 M $KHCO_3$ and 0.001 M EDTA, pH 7.4, then washed three times with RPMI-1640 medium at 4°C. 50 μl of a suspension containing 5×10^6 cells was injected i.p. into neonatal (2-d-old) mice.

Results

We have reported that adult mice produce anti-IgE antibodies with isotypic specificity, as well as antiidiotypic antibodies, after challenge with a conjugate of KLH and syngeneic IgE (KLH-IgE) in CFA; adult mice respond very poorly, however, to unconjugated IgE administered with the same adjuvant (1). The data in Table I confirm those observations and, in addition, show that anti-IgE persists in serum until at least day 217 after the initial inoculation. The mice were inoculated with 200 μg KLH-IgE in CFA on days 0, 14, and 35. The presence of anti-IgE was associated with a greatly reduced serum concentration of IgE, as compared with controls (Table I). It is of interest that mice of group 1, Table I, that received uncon-

TABLE I

Responses of 6-wk-old Mice to Syngeneic IgE, Unconjugated or Conjugated to KLH

Group	Number of mice	Immunogen	μg per inoculation	Concentrations in serum			
				Day 64		Day 217	
				Total IgE	Anti-IgE	Total IgE	Anti-IgE
				ng/ml	*μg/ml*	*ng/ml*	*μg/ml*
1	5	IgE	100	750 ± 490*	0.6 ± 0.2	600 ± 140	<1.0
2	16	KLH-IgE	200	100 ± 30	460 ± 60	90 ± 30	47 ± 5
3	9	KLH	100	4,900 ± 760	<0.5	3,800 ± 1,000	<1.0
4	9	Nonimmunized mice		1,600 ± 350	<0.5	3,400 ± 860	<1.0

Mice were inoculated i.p. on days 0, 14, and 35, with 0.2 ml of a 1:1 emulsion of antigen solution in CFA.
* Mean values ± SEM.

jugated IgE, had substantially reduced levels of serum IgE on day 217, as compared with controls, despite the very low concentration of circulating anti-IgE. IgE levels were, however, much lower in mice of group 2 that received KLH-IgE.

The data in Table II show that neonatal mice also produce anti-IgE antibodies in response to challenge with KLH-IgE in CFA. The anti-IgE titers were higher in adult mice (Table I). The adult mice received three inoculations, however, as compared with two for the neonates. The isotypic specificity of the anti-IgE antibodies was demonstrated by inhibition tests (see Materials and Methods).

As noted above, adult mice responded very poorly or not at all to unconjugated IgE, administered in CFA (1). Fig. 1 shows that, in contrast, mice immunized neonatally with IgE in CFA do produce anti-IgE antibodies. (The mice were inoculated on the day specified in the figure and again 14 d later.) The capacity to respond to unconjugated IgE was rapidly lost as the mice grew older, and minimal responses were observed after 2 wk of age. These responses were detected with the anti-Fc (of IgG) reagent. Very little IgM anti-IgE (<2% of the IgG response) was produced (data not shown).

There was no strict requirement for CFA in the induction of anti-IgE in newborn mice. Alum or IFA were also effective. For example, five 9-d-old mice, immunized with 25 μg of monoclonal IgE in alum and challenged with 50 μg 2 wk later, possessed an average of 6.1 μg/ml of serum anti-IgE 14 d after the second inoculation

TABLE II

Responses of Neonatal Mice to a KLH-IgE Conjugate

Age of mice	Day 28		Day 42		Day 165	
	Anti-IgE	Mean ± SEM	Anti-IgE	Mean ± SEM	Anti-IgE	Mean ± SEM
	μg/ml		*μg/ml*		*μg/ml*	
2 d	0.5,1.0,5.6, 7.0,7.9	4.4 ± 1.5	1.0,3.0,8.0, 21,36	13.8 ± 6.5	9,10,21, 25,27	18.4 ± 3.8
7 d	31,32,34, 47,49	38.6 ± 3.9	32,36,36, 41,51	39.2 ± 3.2	5.7,6.3,20, 23,34	17.8 ± 5.4

A/J mice received 50 μg of antigen in CFA (i.p.) on the day specified in the first column (day 0), and 100 μg on day 14. A conjugate of KLH with mAb SE21.1 was used as the antigen. Each value represents the serum titer of an individual mouse.

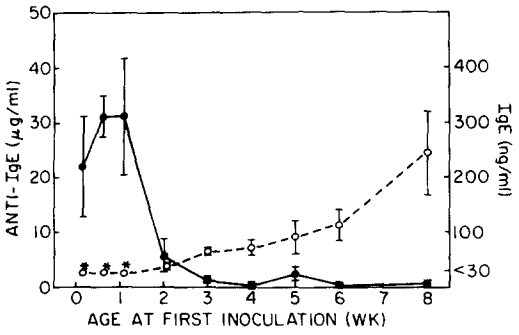

FIGURE 1. Responses of A/J mice to syngeneic monoclonal IgE (SE21.1) administered in CFA. Mice received 25 μg of IgE at the time specified on the abscissa, and 50 μg after 14 d. 14 d later, anti-IgE serum titers were determined, using wells coated with SE20.2 (IgEκ). (*Solid line*) Concentrations of anti-IgE. (*Dashed line*) Serum concentrations of IgE measured just before the first inoculation. The three IgE concentrations marked with an asterisk were values determined with groups of age-matched A/J mice other than those that were immunized; IgE was not detectable in the mice of these three groups (concentrations, <30 ng/ml; *right hand ordinate*). Each point represents the mean value for a group of 5–10 mice.

(data not shown). A group of six 9-d-old mice produced an average of 24 μg/ml of anti-IgE when immunized and tested according to the same schedule, using IFA in place of alum. 5-wk-old mice were unresponsive to IgE administered in alum, IFA, or CFA.

Fig. 1 also shows that serum IgE concentrations before immunization were unmeasurable by our assay until the mice were ~2 wk old and then gradually increased. The average concentration at the age of 8 wk was 245 ng/ml, as compared with values of ~1–3 μg/ml seen in older A/J mice. The data suggest that the ability to respond to monomeric, unconjugated IgE in CFA is lost as IgE is produced by the mice.

The mice used to obtain the results in Fig. 1 were bled again, without further immunization, 156 d after the last inoculation. As seen in Table III, anti-IgE persisted in the sera of mice that were first inoculated at the age of 1 or 8 d; a low but significant concentration of anti-IgE was also present in sera of mice first challenged

TABLE III

IgE and Anti-IgE in Serum, 156 Days After Immunization With IgE in CFA

Group	Number of mice	Age at first inoculation	Day 156 IgE Range	Day 156 IgE Mean ± SEM	Day 156 Anti-IgE Range	Day 156 Anti-IgE Mean ± SEM
			ng/ml		*μg/ml*	
1	5	1 d	all <50		0.8–32	14.4 ± 6.7
2	6	5 d	all <50		5.1–12	8.4 ± 1.1
3	7	8 d	all <50		0.2–35	11.4 ± 4.8
4	8	2 wk	all <50		0.1–17	2.9 ± 2.0
5	10	3 wk	30–1,100	410 ± 130	0–2.6	0.3 ± 0.1
6	8	4 wk	1,000–5,500	2,600 ± 540	0–0.1	0
7	6	5 wk	250–6,300	3,100 ± 1,100	0–0.2	0.1 ± 0.03
8	6	6 wk	270–5,100	3,100 ± 780	0–0.1	0
9	6	8 wk	30–3,100	1,000 ± 550	0–6.9*	1.1 ± 1.0
10	6 (CFA)‡	8 d	620–3,400	1,980 ± 520	0–0.1	0

These are the same mice that are represented in Fig. 1. The data indicate serum titers observed after a long rest period (on day 156).

* All mice but one in this group had unmeasurable titers.

‡ These mice received CFA only (without IgE).

TABLE IV

Dose-response of 6- or 25-d-old Mice to Syngeneic IgE in CFA

Age at first inoculation	Amount of IgE inoculated*	Serum anti-IgE (day 28)‡	Mean ± SEM
d	*μg (μl)*	*μg/ml*	
6	50 (50)	24,28,38,40,47,48,52	40 ± 4
6	10 (50)	6,16,16,27,38,42,50,55	31 ± 6
6	2.5 (50)	7,17,17,19,25,49	22 ± 6
6	0 (50)	all 0 (6 mice)	0
25	100 (100)	0§,0§,1.2,1.7,2.4,5.1	1.7 ± 0.8
25	25 (100)	all 0 (6 mice)	0

* The values in parentheses refer to total volume injected; inocula contained equal volumes of antigen solution and CFA.

‡ Mice were immunized on the day specified in the first column and again 14 d later. They were bled 28 d after the first inoculation.

§ Total volume per inoculation was 200 μl rather than 100 μl.

at the age of 2 wk. Mice that were first inoculated at the age of 3 wk or later had marginal or unmeasurable titers of anti-IgE. Free IgE was in general not detectable by our assay in mice producing anti-IgE (we do not know whether this reflects inhibition of IgE synthesis or simply neutralization by anti-IgE.) It is of interest that mice of group 5, which had very low but probably significant titers of anti-IgE, exhibited substantially depressed serum IgE concentrations.

Dose-response to IgE in CFA of 6- and 25-d-old mice. The question arose as to whether the responsiveness of very young mice to IgE in CFA could be attributed to a relatively high dose per unit of body weight. To approach this question, we challenged 6-d-old mice twice with amounts of IgE (in CFA) ranging from 2.5–50 μg per inoculation (Table IV). For comparison, we used 25 d-old mice. The average body weights of the 6- and 25-d-old groups were 3.2 and 12.1 g, respectively; i.e., the ratio was ~1:4.

The 6-d-old mice responded well to as little as 2.5 μg of IgE in CFA (given twice), whereas the older mice were unresponsive to 25 μg doses; in this comparison, the ratio of weight of antigen to body weight was considerably greater for the unresponsive group. Some 25-d-old mice did produce small amounts of anti-IgE when 100-μg doses of IgE in CFA were administered. However, these responses were marginal and much lower than those of the 6-d-old mice (Table IV).

Binding Affinities of Antiisotypic Anti-IgE Antibodies Induced by Unconjugated IgE. Average binding affinities for IgE were determined with anti-IgE (SE21.1) antisera induced in neonatal mice with unconjugated or conjugated IgE in CFA. ^{125}I-labeled SE20.2 was used as the ligand for these determinations. Groups of six, 5-d-old mice were inoculated with (*a*) 25 μg SE21.1 in CFA or (*b*) 50 μg KLH-SE21.1 in CFA. They were boosted with twice the initial quantity of antigen 14 and 56 d after the first inoculation, and were bled 14 d later. Average binding affinities were determined on pools of antisera from each of the two groups of mice. The average K_a values for groups *a* and *b* were 5.4×10^7 and 4.7×10^7 M^{-1}, respectively. These values are in the range of values obtained upon immunizing adult mice with KLH-IgE in CFA ($0.8–9.0 \times 10^7$; reference 1).

TABLE V

Responses of Neonatal Mice to IgE in CFA after Preinoculation of IgE in Saline

Group	Preinoculation of IgE	Day 45		Day 59	
		Anti-IgE	Mean ± SEM	Anti-IgE	Mean ± SEM
		μg/ml	*μg/ml*	*μg/ml*	*μg/ml*
1	Saline (no IgE)	7.7,8.8,12.6,16.6,18.1	12.8 ± 2.0	5.2,6.0,12.5, 14.2,17.7	11.1 ± 2.4
2	25 μg SE21.1	<0.1,0.1,0.1,0.5	0.2 ± 0.1	<0.1,<0.1, 0.2,1.0	0.3 ± 0.2
3	500 μg SE21.1	0.1,1.1,1.5,2.2,4.5	1.9 ± 0.7	<0.1,0.5,1.0, 2.0,6.0	1.9 ± 1.0
4	25 μg SE17.1	<0.1,<0.1,<0.1,<0.1,2.1	0.5 ± 0.4	<0.1,<0.1,<0.1, 0.3,3.3	0.7 ± 0.6
5	500 μg SE20.2	0.1,0.4,1.6,5.2	1.8 ± 1.1	<0.1,0.9,2.2,3.0	1.5 ± 0.7

2-d-old A/J mice, or 1-d-old mice (group 4), were preinoculated i.p. with saline or with IgE in saline (column 2). They were then inoculated on days 9 and 31 with 25 and 50 μg, respectively, of SE21.1 in CFA. Values shown are serum concentrations for individual mice.

Antiidiotypic Response to Syngeneic IgE. As indicated in Materials and Methods, antiidiotypic antibodies were estimated by coating the wells of microtiter plates with the IgE (SE21.1) used as immunogen and determining the amount of antibody bound in the presence of a large excess of an unrelated IgEκ mAb. Assays were carried out on a total of 29 neonatal mice (up to 2 wk of age). Of these mice, nine were immunized with KLH-IgE in CFA, the remainder with IgE in CFA. The range of ratios of idiotypic to isotypic anti-IgE antibodies ranged from 0.16–0.85, with a mean of 0.43, for mice immunized with KLH-IgE. For mice immunized with IgE, the range was 0.05–1.9 with a mean of 0.61.

We also looked for antiidiotypic antibodies by this method in four pools of sera of mice 3–8 wk of age that were immunized with IgE in CFA. As indicated above, such mice did not produce anti-IgE antibodies with isotypic specificity. We also were unable to detect antiidiotypic antibodies in any of these serum pools.

Induction of Tolerance with Unconjugated IgE. One interpretation of the results presented so far is that the presence of IgE in mice tolerizes them so that they are nonresponsive to syngeneic IgE administered in CFA. If this is so, inoculation of monomeric IgE without adjuvant might tolerize neonatal mice. The data in Table V support this hypothesis. Intraperitoneal inoculation of IgE in saline into 1- or 2-d-old mice greatly reduced their subsequent responses to IgE in CFA, first given at the age of 9 d, at which time the controls (group 1) were responsive. A dose of 25 μg (group 2) appears to be more effective than 500 μg in inducing tolerance. A decreased reponse to IgE in CFA was induced by a saline solution of SE21.1 (used as the subsequent immunogen in CFA) or by different IgEκ mAbs, SE20.2 or SE17.1 (Table V). Thus, the inhibitory effect is evidently not mediated by idiotypic determinants.

Induction of Tolerance in Neonatal Mice by Adoptive Transfer of Adult Spleen Cells. Table VI shows the results of experiments in which splenic leukocytes (spleen cells) were transferred into 2-d-old A/J mice from A/J donors that were 10 d or 8 wk of age. As shown above, only the 8-wk-old mice, of these three groups, are unresponsive to IgE in CFA. The data in Table VI indicate that 2-d-old mice that had received

TABLE VI

Effect of Spleen Cells Transferred from Normal Adult Mice on the Subsequent Response of Neonatal Mice to Syngeneic IgE

Group	Age of donor mice	Serum concentration of anti-IgE (μg/ml) Day 30 Anti-IgE	Day 30 Mean ± SEM	Day 44 Anti-IgE	Day 44 Mean ± SEM	Day 55 Anti-IgE	Day 55 Mean ± SEM
		μg/ml		*μg/ml*		*μg/ml*	
1	No cells transferred	0.1,2.1,2.6,4.7, 5.8	3.1 ± 1.0	0.1,4.6,5.7 15.0,15.2	8.1 ± 3.0	0.1,4.8,5.1,13.2, 14.5	7.5 ± 2.7
2	8 wk	<0.1,<0.1,<0.1, 0.3,0.3,0.4,0.6	0.3 ± 0.1	0.1,0.2,0.2,0.2 1.0,2.1,2.1	0.8 ± 0.3	<0.1,<0.1,<0.1, <0.1,1.7,1.8,2.1	0.9 ± 0.4
3	10 d	0.2,1.0,2.3,5.5, 16.6	5.1 ± 3	0.7,1.6,10.4, 10.6,15.7	7.8 ± 2.9	0.5,1.3,6.0,10.4, 14.4	6.5 ± 2.7

2-d-old A/J mice received 5 × 10^6 spleen cells from the donor mice (column 2). They were immunized i.p. on days 9 and 30 with 25 and 50 μg, respectively, of IgE (SE21.1) in CFA. The day 30 bleeding was taken before the day 30 immunization. Each value represents the serum titer of an individual mouse.

5×10^6 spleen cells from 8-wk-old donors produced much lower titers of serum anti-IgE than control mice after challenge with IgE in CFA. In contrast, cells from 10-d-old mice did not have this inhibitory effect (Table VI, group 3). These results are consistent with the ability of 10-d-old, but not 8-wk-old mice, to respond to IgE in CFA (Fig. 1 and Table III), and suggest that some type of active suppression occurs in the 8-wk-old mice.

Discussion

We have shown previously that adult A/J or BALB/c mice produce substantial amounts of anti-IgE antibodies with isotypic, as well as idiotypic specificity upon challenge with monoclonal syngeneic IgE conjugated covalently to KLH, but not with unconjugated IgE (1). This indicates that B cells with anti-IgE specificity are present in the mice. The failure to respond to unconjugated IgE suggests that a state of tolerance may exist at the level of T cells; conjugation with KLH may provide the T cell help requisite for an anti-IgE response.

The present results show that, in contrast to adult mice, A/J mice that are <2 wk old respond well to unconjugated IgE in CFA (as well as to KLH-IgE), producing anti-IgE antibodies with isotypic and idiotypic specificity. The capacity to produce either antiisotypic or antiidiotypic antibodies to IgE is greatly diminished by the time the mice reach the age of 3 wk. There is an inverse correlation of the ability to respond to unconjugated IgE with the presence of detectable IgE in serum before immunization. A/J mice that are 10 d old or younger had serum IgE levels that are undetectable by our assay (<30 ng/ml; Fig. 1). At the age of 3 wk, the average concentration is 62 ng/ml and serum IgE levels continue to rise with increasing age. Adult A/J mice eventually exhibit serum concentrations of 1–3 μg/ml.

The possibility that the reduced titer of anti-IgE in older mice may simply be due to absorption of anti-IgE by IgE is not consistent with our data. Mice immunized with IgE 8 d after birth were responsive, whereas 21-d-old mice were not. Both groups were boosted 14 d later. This difference still persisted at day 156 (Table III). By that time, differences in exposure to endogenously synthesized IgE were negligible, particularly since IgE levels are very low during the first 6 wk of life (Fig. 1).

These observations are consistent with the hypothesis that tolerance at the T cell level is acquired as a consequence of synthesis of IgE by the mouse. This view is supported by the results of intraperitoneal inoculation of IgE in saline into 2-d-old mice (Table V). Mice that received 25 or 500 μg of unconjugated monoclonal IgE in saline were much less responsive than control mice to IgE in CFA, administered at the age of 9 d. 25 μg of IgE in saline was more effective than 500 μg. It is highly unlikely that the observed effects can be attributed simply to neutralization of anti-IgE by the IgE that was injected into 2-d-old mice. The half-life of IgE in serum is 5–8 h (8) and anti-IgE titers were measured at days 45 and 59. Also, when large doses of radiolabeled IgE (>100 μg) were inoculated into an adult mouse, 99% was entirely cleared from the mouse within 48 h (8). We considered the possibility that the reduced responsiveness of older mice to IgE in CFA might be related to their greater body weight in relation to the dose of antigen. However, when 3-wk-old (unresponsive) and 6-d-old (responsive) mice were compared, it was found that the differences persisted even when the older mice received amounts of IgE that were con-

siderably greater (expressed as a ratio to body weight) than those administered to the 6-d-old mice (Table IV).

The tolerizing effect of IgE in saline, given to neonatal mice, as well as the inverse relationship between serum IgE levels and anti-IgE responses, suggest that tolerance is induced, by the age of 3 wk, by the presence of adequate amounts of IgE in the mouse. The fact that both neonatal and adult mice are responsive to KLH-IgE suggests that B cells are present and that tolerance may be caused by the absence or suppression of helper T cells with specificity for IgE. That such T cells are specific for isotype, rather than idiotype is indicated by the fact that inoculation of an IgE mAb (either SE20.2 or SE17.1) in saline induced tolerance to an idiotypically unrelated IgE mAb (SE21.1; Table V).

As an initial exploration of the mechanism of tolerance induction, we transferred 5×10^6 spleen cells from 8-wk-old A/J mice (tolerant to IgE in CFA) into 2-d-old (responsive) A/J recipients. The recipient mice showed a greatly decreased capacity to respond to syngeneic IgE in CFA (Table VI). As a control, 10-d-old (responsive) mice were used as cell donors; no significant change in the capacity to respond to IgE in CFA was noted in the 2-d-old recipients. Thus, the tolerant state can be induced by transfer of spleen cells from a tolerant donor. The results do not prove that IgE-specific suppressor T cells are present in the 8-wk-old mice. It is possible, for example, that the anergic state is mediated by IgE produced by B cells. Further studies are needed to evaluate the mechanism of induction of tolerance. It seems, however, that helper T cells are either absent or inactivated in the unresponsive mice, since tolerance can be overcome by inoculation of a conjugate of IgE to an immunogenic molecule (KLH).

The appearance of anti-IgE in serum was always accompanied by greatly reduced levels of serum IgE. A striking example of this is provided by mice of group 4, Table III, which exhibited a markedly diminished average level of serum IgE at day 156 despite the presence of very low levels of anti-IgE in the mice. Our data do not at this point permit a conclusion as to whether the reduced levels of serum IgE are due to inhibition of its synthesis or simply to neutralization by anti-IgE. This question is being investigated.

Marshall and Bell (9) were able to induce anti-IgE in rats by immunization with a rat IgE myeloma protein. Several attempted methods of immunization were unsuccessful; these included the use of CFA with the intact myeloma protein; CFA, alum, or Bordetella pertussis with epsilon chains; and epsilon chains alone. Successful immunization was, however, accomplished with the intact protein precipitated with alum. Concentrations of serum anti-IgE produced were not specified; the amount of developing antibody (sheep anti-rat-IgG) bound to IgE-coated wells in RIAs was of the order of 0.1–1 μg (corrected for dilution) per ml of anti-IgE serum. Our results are in agreement with theirs in indicating that the presence of detectable anti-IgE was always accompanied by a marked reduction in the IgE levels determined by immunoassay (Tables I and III).

The belated appearance of IgE during ontogeny provides a convenient system for studying immune tolerance and its converse, autoimmunity. Other related models have proven useful for such studies. One involves two congenic strains of mice, one of which is deficient in C5. Using these strains, it was shown by Borel and collaborators that tolerance in the "C5-sufficient" mice resides in the T cell compartment

and is maintained by cells that suppress the response to C5. Both congenic strains contain responsive B cells (10, 11). Another interesting model makes use of a cytoplasmic liver protein (protein F), that occurs in two forms (F1 and F2) in different strains of mice. Mice do not respond to immunization with their own F protein; however, some strains respond to the allo-F protein by producing antibodies that react with determinants common to F1 and F2, suggesting that tolerance resides in T cells (12). It appears that the self-reactive helper T cells are eliminated or inactivated by suppressor T cells (13). Another relevant observation is that of Maniatis et al. (14), who showed that tadpoles can produce antibodies against a form of hemoglobin that is absent in tadpoles but present in mature frogs; the cellular basis has not been investigated.

An interesting and somewhat related model is that of tolerance of mice to mouse thyroglobulin (15, 16). Adult mice, which have very low levels of serum thyroglobulin (nanogram quantities) produce anti-thyroglobulin antibodies after challenge with that protein in CFA. However, the mice can be tolerized by prior inoculation of large amounts (2×200 μg) of thyroglobulin in saline; tolerance is accompanied by the appearance of specific suppressor T cells (15).

Mechanisms implicated in self-tolerance (17–19) include clonal deletion of T or B cells or maintenance of tolerance by suppressor cells (20–28). Much of that work, as well as the present study, suggests that, in T-dependent humoral responses, tolerance is frequently maintained in T cells, while B cells remain responsive. This conclusion is supported, for example, by the ability of nonidentical crossreactive antigens to break tolerance (24), and by the production of many autoantibodies, in vivo or in vitro, upon stimulation with polyclonal B cell activators (29, 30).

The model presented here is rather unique because of the age-dependent occurrence of tolerance, associated with appearance of the antigen in serum. A closely related observation is the clonal elimination of I-E-reactive T cells that occurs upon maturation of thymocytes in mice (fetal or adult) that express the I-E antigen, presumably owing to exposure to I-E during the maturation process (31). The availability of transgenic mice provides another approach to the question of time-dependent acquisition of tolerance. For example, Adams et al. (32) have shown that transgenic mice, expressing the SV40 T antigen under control of the insulin gene regulatory region, exhibit immunity to the T antigen only when its expression is delayed during ontogeny.

Further study of such systems, including ours, should help to elucidate mechanisms in self tolerance and to resolve the still unsettled question as to the relative contributions of clonal deletion and active suppression of T cells in various tolerant states. Studies on IgE regulation may also be useful in designing improved methods for immunotherapy of allergy.

Summary

We have previously shown that adult A/J mice produce high titers of anti-IgE with isotypic or idiotypic specificities in response to challenge with a conjugate of KLH with syngeneic monoclonal IgE. Thus, B cells that can synthesize anti-IgE are present in the mice. Adult mice are unresponsive to unconjugated IgE in CFA, suggesting that tolerance exists at the level of T cells. The present study shows that neonatal mice produce anti-IgE antibodies in response to unconjugated IgE in CFA,

but that this capacity is lost after the age of 2–3 wk. The loss of responsiveness corresponds closely with the appearance of detectable IgE in serum, suggesting that the IgE may induce tolerance. The affinities of anti-IgE antibodies produced by neonatal mice fall in the range of values obtained with KLH-IgE in adult mice. Tolerance to unconjugated IgE in CFA can be induced in neonatal mice by administration of IgE in saline. In addition, the tolerant state can be induced by adoptive transfer of spleen cells from adult mice. The time-dependent acquisition of tolerance provides a useful model for studying mechanisms of tolerance and autoimmunity.

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