

# IgE ANTIBODY AND RESISTANCE TO INFECTION

## I. Selective Suppression of the IgE Antibody Response in Rats Diminishes the Resistance and the Eosinophil Response to *Trichinella spiralis* Infection\*

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High levels of IgE antibody (1–3) and marked blood and tissue eosinophilia (4–7) are two characteristics of helminth infections. Recent reports strongly suggest that eosinophils play an important role in the destruction of invading parasites (8–10). By contrast, there is at present no definitive evidence that IgE antibodies are critical to resistance against helminth or any other infections. The constant association in these parasitic diseases between eosinophilia and high reaginic titers has suggested that IgE antibodies might be responsible for the elevated eosinophilia. This hypothesis is supported by observations showing that in the presence of relevant antigens, IgE antibody triggers the release by mast cells of mediators capable of mobilizing eosinophils (11–14). However, several IgE-independent mechanisms could also regulate the eosinophils' response, and it is not known if any of these plays a major part in determining the level of blood and tissue eosinophilia during the course of a natural infection. This study addresses that question.

We examined the consequence of an in vivo depletion of the IgE antibody response on the magnitude of tissue and blood eosinophilia following oral infection of rats by the nematode *Trichinella spiralis*. Suppression of the IgE antibody response resulted in a marked reduction of tissue eosinophilia and in an abbreviated blood eosinophilia. Moreover, IgE-suppressed rats were much less resistant to infection by this nematode than their control littermates.

### Materials and Methods

*Media and Reagents.* The L15HS medium is L15<sup>1</sup> medium (Microbiological Associates, Walkersville, Md.) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mM Hepes buffer, and 10% heat-inactivated fetal calf serum (all obtained from Grand Island Biological Co., Grand Island, N. Y.). The low magnesium medium (LMM) contained (per liter): 8 g NaCl, 0.4 g KCl, 0.19 g Na<sub>2</sub>HPO<sub>4</sub>, and 0.06 g KH<sub>2</sub>PO<sub>4</sub>, 0.15 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 20 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 1 g/liter glucose, 0.1 g/liter phenol red, and amino acids, vitamins,

\* Supported by grants from the Edna McConnell Clark Foundation, the Rockefeller Foundation, and grant CA 14723 from the National Cancer Institute.

‡ Supported by training grant 5 T32 CA 09141 from the National Cancer Institute.

<sup>1</sup> Abbreviations used in this paper: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay, L15, Microbiological Associates L 15 medium (Leibovitz); LMM, low magnesium medium; OA, chicken egg albumin; PBS, phosphate-buffered saline, pH 7.1; PTS, L-α-phosphatidyl-L-serine.

and glutamine at the concentrations in Eagle's minimum essential medium (MEM; Grand Island Biological Co.), antibiotics and Hepes as above, and 1% heat-inactivated normal rat serum. The pH was adjusted to  $7.3 \pm 0.1$ .

Aliquots of a solution of L- $\alpha$ -phosphatidyl-L-serine (PTS) in a 95% chloroform:5% methanol ratio (Sigma Chemical Co., St. Louis, Mo.) were dried in air before use and resuspended in Dulbecco's phosphate-buffered saline (PBS; Grand Island Biological Co.) by sonication. The ionophore A23187 (a gift of the Eli Lilly Co., Indianapolis, Ind.), dissolved in dimethyl sulfoxide at a concentration of 10 mg/ml, was diluted in LMM immediately before use.

**Animals.** 14-d-pregnant female Wistar/Lewis rats were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. Hooded Lister rats were from our own colony. No more than 10 newborn rats were kept per litter.

**Antisera.** Anti- $\epsilon$  chain antisera and control sera: purified rat IgE and rat  $\epsilon$ -chain were a gift of Dr. H. Metzger (National Institutes of Health, Bethesda, Md.). Groups of four rabbits received injections into the footpads and the back either with 1 mg IgE or  $\epsilon$ -chain emulsified in complete Freund's adjuvant (CFA; H37 Ra, Difco Laboratories, Detroit, Mich.). The animals were boosted 2 wk later and thereafter at 3-mo intervals with 200–500  $\mu$ g of IgE or  $\epsilon$ -chain emulsified in CFA. Sera from rabbits immunized with the  $\epsilon$ -chain were not pooled with sera from rabbits immunized with the whole IgE molecule. Control rabbit sera were obtained by priming and repeated boosting of three rabbits with CFA. The IgG fractions of anti-IgE, anti- $\epsilon$ , and control sera were extracted by  $\text{Na}_2\text{SO}_4$  precipitation; anti-light chain activity was removed by extensive adsorption on rat IgG-coupled Sepharose. The specificity of the antisera for the  $\epsilon$ -chain of the IgE antibody molecule was assessed by Ouchterlony technique and immunoelectrophoresis. Thereafter, the sera was sterilized by filtration, and the protein concentration of the globulin fractions was adjusted to 10 mg/ml. All sera were kept at  $-20^\circ\text{C}$ .

Goat anti-rat IgG2a, goat anti-rat IgG2c, rabbit anti-rat IgG2b, rabbit anti-rat IgM, sheep anti-rat IgG1, peroxidase-conjugated IgG fraction of goat anti-rabbit IgG, rabbit anti-goat IgG, and rabbit anti-sheep IgG were purchased from N. L. Cappel Laboratories, Inc., Cochranville, Pa.

**Treatment Protocol for Suppression of the IgE Antibody Response.** Neonatal rats were randomly assigned to one of two treatment groups within their respective litters. Treatment consisted of intraperitoneal injections of anti- $\epsilon$  chain (globulin fraction) or control sera (globulin fraction). Rats were given 0.05 ml of serum every 5 d for the 1st 2 wk of life. This volume was gradually increased up to 0.25 ml during the next 5 wk. Then the rats were infected with *T. spiralis* larvae and injected with 0.3 ml of serum every 4 d.

#### Antibody Titration

**IgE ANTIBODY.** IgE anti-*T. spiralis* antibodies were titrated by passive cutaneous anaphylaxis method as previously described (15). Rats were challenged 48 h after skin sensitization with 1 mg of an extract of *T. spiralis* (muscle stage) larvae.

**IgM, IgG1, IgG2a, 2b, AND 2c ANTIBODIES.** IgM, IgG1, IgG2a, 2b, and 2c anti-*T. spiralis* antibodies were titrated by enzyme-linked immunosorbent assay (ELISA) (16). For this assay, microtiter plates (Cooke 1-220-295; Cooke Engineering Co., Alexandria, Va.) were coated with 100  $\mu$ g/ml of *T. spiralis* extract in carbonate buffer, pH 9.4. Before use, plates were washed four times with 0.05% Tween 20 (Fisher Scientific Co., Pittsburgh, Pa.) phosphate-buffered saline (PBS). Then, control and *T. spiralis*-infected rat sera, diluted in 1% bovine serum albumin (BSA)-PBS, were added. After 1 h incubation at  $37^\circ\text{C}$ , plates were washed four times and a 1:600 dilution in 1% BSA-PBS of sheep anti-rat IgG1, goat anti-rat IgG2a, goat anti-rat IgG2c, rabbit anti-rat IgG2b, or rabbit anti-rat IgM was added. After a further 1-h incubation at  $37^\circ\text{C}$ , the plates were washed, and peroxidase-conjugated goat anti-rabbit IgG or rabbit anti-goat IgG or rabbit anti-sheep IgG, diluted 1:500 in Tween PBS containing 1% BSA and 1% normal rat serum, were added. The plates were then incubated for 1 h, washed, and the peroxidase substrate was added. The reaction was stopped with 1  $\text{NH}_2\text{SO}_4$  and read at 490 nm. Results are expressed as the reciprocal of the highest dilution of immune sera producing the same reaction as a 1:50 dilution of a pool of normal rat sera.

*In Vitro Mast Cell Degranulation Assay*

**ISOLATION AND RADIOLABELING OF RAT MAST CELLS.** Peritoneal cells were harvested by washing the peritoneal cavity with 15 ml L15HS. Mast cells, 80–95% pure, were obtained as previously described (17). The yield from each rat ranged from 1 to  $5 \times 10^6$  mast cells. To monitor mast cell mediator release and cytolysis simultaneously, double-labeling<sup>2</sup> with [<sup>14</sup>C]-serotonin and <sup>51</sup>Cr was employed. These markers selectively label two discrete intracellular compartments of the mast cells: [<sup>14</sup>C]serotonin concentrates in histamine-containing granules, whereas <sup>51</sup>Cr is largely excluded from these granules.  $10^4$  double-labeled mast cells released 1,500–4,800 cpm of <sup>51</sup>Cr, and 1,800–4,400 cpm of <sup>14</sup>C upon freezing overnight at  $-15^\circ\text{C}$  and thawing.

**IN VITRO SENSITIZATION OF MAST CELLS.** The desired sensitizing serum was added (1:10) to double-labeled mast cells in LMM, and the cells were incubated at  $37^\circ\text{C}$  for 2 h with occasional mixing. Cells were then washed three times at room temperature and resuspended in LMM. Control cells were treated with LMM instead of serum.

**ISOTOPE RELEASE ASSAY.**  $3\text{--}5 \times 10^3$  double-labeled mast cells and PTS (7.5  $\mu\text{g}/\text{ml}$ ) were added to 12- $\times$  75-mm plastic tubes (Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, Calif.), gently mixed, and warmed for 5 min at  $37^\circ\text{C}$ . Putative secretagogues were then added, and the tubes were incubated at  $37^\circ\text{C}$  for 2 min (when A23187 was the secretagogue) or for 15 min (when anti-IgE or *T. spiralis* extract were the secretagogues). Control tubes received an aliquot of LMM instead of a secretagogue. The final reaction mixtures had a volume of 0.15 ml. The release reaction was terminated by removing tubes to ice, adding 1 ml cold saline, and gently vortexing.

**DETERMINATION OF PERCENTAGE ISOTOPE RELEASE.** Tubes were centrifuged at 800 *g* for 10 min at  $4^\circ\text{C}$ . <sup>14</sup>C and <sup>51</sup>Cr released in the supernate were counted simultaneously with a Beckman model LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Results are expressed as percentage of corrected isotope release:

$$\% \text{ corrected isotope release} = 100 \times \frac{(\text{cpm released in sample}) - (\text{cpm released in control})}{(\text{cpm released by freezing and thawing})}$$

**Leukocyte Determination.** Blood samples from rats were obtained by cutting the tail. Total leukocyte counts were determined on a 1:20 dilution of blood in 0.03 M acetic acid, 0.01% crystal violet diluent using an improved Neubauer counting chamber. Peripheral blood eosinophils were counted in a 1:10 dilution in Discomb's fluid (18) using a Fuchs-Rosenthal counting chamber. Differential leukocyte counts were performed on smears treated with Wright's stain; 500 cells were counted on each smear.

**Histology.** Control and IgE-suppressed rats were orally infected with *T. spiralis* larvae. Rats were sacrificed between day 28 and 33 because the inflammatory reaction around the muscle larvae reaches its maximum during this period. Diaphragms were excised and cut into six pieces. Pieces were fixed immediately in Bouin's Holland fixative (19), dehydrated in alcohol, and embedded in paraffin. 5- $\mu\text{m}$  sections were stained with either hematoxylin and eosin or with the Litt modification of Dominici's stain (20). The eosinophil infiltration was determined by counting the number of eosinophils present in the inflammatory reaction around the encysted larvae. One section of each piece of diaphragm was examined. All encysted larvae were scored on each section. A total of 40–70 larvae/rat were counted.

*Parasitological Procedures*

**INFECTION.** Rats were starved for 12 h and then infected via the gastrointestinal route with a 3-ml syringe to which a polystyrene tubing was connected. Great care was taken to inject the larvae directly into the stomach and not into the lungs.

**LARVAL RECOVERY.** *T. spiralis* larvae were recovered by pepsin digestion (21) of the carcasses of Wistar/Lewis rats infected 1–2 mo before with 20–25 larvae/g body wt. In experiments requiring quantitative larval recovery, the entire carcass of individual animals with the

<sup>2</sup> Parker, W. L., and E. Martz. Calcium ionophore A23187 as a secretagogue for rat mast cells. Does it bypass inhibition by flux blockers? Manuscript submitted for publication.

exception of head and feet was digested, and larvae were carefully filtered and concentrated to 50 ml, and 10 100- $\mu$ l samples were counted.

**T. SPIRALIS EXTRACT.** *T. spiralis* larval antigens were prepared by repeated freezing and thawing of muscle stage larvae in PBS. After centrifugation at 10,000 *g* for 1 h, the protein concentration of the supernatant fluid was adjusted to 10 mg/ml, and the extract was stored at  $-20^{\circ}\text{C}$ .

## Results

*IgE Antibody Response to Ovalbumin (OA) in Rats Given Anti- $\epsilon$  Chain Antibodies (Table I).* Hooded Lister rats were injected with the globulin fraction of control or anti-rat  $\epsilon$ -chain rabbit sera from birth until 5 wk of age. 1 or 3 wk after the last serum injection, they were primed with 10  $\mu$ g ovalbumin in *Bordetella pertussis* adjuvant and boosted 15 d later. As is shown in Table I, neither primary nor secondary IgE anti-OA antibody response was detectable in rats given anti- $\epsilon$  chain antibodies. In contrast, rats given control sera mounted a significant IgE anti-OA antibody response.

TABLE I  
*IgE Antibody Response to Ovalbumin in Rats Given Anti- $\epsilon$  Chain Antibodies*

Interval between treatment and priming	IgE anti-ovalbumin antibody (PCA) titer*			
	Control rats		IgE-suppressed rats	
	Day 14	Day 32	Day 14	Day 32
<i>wk</i>				
1	30 $\pm$ 10	160 $\pm$ 50	<5	<5
3	160 $\pm$ 20	320 $\pm$ 50	<5	<5

Hooded Lister rats were injected from birth until 6 wk of age with rabbit anti-rat  $\epsilon$ -chain antibodies or rabbit control immunoglobulins as described in Materials and Methods. Four rats in each group were primed (day 0) with a mixture of 10  $\mu$ g chicken ovalbumin and  $10^{10}$  *Bordetella pertussis* organisms 1 or 3 wk after the last antiserum injection. Animals were boosted 15 d later (day 15) with 10  $\mu$ g ovalbumin in saline.

\*  $\pm$ SE.

TABLE II  
*Specific Suppression of the IgE Antibody Response in T. spiralis-infected Rats*

	Anti- <i>T. spiralis</i> antibody titer*					
	Control rats			IgE-suppressed rats		
	Day 15	Day 25	Day 33	Day 15	Day 25	Day 33
IgE	20 $\pm$ 10	80 $\pm$ 40	360 $\pm$ 60	<2	<5	<5
IgM	NDc‡	NDc	880 $\pm$ 200	NDc	NDc	1,000 $\pm$ 200
IgG1	500 $\pm$ 150	2,200 $\pm$ 400	1,200 $\pm$ 300	600 $\pm$ 200	5,000 $\pm$ 1,000	1,400 $\pm$ 200
IgG2a	300 $\pm$ 150	1,400 $\pm$ 160	1,400 $\pm$ 300	400 $\pm$ 100	1,600 $\pm$ 360	2,100 $\pm$ 300
IgG2b	300 $\pm$ 120	1,100 $\pm$ 200	1,600 $\pm$ 250	300 $\pm$ 150	1,800 $\pm$ 260	2,000 $\pm$ 400
IgG2c	300 $\pm$ 140	1,200 $\pm$ 200	2,100 $\pm$ 300	250 $\pm$ 60	2,600 $\pm$ 860	3,000 $\pm$ 800

Wistar/Lewis rats were injected from birth until the end of the experiment with rabbit anti-rat  $\epsilon$ -chain antibodies or rabbit control immunoglobulins (see Materials and Methods). At 2 mo of age, 12 rats in each group were orally infected with *T. spiralis* larvae (4 larvae/g of body wt) and bled 15, 25, and 33 d later. Anti-*T. spiralis* antibodies were titrated as indicated in Materials and Methods.

\*  $\pm$ SE.

‡ NDc, not detectable.

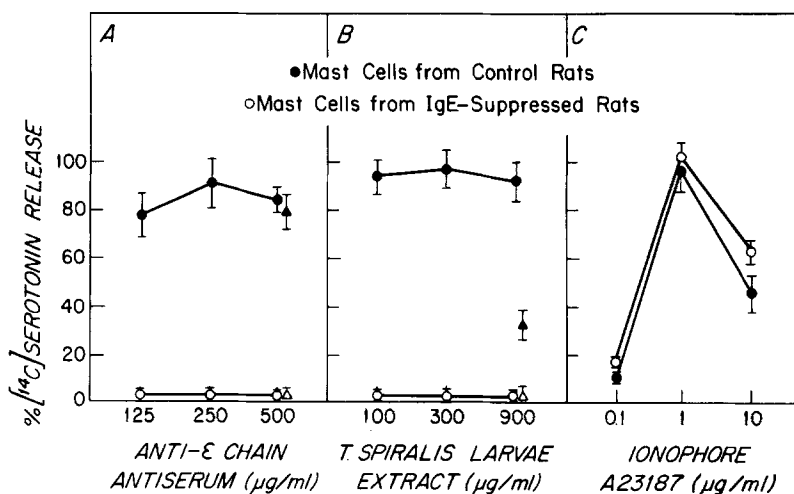


FIG. 1. Failure to detect IgE antibodies on peritoneal mast cells recovered from IgE-suppressed rats. Control or IgE-suppressed Wistar/Lewis rats were orally infected with *T. spiralis* larvae (7 larvae/g of body wt) and sacrificed 28 d later. Mast cells were harvested from the peritoneal cavities and their susceptibility to degranulation by anti- $\epsilon$  antibodies, *T. spiralis* extract, or ionophore A23187 was assayed as described in Materials and Methods. Mast cells from IgE-suppressed rats were also sensitized in vitro with sera from 4-wk-infected control ( $\blacktriangle$ ) or IgE-suppressed rats ( $\triangle$ ) and assayed for degranulation by anti- $\epsilon$  antibodies or *T. spiralis* extract. The percentage of [ $^{14}$ C]-serotonin released in the absence of secretagogue ranged from 5 to 10%.  $^{51}$ Cr release did not exceed 7% when anti- $\epsilon$  chain antibodies or *T. spiralis* extract were the secretagogue nor 16% when A23187 was the secretagogue. This shows that [ $^{14}$ C]serotonin release is due to mast cell degranulation and not to cytolysis. Each point represents the mean  $\pm$  SE of duplicate determinations with mast cells from three different rats.

*Specific Suppression of the IgE Antibody Response in T. spiralis-infected Rats (Table II and Fig. 1).* Preliminary experiments showed that the injection of anti- $\epsilon$  antibodies must be continued during the course of the *T. spiralis* infection in order to maintain total suppression of the IgE antibody response. Therefore, rats were treated with injections of anti- $\epsilon$  chain antibodies from birth until the end of the experiments. Control rats were injected with the globulin fraction of a pool of sera from rabbits primed and boosted with CFA. During the course of this study, 300 rats were given anti- $\epsilon$  antibodies,  $80 \pm 10\%$  of which survived the treatment. The percent survival was the same in rats given control antibodies. The anti-*T. spiralis* antibody response during the course of the infection is shown in Table II. In 10 separate experiments, no IgE antibody could be detected at any time in the rats given anti- $\epsilon$  antibodies. In contrast, IgE levels in control rats reached significant levels after infection. The suppression was specific for the IgE antibody class because the anti-*T. spiralis* IgM, IgG1, 2a, 2b, and 2c antibody levels were not lower in IgE-suppressed rats than in control rats.

To confirm the absence of IgE antibodies in anti- $\epsilon$ -treated rats, peritoneal mast cells were recovered 3 wk after infection, washed to remove bound IgG2a antibodies (22), and tested for susceptibility to degranulation by anti- $\epsilon$  antisera or by *T. spiralis* extracts (Fig. 1a and b). Mast cells from control rats were easily degranulated under these conditions. By contrast, no degranulation occurred in mast cells from anti- $\epsilon$  sera-treated rats. Capacity of the mast cells to degranulate was shown in experiments where mast cells from both groups of rats degranulated during incubation with the

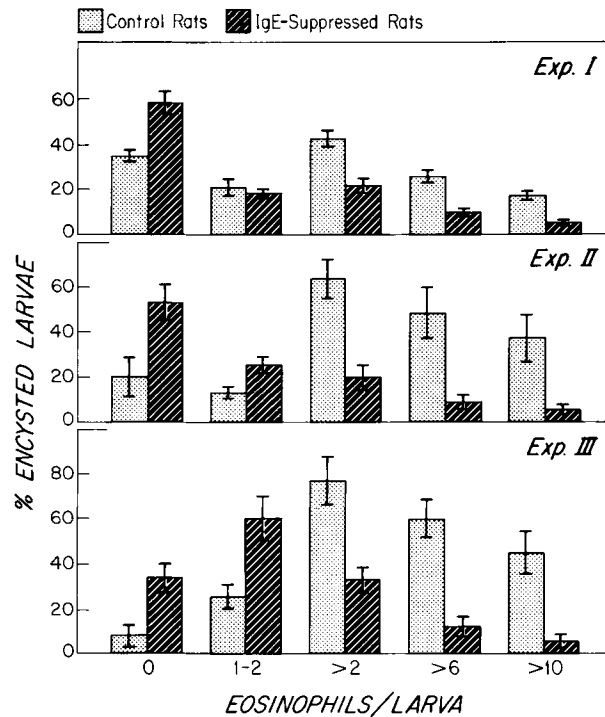


FIG. 2. Tissue eosinophilia in control and IgE-suppressed rats infected with *T. spiralis*. Control and IgE-suppressed Wistar/Lewis rats were infected with 4, 7, and 11 *T. spiralis* larvae/g of body wt (experiments 1, 2, and 3, respectively). The animals were sacrificed 30 (experiment 1), 33 (experiment 2), 28 (experiment 3) d later, the diaphragms were excised, fixed, and stained, and the eosinophilia around the nurse cells determined as indicated in Materials and Methods. Results are expressed as the percentage of *T. spiralis* containing nurse cells surrounded by an inflammatory reaction containing 0, 1, or 2, >2, >6, or >10 eosinophils. Each bar represents the mean ( $\pm$ SE) of determinations performed on the diaphragms of six rats. Statistical analysis using a paired *t* test has been performed to compare tissue eosinophilia in control vs. IgE-suppressed rats. In these three experiments for 0 eosinophil:  $P < 0.05$  (Experiments 1, 2, and 3), for 1-2 eosinophils:  $P > 0.05$  (experiment 1),  $P = 0.04$  (experiments 2 and 3), for >2, >6, >10, eosinophils:  $P < 0.001$  (experiments 1, 2, and 3).

ionophore A23187 (Fig. 1c). Moreover, *in vitro* sensitization of mast cells from IgE-suppressed rats with sera obtained from 4-wk-infected control rats restored their susceptibility to degranulation by anti- $\epsilon$  antibodies or *T. spiralis* antigens (Fig. 1a and b, triangles). Such sensitization could not be achieved with sera obtained from 3- or 4-wk-infected IgE-suppressed rats.

*Tissue Eosinophilia around Encysted Larvae in Control and IgE-suppressed Rats* (Fig. 2). The intensity of eosinophil infiltration around larvae encysted in the diaphragms of control and IgE-suppressed rats was examined in three experiments. The intensity of the inflammatory reaction around the nurse cells (larva-containing muscle cell) varies greatly from one larva to another, ranging from a multilayer inflammatory focus, mainly mononuclear cells, to few cells scattered at the periphery of the nurse cell. Although more larvae were present in the diaphragms of IgE-suppressed rats, fewer eosinophils were seen among the cells surrounding the larvae in the diaphragms of these IgE-suppressed animals (Fig. 2). A large fraction (26-60%) of

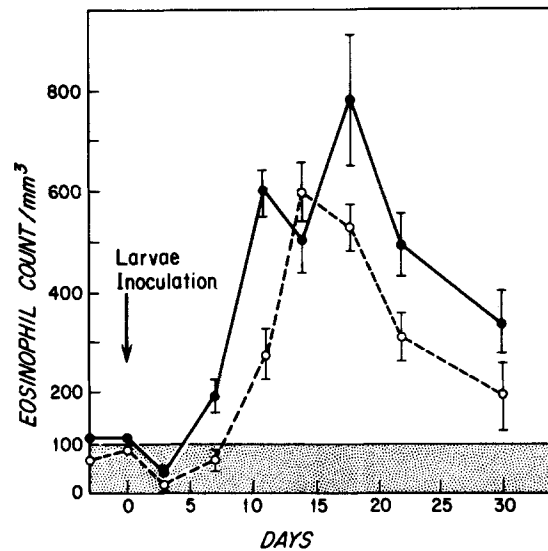


FIG. 3. Blood eosinophilia in control and IgE-suppressed rats following infection by *T. spiralis*. Control (●) or IgE-suppressed (○) rats were infected with 7–9 *T. spiralis* larvae/g of body wt at 6 wk of age. Cell counts were monitored as described in Materials and Methods. Blood eosinophilia is expressed as the mean  $\pm$ SE of the eosinophil counts from 26 rats from three separate experiments. Normal eosinophil levels, in the absence of infection, varied between 75–100 eos/mm<sup>3</sup>. Differences in eosinophil counts between control and IgE suppressed rats were significant ( $P < 0.05$ ) on day 8, 11, 22, and 30.

TABLE III

Total and Differential Blood Leukocyte Counts After *T. spiralis* Infection in IgE-suppressed and Control Rats

Days after inoculation	Mean leukocyte counts/mm <sup>3</sup>					
	Control rats			IgE-suppressed rats		
	Total	Neutrophil	Eosinophil	Total	Neutrophil	Eosinophil
0	10,100	1,510	100	9,500	1,560	90
7	13,000	2,080	200	11,000	1,870	60
10	13,500	2,560	500	11,500	2,870	190
14	12,500	2,500	500	13,000	2,900	600
18	14,000	2,500	800	12,000	1,800	450
25	14,000	1,960	450	11,500	1,610	180

The results reported here correspond to experiments presented in Fig. 4. Total and differential leukocyte counts were monitored as indicated in Materials and Methods.

the larvae in the diaphragms of control rats were surrounded by more than six eosinophils, and among these, 17–46% attracted a large number of eosinophils (10–200). By contrast, no eosinophils or very few (1–2 eosinophils) were observed around 70–90% of the larvae in the diaphragms of IgE-suppressed rats. Few inflammatory foci (9–12%) contained more than six eosinophils, and the large eosinophil infiltrations seen in control rats were rarely seen in the diaphragms of IgE-suppressed rats (3–6%).

*Blood Eosinophilia in Control and IgE-suppressed Rats (Fig. 3).* The finding that fewer eosinophils are attracted to larvae in tissue of IgE-suppressed rats led us to follow the

pattern of blood eosinophilia in control and IgE-suppressed rats. In eight experiments, three of which are presented in Fig. 3, both IgE-suppressed and control rats mounted a significant blood eosinophil response to *T. spiralis* infection. Blood eosinophilia appeared earlier and lasted longer in control than in IgE-suppressed rats, but there was no consistent difference in the peak response in these animals.

In most experiments, suppression of IgE antibody response did not affect total leukocyte or neutrophil counts as a response to infection (see Table III).

*IgE-suppressed Rats Are More Susceptible to T. spiralis Infection (Table IV).* Trichinella larvae encysted in muscles were recovered from carcasses of IgE-suppressed and control rats 3–5 wk after infection. In four out of five experiments (Table IV), the number of muscle stage larvae recovered from IgE-suppressed rats was two to three times higher than the number of larvae obtained from control rats. The variation observed between individuals in a representative experiment is shown in Fig. 4.

### Discussion

The results presented here support three conclusions. First, isotype-specific suppression of IgE antibody response can be achieved in rats by the injection of rabbit anti-

TABLE IV  
*Susceptibility of Control and IgE-suppressed Rats to T. spiralis Infection*

Experiment	Animals per group	Inf. dose larvae/g	Day of recovery	Larvae recovery/rat*		
				Control rats	IgE-suppressed rats	P
1	6	2	36	2,700 ± 900	14,000 ± 3,500	0.04
2	12	4	33	25,000 ± 2,600	61,000 ± 7,800	0.008
3	6	6	25	18,500 ± 2,000	60,500 ± 2,800	0.01
4	10	11	29	79,000 ± 6,000	160,000 ± 15,000	0.04
5	4	15	20	8,000 ± 2,000	14,000 ± 3,000	0.1

Control and IgE-suppressed rats (see Materials and Methods) were orally infected between 6–8 wk of age with *T. spiralis* larvae. Rats were killed 20–36 d later, and muscle stage larvae were recovered by pepsin digestion.

\* ±SE.

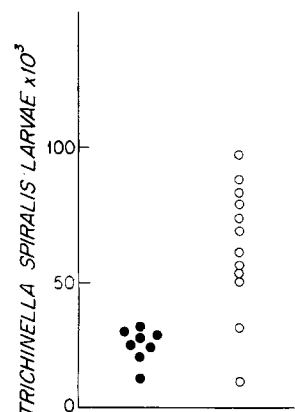


FIG. 4. Susceptibility of control (●) and IgE-suppressed (○) rats to *T. spiralis* infection: variations in larval recovery between individual rats. This experiment corresponds to experiment 2, Table III; only larval recovery from rats killed and digested the same day under identical conditions have been represented. Each point represents the number of larvae recovered from one individual rat.



rat  $\epsilon$ -chain antibodies. Infection with *T. spiralis*, which in normal rats results in strong stimulation of IgE synthesis, does not overcome this suppression if injections of the anti-rat  $\epsilon$ -chain antibodies are continued during the course of the infection. Second, suppression of the IgE antibody response results in a marked reduction of tissue eosinophilia occurring around *T. spiralis* larvae that have invaded striated muscle. Although blood eosinophilia reaches normal peak levels, it is delayed and terminates earlier in IgE-suppressed rats than in control rats. Third, IgE-depleted rats are more susceptible to *T. spiralis* infection than their control litter mates, as is shown by the fact that two to three times more larvae were found encysted in muscles of IgE-suppressed rats.

The suppression of the IgE response in rats injected with rabbit anti-rat  $\epsilon$ -chain antibodies is demonstrated by the absence of detectable specific IgE antibodies in the sera of these rats after immunization with ovalbumin or after infection with *T. spiralis*. Moreover, IgE antibodies could not be detected on the surface of mast cells obtained from IgE-suppressed and *T. spiralis*-infected rats. This suppression was selective since levels of IgM, IgG1, IgG2a, IgG2b, and IgG2c anti-*T. spiralis* antibodies were similar in IgE suppressed and control rats.

Injections of anti- $\mu$  chain antibodies in mice and rats suppress production of most classes of antibody (23–26), probably by neutralizing the common  $\mu^+$ B cell precursors. Selective suppression of certain allotypes has been achieved by perinatal exposure to maternal antipaternal allotype antibodies (27) or by neonatal injections of antibodies against certain allotypic specificities (28, 29). The selective suppression of the IgE response by anti- $\epsilon$  chain antibodies is shown here for the first time. Possibly, neonatal injections of anti- $\epsilon$  chain antibodies result in the development of isotype-specific suppressor cells (15, 30–32). However, it is unlikely that such suppressor cells could account totally for the suppression of the IgE antibody response after *T. spiralis* infection, because helminthic infections strongly stimulate IgE-specific B cell activity (33, 34), usually overcoming most negative regulations exerted on IgE antibody synthesis. It is more likely that anti- $\epsilon$  chain antibodies block the differentiation or the secretion of IgE-producing B cells by direct interaction with  $\epsilon$ -chain determinants on the surface of IgE-producing cell precursors.

Immune complexes generated by repeated infections of foreign material may interfere with the eosinophil response and with host immunity. However, neonatal injections should induce a state of tolerance against most of the antigenic determinants on rabbit immunoglobulins and likely should block the differentiation of IgE B cells, thus preventing the formation of IgE anti-IgE immune complexes. Moreover, two experiments carried out with untreated animals infected with *T. spiralis* showed that blood and tissue eosinophilia as well as larvae recovery were similar in untreated animals and in rats given control immunoglobulins (data not shown).

Tissue invasion by the newborn larvae occurs and is terminated at the same time in both normal and IgE-suppressed rats (A. J. Dessein et al., manuscript in preparation). Therefore, results presented in Fig. 3 show the magnitude of the eosinophil infiltration around larvae which encysted in the diaphragms of both groups of rats at a comparable time. Our observation that the number of eosinophils in the tissues around encysted larvae is diminished in IgE-suppressed rats directly supports the hypothesis that mast cell mediators such as chemotactic factor (11–14, 35–37), released

following immediate hypersensitivity reactions, play an important part in recruiting eosinophils into the tissues (38–40).

Several eosinophil chemotactic factors are generated independently of IgE antibodies during the course of a *T. spiralis* infection. Those include lymphokines (41), complement split products (42, 43), and even mast cell mediators, the release of which can also be triggered by Ig2a antibodies or by anaphylatoxins. These factors, released by IgE-independent mechanisms, may account for the residual eosinophil infiltration seen around the larvae in IgE-suppressed rats. However, a major conclusion from this study is that tissue eosinophilia in *T. spiralis*-infected rats is highly IgE dependent. This central role of IgE antibodies is likely to be due to their unique ability to bind and to remain firmly bound to mast cells for a long time.

This report also shows that blood eosinophilia in IgE-suppressed rats is reduced in duration, appearing and terminating earlier than in control rats. Whether the depression of blood eosinophilia and the reduction of tissue eosinophilia are related phenomena is not established in this study. Reports by others (44–45) suggest that blood eosinophil counts may be directly related to the level of tissue eosinophilia when massive accumulation of eosinophils occur in tissues consecutively to widespread mast cell degranulation.

IgE-suppressed rats are much less resistant to *T. spiralis* infection than control animals. This is the first direct evidence that IgE antibodies may be critical to immune protection against certain parasites. This aggravated susceptibility to *T. spiralis* infection might be a consequence of the reduction of the early eosinophil response. As shown by Grove et al. (46) in eosinophil-depleted animals, newborn larvae are vulnerable to attack by eosinophils when they migrate from the gut to the muscle cells. This migration occurs in Wistar/Lewis rats (A. J. Dessein et al., manuscript in preparation) between day 5 and 11 precisely when IgE-suppressed rats fail to mount a significant blood eosinophilia.

Other IgE-dependent mechanisms could also be involved in the destruction of the *T. spiralis* larvae. Capron et al. (47–48) reported the killing of *Schistosoma mansoni* larvae by IgE-armed macrophages. Although it has not been demonstrated that this observation is relevant to an in vivo immunity, it suggests a direct involvement of IgE antibodies in the cell-mediated destruction of certain parasites. Depletion of the IgE antibody response may also affect the expulsion (or the fecundity) of adult *T. spiralis* worms: like IgE antibody synthesis, this expulsion is strictly T cell dependent (49); moreover, it is accelerated by transferring mesenteric B and T lymph node cells and/or sera from 10–16-d-infected rats (50–52) and follows an impressive increase in the number of intestinal mast cells (53). Preliminary experiments show that the difference in larval recovery observed between control and IgE-suppressed rats is detectable as early as 14 d after injection, confirming that anti-*T. spiralis* immunity is impaired at an early stage in IgE-suppressed rats. Experiments are presently being undertaken to identify the IgE-dependent immune mechanism(s) acting against *T. spiralis* adult worms and/or *T. spiralis* newborn larvae.

Finally, the conclusions presented here support the hypothesis that IgE-sensitized mast cells, located at the portal entry of helminths, below the epithelial and epidermal surfaces and around endothelial cells, are essential alert units of the immune host. Triggering of these units by the invading parasite immediately initiates the local

recruitment of cells such as eosinophils able to damage and kill the parasite before it has time to evade the immune system.

### Summary

Selective suppression of the total IgE antibody response has been achieved in rats by injection of rabbit anti-rat  $\epsilon$ -chain antibodies. This IgE-specific suppression was maintained during the course of a natural infection by the nematode *Trichinella spiralis*. Depletion of the IgE antibody response resulted in a marked reduction of the number of eosinophils attracted to the *T. spiralis* larvae encysted in striated muscle. Blood eosinophilia following *T. spiralis* infection, although reaching normal peak levels, was abbreviated in IgE-suppressed animals. Moreover, IgE-depleted animals were more susceptible to the infection; they harbored two to three times more larvae encysted in their muscles than their control litter mates.

We gratefully acknowledge the excellent assistance of Kimeri Brown. We thank Doctor Baruj Benacerraf for his support and his helpful advice during this study. We also thank Doctors Ronald Germain, Eric Martz, Jim Byron, and Alan Sher for their criticisms and discussions.

*Received for publication 14 October 1980.*

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