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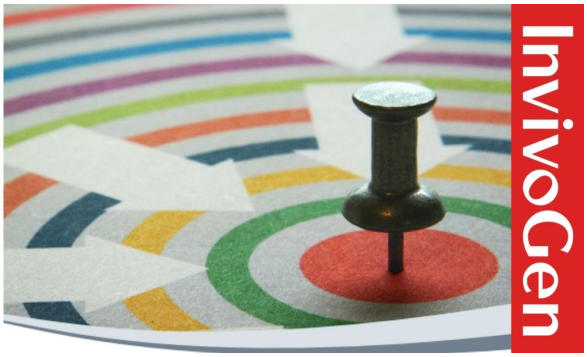

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INDUCTION OF TOLERANCE TO SYNGENEIC IgE IN NEONATAL MICE[1]

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We have previously shown that adult A/J mice are tolerant to syngeneic IgE at the level of T cells, but not B cells. T cells of mice are responsive until the age of 2 to 3 wk, which correlates with the time of appearance of serum IgE. Tolerance can be induced earlier by neonatal administration of IgE in saline. We report here that purified nonimmune adult B cells, but not T cells, can transfer the state of tolerance to neonatal mice. As few as 2 × 10^6 B cells are effective. If IgE-bearing or IgE-secreting cells prove to be responsible, the amount of cell-bound IgE that can induce tolerance must be very small. The results also indicate that suppressor T cells do not have a major role in maintenance of self-tolerance to IgE.

Adult A/J mice are tolerant to syngeneic IgE at the level of T cells but not B cells. Inoculations, in CFA, of KLH[3] conjugated to a syngeneic IgE mAb induced high titers (up to 1 mg/ml) of anti-IgE with isotypic specificity (as well as antiidiotypic antibodies). In contrast, unconjugated IgE in CFA was not immunogenic in adult mice (1, 2). The results can be interpreted on the basis that KLH provides T cell help, which is not available when unconjugated IgE is the immunogen. B cells with anti-IgE specificity are obviously present and active in adult mice (1, 2). Working with adult inbred rats, Marshall and Bell (3) have induced anti-IgE antibodies at relatively low titers by inoculation of an allogeneic IgE myeloma protein.

In normal A/J mice, IgE is undetectable by our assay (<30 ng/ml) until the mice are approximately 3 wk old. We observed that, in contrast to adult mice, anti-IgE can be induced in perinatal mice by unconjugated IgE in CFA, and that the mice become tolerant at the approximate age of 2 to 3 wk. The results suggested that IgE-specific T cells are tolerized in young mice as a consequence of the initial synthesis of IgE. This view was supported by the induction of tolerance in 0- to 1-day-old mice by 25 to 500 μg of syngeneic IgE mAb, inoculated i.p. in saline (4).

In adoptive transfer experiments, we found that 5 × 10^6 nonimmune adult spleen cells conferred tolerance to IgE when inoculated i.p. into 0- to 1-day-old mice (4). We report here that splenic B cells, but not T cells, are responsible for the induction of tolerance and that as few as 2 × 10^6 B cells are effective. The results are discussed in terms of the mechanism of tolerance induction.

MATERIALS AND METHODS

Mice. BALB/c and strain A mice were obtained from The Jackson Laboratory, Bar Harbor, ME. CAF_1 mice were bred in our laboratory from female BALB/c and male A/J mice. CAF_1 neonatal mice, rather than A/J mice, were used because of their greater survival rates.

Monoclonal IgE Antibodies. mAb SE17.1, SE1.3, and SE20.2 (all IgEk, anti-Ar) have been described (1, 5). SE20.2, but not SE1.3 or SE17.1, carries the major idiotype of strain A anti-Ar antibodies.

Polyclonal Antibodies. Rabbit antimouse IgE was prepared by immunization with mAb TIB-142 (IgEκ, anti-TNP); the hybridoma was obtained from the American Type Culture Collection, Rockville, MD (donated by M. Wabl). The antiserum was adsorbed on normal, pooled mouse IgG conjugated to Sepharose 4B. Anti-IgE recovered in the eluate was affinity purified on mAb SE1.3 conjugated to Sepharose 4B, with 3 M NaSCN as eluant; the recovered protein was dialyzed immediately (5). Goat anti-mouse Fc was prepared against the Fc fragment of papain-digested mouse IgG, absorbed with mouse F(ab), and affinity purified on mouse IgG-Sepharose. The purified antibody reacted with mouse IgG but not with IgE in a solid-phase RIA (2).

B cells. Single-cell suspensions of spleen were prepared in cold RPMI 1640 medium, and the erythocytes were lysed (6). B cells were positively selected by panning on plastic surfaces coated with anti-mouse F(ab) (6). Splenic leukocytes, in RPMI 1640 medium supplemented with 5% FCS, were placed on 100-mm Petri dishes (10^8 cells/5 ml medium/dish) precoated with a mixture of rabbit anti-mouse F(ab) (10 μg/ml) and nonspecific rabbit IgG (90 μg/ml). The cells were allowed to stand for 40 min at 4°C and, after gentle agitation, for an additional 40 min. Nonadherent cells were resuspended and removed. The plates were washed four times with cold PBS containing 3% FCS. Adherent cells were released into 10 ml PBS containing 3% FCS by vigorous pipetting at room temperature, then treated with anti-Thy-1.2 and "Low-Tox" rabbit complement (Cedarlane Laboratories, Hicksville, NY). Recovery of enriched B cells was 25 to 30% of the original population, and >95% were viable. A second treatment with anti-Thy-1.2 + C resulted in nondetectable killing. When 4% of enriched T cells were artificially added to such an enriched B cell population, we were able to detect killing significantly above background.

T cells. To enrich for T cells, B cells were removed by two cycles of panning on anti-mouse F(ab)-coated dishes. For the first cycle, dishes were coated as described above. For the second cycle, they were coated with 200 μg/ml rabbit anti-mouse F(ab); 1 × 10^8 or 6 × 10^7 cells/5 ml/dish were plated, respectively, for the two cycles. Recovery of nonadherent cells was 20 to 25% of the original spleen cell population; 75 to 85% of these cells were killed by monoclonal anti-Thy-1.2 + C.

To further purify T cells, the nonadherent cells were positively selected on Petri dishes precoated with anti-Thy-1.2 mAb; the adherent cells, released by vigorous pipetting, were >95% viable, and >95% were killed by anti-Thy-1.2 + C. Approximately 50% of the B cell-depleted cells were recovered as the positively selected adherent (T cell) fraction; ~10 to 15% were recovered as cells nonadherent to anti-Thy-1.2-coated dishes.

Alternatively, T cells were enriched by passage over Sephadex G-10 (see below) followed by panning on an anti-F(ab)-coated dish; the nonadherent cells were used.

Sephadex G-10 treatment. Passage of spleen cells through a Sephadex G-10 column was performed according to Ly and Mishell (7). This treatment was reported by the authors to remove antibody-

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[3] Abbreviations used in this paper: KLH, keyhole limpet hemocyanin; Ar, *p*-azobenzenearsonate; CAF_1, (BALB/c × A/J)F_1.

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secreting cells as well as accessory cells.

Assay for anti-IgE in serum. Polyvinylchloride microtiter plates were coated with mAb SE20.2 (1 μg in 0.1 ml/well) overnight, then saturated with 2.5% horse serum (1). After exposure to 50 μl of the test sample for 6 h at room temperature, the wells were washed and developed with ^{125}I-labeled affinity purified goat anti-mouse Fc (100 ng in 0.1 ml). After overnight incubation at room temperature, wells were washed and counted. As a standard for the assay, we used pooled hyperimmune mouse anti-KLH-IgE antiserum whose anti-IgE content had been determined by measuring its maximum IgE-binding capacity (1).

Enumeration of IgE-secreting cells in spleen. This was carried out as described previously (8).

Conjugation of IgE to KLH. This procedure was carried out as described previously (1), using glutaraldehyde and a 1:1 weight ratio of the two proteins.

RESULTS

Transfer of adult lymphoid cells into 0- to 1-day-old recipients. Table I shows the effects of adoptive transfers of adult lymphoid cells into neonatal mice on their subsequent responses to IgE in CFA. Cells were transferred within 1 day after birth (day 0), and mice were immunized with 25 μg IgE in CFA on day 8, and with 50 μg on day 36. Mice were bled on days 35 and 50 and anti-IgE serum levels determined. Mice that received no cells (controls: experiment 1, group 1 and experiment 2, group 5) had anti-IgE titers of 6.2 to 32.2 μg/ml (mean values); titers were higher in the second bleeding. The transfer of 5×10^6 unfractionated adult spleen cells resulted in significant decreases in anti-IgE levels (group 2, both bleedings and group 6, first bleeding). In both experiments, 5×10^6 enriched B cells caused significant decreases in anti-IgE titers at days 35 and 50, whereas the same number of enriched T cells was not significantly inhibitory. In experiment 1 the T cells were purified by exposure of cells to anti-F(ab)-coated plates; the nonadherent T cells were then positively selected on an anti-Thy-1.2-coated plate (see *Materials and Methods*). In experiment 2, the T cells were not positively selected; however, cells were treated by passage over Sephadex G-10, to remove accessory cells and antibody-secreting B cells (7), followed by panning on an anti-F(ab)-coated dish; the nonadherent cells were used. Note that the B cell population in experiment 2, but not experiment 1, was passed over Sephadex G-10 before positive selection, in each case, on anti-F(ab). In summary, unfractionated adult spleen cells or B cells inhibited anti-IgE responses when administered to neonates, whereas T cells were not inhibitory.

Transfer of adult lymphoid cells into 5-day-old recipients. The experiments in groups 1 to 4 (Table I) were repeated with 5-day-old, in place of 0- to 1-day-old, recipient mice; again, 5×10^6 adult cells were transferred. Significant inhibition was not observed in any of the groups on days 35 or 50; p values were >0.2 in each case (data not shown).

Dose response of the inhibitory effect of adult spleen cells. Table II shows the inhibitory effects of varying numbers of T-depleted adult spleen cells on the anti-IgE responses of recipient neonatal mice (0 to 1 day old). T-depleted spleen cells from 9-day-old donors were also tested. Recipient mice were immunized with IgE in CFA on days 8 and 36 and bled on days 35 and 50.

Control mice that received no cells had mean anti-IgE titers of 4.9 and 20.0 μg/ml after the first and second bleedings, respectively. All mice that received adult T-depleted spleen cells failed to express anti-IgE in the first bleeding; the number of cells transferred varied from 1×10^6 to 1×10^7. Significant inhibition was also observed in the second bleeding although here a dosage effect can be seen; i.e., 1×10^6 cells were less effective than 1×10^7 cells. In contrast 5×10^6 T-depleted cells from 9-day-old donors did not have a significant inhibitory effect on

TABLE I

Effect of adoptive transfer of adult spleen cells on responses of neonatal mice to IgE in CFA[a]

Group	Treatment of Transferred Spleen Cells	Anti-IgE in Serum (μg/ml)					
		Day 35	Mean ± SEM	p[b]	Day 50	Mean ± SEM	p[b]
Experiment 1							
1	Control (no cells)	2.0, 2.8, 10.5, 13.4, 17.1, 22.3, 22.6	13.0 ± 3.2		3.5, 16.1, 19.2, 30.8, 36.4, 40.2, 80.2	32.3 ± 9.3	
2	Unfractionated	0.2, 0.2, 0.2, 0.5, 1.0, 1.6, 3.5	1.0 ± 0.5	0.011	<0.15, 0.2, 0.3, 2.8, 3.2, 6.0, 6.4	2.7 ± 1.0	0.019
3	T cells[c] (pos. selection)	0.5, 1.3, 4.0, 5.5, 6.6, 8.9, 13.2, 14.3	6.7 ± 1.8	0.105	5.2, 8.2, 10.2, 15.6, 25.5, 39.2, 40.5, 52.5	24.6 ± 6.2	0.492
4	B cells[d] (pos. selection)	<0.15, <0.15, <0.15, 0.4, 0.6, 0.6, 1.1, 5.1	1.0 ± 0.6	0.008	0.2, 0.2, 0.6, 1.3, 3.5, 4.8, 5.0, 47.7	7.9 ± 5.7	0.038
Experiment 2							
5	Control (no cells)	1.0, 1.7, 3.7, 4.7, 4.9, 5.0, 13.0, 15.7	6.2 ± 1.8		3.1, 6.9, 13.0, 13.2, 17.6, 30.0, 31.2, 56.6	21.5 ± 6.1	
6	Unfractionated	<0.15, <0.15, <0.15, 0.2, 0.2, 0.3, 0.6, 2.0	0.4 ± 0.2	0.018	<0.15, 0.2, 0.7, 1.4, 3.3, 10.5, 11.2, 23.2, 30.2	9.0 ± 3.7	0.105
7	Sephadex G-10 (nonadherent)	<0.15, <0.15, 1.4, 1.6, 1.6, 1.8, 5.0	1.7 ± 0.7	0.046	0.3, 1.3, 6.4, 7.0, 13.1, 14.9, 25.5	9.8 ± 3.3	0.120
8	Group 7, enriched T cells[e]	1.6, 2.3, 2.8, 5.8, 7.4, 8.4, 8.5, 14.0, 27.6	7.6 ± 2.6	—[g]	15.4, 16.7, 17.3, 29.7, 37.1, 41.2, 50.3, 50.5, 58.4	35.2 ± 5.4	—[g]
9	Group 7, enriched B cells[f]	<0.15, <0.15, <0.15, <0.15, 0.2, 0.5, 0.7, 3.2	0.7 ± 0.4	0.019	0.3, 0.3, 0.8, 1.2, 1.4, 3.4, 4.6, 5.9	2.2 ± 0.8	0.017

[a] Donor (CAF_1) mice were 15–18 wk old; recipients 0–1 day old. In each experiment (except controls, groups 1 and 5) 5×10^6 cells were transferred. Recipients were immunized with 25 μg IgE (mAb SE17.1) in CFA on day 8 after the transfer and with 50 μg on day 36. Mice were bled on days 35 and 50. Each value in the table represents an individual mouse.

[b] As compared to controls (group 1, expt. 1; group 5, expt. 2).

[c] Cells that were nonadherent to anti-F(ab) were positively selected on an anti-Thy-1.2-coated Petri dish.

[d] Purified by panning on an anti-F(ab)-coated Petri dish. Cells eluted from the dish were further treated with anti-Thy-1.2 + C.

[e] Sephadex G-10-nonadherent cells were further depleted of B cells by panning on an anti-F(ab)-coated Petri dish.

[f] Sephadex G-10-nonadherent cells were purified as described for group 4.

[g] The mean value exceeds that of the controls (group 5).

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TABLE II

Tolerizing effect of transfer of varying numbers of adult or perinatal T-depleted spleen cells into neonatal recipients[a]

No. of Cells Transferred	Donors: Adult (A) or Perinatal (P)[b]	Anti-IgE in serum (μg/ml)					
		Day 35	Mean ± SEM	p[c]	Day 50	Mean ± SEM	p[c]
None (control)		0.8, 1.0, 1.0, 2.2, 3.1, 5.9, 6.6, 18.4	4.9 ± 2.1		0.8, 1.5, 2.9, 14.8, 21.4, 27.9, 40.1, 50.6	20.0 ± 6.6	
1×10^7	A	0, 0, 0, 0, 0	<0.1	<0.05	0, 0, 0.1, 0.7, 1.1	0.4 ± 0.2	0.021
5×10^6	A	0, 0, 0, 0, 0, 0.2	<0.1	<0.05	0, 0, 0, 0, 0.3, 3.0	0.6 ± 0.6	0.022
2×10^6	A	0, 0, 0, 0, 0, 0.1	<0.1	<0.05	0, 0, 0.1, 0.1, 4.8, 17.3	3.7 ± 2.8	0.047
1×10^6	A	0, 0, 0, 0, 0	<0.1	<0.05	0.4, 1.5, 3.0, 6.5, 14.4	5.2 ± 2.6	0.065
5×10^6	P	0, 0.8, 0.8, 2.5, 5.8, 5.9	2.6 ± 1.1	0.36	4.9, 5.7, 8.5, 10.3, 11.4, 18.2	9.8 ± 2.0	0.18

[a] Cells were treated with anti-Thy-1.2 + C. Recipients were 0–1 day old. They were immunized with IgE in CFA as described in Table I.
[b] P donors were 9 days old.
[c] As compared to the control group.

the anti-IgE response in either bleeding (p = 0.36 and 0.18).

An experiment was carried out to determine whether exposure to IgE of T-depleted cells from 9-day-old donors rendered them tolerogenic to newborn mice. The cells were treated with lightly labeled [^{125}I]IgE (10 μg/ml) in phosphate-buffered saline at room temperature for 1 h before adoptive transfer of 5×10^6 cells into each of 8 mice on their day of birth. The mice were challenged i.p. with IgE in CFA on days 8 and 36 and bled on days 35 and 50 (as in experiments described in Table II). All mice produced significant titers of anti-IgE (0.2 to 24.9 μg/ml on day 50, with a mean of 9.5 ± 3.0 μg/ml). A control group of 8 mice received 5×10^6 T-depleted cells, not treated with IgE, from 9-day-old mice. After immunization as described above, the mean anti-IgE titer on day 50 was 12.3 ± 5.0 μg/ml. Thus, the experimental and control groups did not differ significantly; i.e., the treatment with IgE did not cause the cells to become tolerogenic. This may correlate with the observation that the cells took up very little IgE (<0.6 ng/5×10^6 cells). In contrast 5×10^6 T-depleted cells from 2- to 3-month old mice adsorbed ~5 ng of IgE when similarly treated.

Time of appearance of IgE-secreting cells. We have previously shown that IgE becomes detectable in the sera of mice at the approximate age of 3 wk (4). In view of the age-dependent tolerizing ability of B cells, we enumerated IgE-secreting cells in spleen as a function of age. Groups of 4 mice were sacrificed and tested individually at the ages of 8 to 10 days, 2 wk, 3 wk, and 4 wk. The mean values, expressed as number of IgE-secreting cells per spleen, were 0, 1 ± 1, 60 ± 12, and 90 ± 13, respectively, for the four groups. Thus, IgE-secreting cells appear at roughly the same time after birth as detectable serum IgE.

Tolerizing effect on neonates of IgE in saline. The data in Table III indicate the results of i.p. inoculation of monomeric IgE mAb (SE20.2), dissolved in saline, into mice within 24 h after birth or at the age of 5 days. All recipients were challenged at the ages of 8 and 36 days with IgE in CFA and were bled when they were 35 and 50 days old.

Inoculation of 5 to 250 μg of IgE in saline into newborn mice significantly inhibited their anti-IgE responses, as measured in either bleeding. One μg of IgE did not cause significant inhibition. In contrast, the responses of 5-day-old recipients were not inhibited by inoculation of IgE in saline (25 to 250 μg) before immunization with IgE in CFA. In an additional experiment (not shown) we inoculated 4 mice with 100 μg IgE in saline three times, on days 5, 7, and 9 after birth. Mice were challenged on days 10 and 36 with 25 μg and 50 μg, respectively, of IgE in CFA. Significant titers of serum anti-IgE were present in both bleedings (8.3 ± 1.9 and 13.8 ± 4.5 μg/ml, respectively). These were not significantly different from the control group of 5 mice (5.7 ± 2.1 and 12.5 ± 3.9 μg/ml). Controls did not receive IgE in saline.

Nature of cells rendered tolerant by administration of IgE in saline. This question was approached by immunizing 4 mice, tolerized neonatally by 250 μg of IgE in saline as described in the previous paragraph, with a KLH-IgE conjugate. Mice were challenged i.p. at the ages of 8 and 36 days with 50 and 100 μg, respectively, of the conjugate emulsified in CFA and were bled on day 50. Nontolerized controls (4 mice) were immunized by the same procedure. The mean anti-IgE responses (±SEM) of the experimental and control groups were 22 ± 8.8 and 8.4 ± 2.5 μg/ml, respectively. These results indicate that tolerance does not reside in the B cell compartment and therefore suggest that T cells are tolerized by IgE in saline. The results correspond to those obtained with normal adult mice (1).

DISCUSSION

Data discussed in the introduction indicate that adult mice are tolerant to syngeneic IgE, that the tolerance resides in T cells, and that B cells that can respond to IgE are present. Neonatal mice are responsive to unconjugated IgE in CFA until they begin to produce IgE in amounts detectable in serum (at 2 to 3 wk of age), suggesting that T cell tolerance is induced by the endogenous IgE (1, 2, 4). This delayed onset of tolerance appears quite unique and permits experimental manipulations during the neonatal responsive period.

Tolerance to IgE (administered in CFA) can be induced in neonates by i.p. inoculation of monomeric IgE mAb in saline (4). Our present experiments, showing that KLH-IgE is immunogenic in these mice, indicate that the neonatal treatment induces tolerance in T cells but not in B cells. The experiments described in Table III establish the minimum tolerizing dose (between 1 and 5 μg). Tolerance can also be induced by the adoptive transfer of 5×10^6 unfractionated, nonimmune adult splenic leukocytes; leukocytes from 10-day-old (nontolerant) mice did not induce tolerance (4). Experiments were done to identify the cell population(s) responsible for the transfer of tolerance. We found that purified nonimmune adult B cells were highly effective in transferring tolerance, whereas T cells were ineffective. Splenic B cells were positively selected on an anti-F(ab)-coated Petri dish,

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TABLE III

Induction of tolerance in newborn or 5-day-old mice by inoculation of various amounts of IgE in saline[a]

μg tolerogen (IgE mAb)	Age of Recipients (days)	Anti-IgE in serum (μg/ml)					
		Day 35	Mean ± SEM	p[b]	Day 50	Mean ± SEM	p[b]
None (control)		0.3, 1.1, 1.6, 1.7, 2.1, 2.3, 2.3, 3.4, 5.2, 7.0, 7.2	3.1 ± 0.7		2.9, 3.3, 6.1, 10.2, 17.2, 21.3, 31.8, 36.1, 36.7, 38.9, 42.1	22.4 ± 4.6	
250	0–1	0, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.2, 0.8	0.3 ± 0.1	0.003	0.4, 0.9, 1.1, 1.2, 1.5, 1.8, 2.3, 2.6	2.8 ± 1.3	0.002
100	0–1	0, 0.1, 0.1, 0.3, 0.3, 0.7, 2.7, 3.0	0.9 ± 0.4	0.017	1.2, 1.5, 1.6, 1.6, 3.6, 9.0, 10.2, 14.3	5.4 ± 1.8	0.004
25	0–1	0, 0, 0.1, 0.1, 0.1, 0.3, 0.3, 0.9	0.3 ± 0.1	0.003	0.1, 0.1, 0.7, 0.9, 0.9, 6.0, 6.1, 16.3	3.9 ± 2.0	0.002
5	0–1	0.1, 0.1, 0.3, 0.5, 0.6, 1.4, 1.6, 2.2, 3.0	1.1 ± 0.3	0.018	0.5, 0.6, 1.7, 2.4, 3.9, 5.1, 6.5, 14.8, 27.6	7.0 ± 3.0	0.011
1	0–1	0.2, 0.2, 0.3, 0.4, 0.4, 0.8, 3.9, 10.4	2.1 ± 1.3	0.46	2.6, 3.3, 4.2, 6.0, 7.5, 12.5, 21.6, 39.1	12.1 ± 4.4	0.14
250	5	1.7, 4.8, 5.1, 8.1, 8.1	5.5 ± 1.2	–[c]	5.8, 8.2, 10.0, 26.7, 34.2	17.0 ± 5.7	0.50
100	5	5.0, 7.0, 7.5, 8.5, 14.4	8.5 ± 1.6	–[c]	5.7, 12.2, 16.3, 18.3, 18.5	14.2 ± 2.4	0.13
25	5	1.8, 7.7, 10.0, 15.0, 17.7	10.4 ± 2.8	–[c]	13.5, 22.6, 24.8, 25.8, 51.8	27.4 ± 6.4	–[c]

[a] The IgE sample injected (i.p.) was mAb SE20.2. Recipients were immunized with IgE in CFA as described in Table I.
[b] As compared to the control group.
[c] The mean value exceeds that of the control group.

then treated with anti-Thy-1.2 + C. T cells were enriched by removing B cells on anti-F(ab) after passage of spleen cells through Sephadex G-10; or by positive selection on an anti-Thy-1.2-coated dish after the removal of B cells by panning on anti-F(ab). We found that passage over an anti-F(ab)-coated dish, without exposure to Sephadex G-10, left residual tolerizing activity in the enriched T cell population (data not shown), possibly because some antibody-secreting cells were not bound by the anti-F(ab). (It was necessary also to test T cells purified without the use of Sephadex G-10, because of the possibility that this treatment removes suppressor T cells).

As few as 2×10^6 adult B cells conferred tolerance to IgE when inoculated into neonatal mice. Although we as yet have no direct evidence bearing on this question, it seems possible that IgE-bearing or IgE-secreting B cells are responsible for the induction of tolerance, since we have shown that IgE can induce tolerance [(4), and present results]. If so, the amount of cell-bound IgE needed to confer tolerance must be very small. If one assumes that the normal B cells have about 5,000 FcεRII receptors per cell (9), the amount of IgE bound would be <5 ng, even if all receptors were occupied. A smaller contribution is probably made by IgE present as receptors on B cells. Kehry and Yamashita (10) have shown that IgE-antigen complexes can be internalized via the FcεRII receptor with the consequent surface presentation of peptides of the antigen. It seems possible that peptides of IgE might be presented, in association with an MHC product, through a similar mechanism and act as a tolerogen. Internalization of IgE via FcεRII has been demonstrated by Richards and Katz (11).

The fact that passage over Sephadex G-10, believed to deplete antibody-secreting cells (7), did not remove the tolerizing capacity of enriched B cells suggests the possibility that secreted antibody may not be exclusively responsible for tolerance induction; however, some differentiation into antibody-secreting cells may have occurred after the adoptive transfer.

Our results argue against a role for suppressor T cells in the maintenance of tolerance to IgE in adult mice, and are consistent with recent reports that emphasize neonatal clonal deletion of T cells (12–15) or clonal anergy (16) as important mechanisms in T cell tolerance to normal antigens or to the products of transgenes.

Another observation was the failure to induce tolerance to IgE, with lymphoid cells or with monomeric IgE in saline, administered 5 days after birth, or administered three times on days 5, 7, and 9, rather than on the day of birth. This confirms the relative sensitivity of 0- to 1-day-old mice to tolerance induction. It contrasts with the induction of tolerance at a later age (2 to 3 wk) that takes place in normal, untreated mice, coincident with the appearance of IgE in serum. A possible explanation for this apparent inconsistency is the continuous exposure to IgE that occurs after the age of 2 to 3 wk in untreated mice. It is also possible that, after day 1, passively administered IgE (or adult B cells) do not have access to all physiologically relevant sites.

It is conceivable that 5- to 10-day-old mice possess cells that actively suppress tolerance induction. This possibility can be tested by appropriate adoptive transfer experiments.

We have recently reported (8) that inoculation of IgE in saline, as well as in CFA, IFA, or alum (4), in mice between the ages of 3 and 12 days results in the induction of anti-IgE antibodies, which appear to play a major role in the prolonged inhibition of IgE synthesis or secretion caused by perinatal injection of IgE (17).

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