Protective immunity and eosinophilia in IgE-deficient SJA/9 mice infected with *Nippostrongylus brasiliensis* and *Trichinella spiralis*

(nematode/parasite)

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ABSTRACT   To elucidate the roles of IgE antibody in host responses to helminth infection, selective IgE-deficient SJA/9 mice were infected with the nematode parasites *Nippostrongylus brasiliensis*, which elicits remarkable potentiated IgE production, and *Trichinella spiralis*, which induces strong anti-*Trichinella* IgE antibody production in normal mice. The kinetics of blood eosinophilia, worm burden after primary infection, and resistance to secondary infection in SJA/9 mice were the same in both infections as those in congenic SJL/J mice used as an IgE-producing control. These results indicate that the host responses examined here operate under IgE-independent mechanisms.

Enhancement of IgE production (1, 2) and eosinophilia in tissues and blood (3) are prominent features in hosts with helminth infections. It has been reported that IgE antibody has a function to induce eosinophilia (4) and protective immunity (5, 6).

In the course of study on IgE regulation in SJL/J mice (7), we have established that SJA/9 mice are congenitally deficient in IgE production (8). The SJA/9 mouse is an allotype congenic strain of SJL/J, whose IgG heavy-chain gene is replaced by the corresponding gene from BALB/c mice (9). The mechanisms of IgE deficiency in SJA/9 mice appear to relate to a defect of a subset of T cells that seems to induce interleukin 4 (10, 11). The defect seems to impair the induction of Fc_ε receptor-bearing lymphocytes (12). The discovery of mice congenitally deficient in IgE prompted us to use these mice to examine the roles of IgE in helminth infections.

Two nematode parasites were used. The first was *Nippostrongylus brasiliensis* (Nb), which elicits a striking elevation in total IgE levels in the serum of normal mice; this IgE results from polyclonal IgE B-cell activation and is not specific for Nb antigens (13, 14). In contrast, the other nematode, *Trichinella spiralis* (Ts), induces Ts antigen-specific IgE antibody production in normal mice (15). In the present report, we describe the kinetics of eosinophilia and worm burden following infection with Nb or Ts in IgE-deficient SJA/9 mice and in IgE-producing SJL/J mice.

MATERIALS AND METHODS

Infection of Mice with Parasites. SJA/9 and SJL/J mice were obtained from L. A. Herzenberg (Stanford University, Stanford, CA) and bred at Ohmura Laboratory Animals (Kanagawa, Japan). The mice were injected subcutaneously with 750 third-stage larvae of Nb (16) or inoculated orally with 100 infective larvae of Ts.

Measurement of Total IgE. Total IgE levels in the serum were measured by ELISA using monoclonal rat anti-mouse

IgE antibodies produced by ourselves (17, 18). Immuron II plates (Dynatech, Alexandria, VA) were coated with 50 μl of anti-mouse IgE monoclonal antibody (HMK 12) and blocked with 1% bovine serum albumin/phosphate-buffered saline (PBS). After application of unknown samples or standards, biotinylated monoclonal anti-mouse IgE antibody (6HD5), which recognizes an epitope on the IgE molecule unrecognized by HMK 12, was added. Avidin/peroxidase was added; this was followed by addition of H_2O_2 and the substrate *p*-phenylenediamine. The incubation period for each step was 1 hr and three washings with 0.05% Tween 20/PBS were performed after every step. The colorimetric reaction was measured at 490 nm.

Passive Cutaneous Anaphylaxis (PCA). Anti-Nb and anti-Ts IgE antibodies were detected by using the PCA reaction (19, 20). For each serum, 0.1-ml aliquots of a series of dilutions were injected intradermally into normal Wistar rats. The rats were challenged intravenously with 1–4 mg of worm antigens in 1 ml of 0.5% Evans blue 2 hr after the intradermal injections. The reaction was examined 30 min after challenge. Titer was expressed as the highest dilution eliciting a reaction. Nb antigen was a soluble extract of Nb adult worms, whereas Ts antigen was a soluble extract of muscle larvae. Worms were washed extensively before extracts were made. The worms were homogenized and dialyzed against borate-buffered saline. The antigens were obtained after centrifugation at 10,000 rpm for 30 min at 4°C.

Eosinophil Counting. The number of eosinophils in peripheral blood was counted under a microscope following staining with Hinkelman solution.

Worm Burden. To count Nb adult worms, intestines were removed from the mice and the number of adult worms was directly counted. For Ts counting, mice were sacrificed and skinned. After removal of visceral organs, the remaining tissue was cut into small pieces with scissors and digested in 200 ml of 0.5% pepsin at 37°C for 2–4 hr until the pieces of muscle were not detectable (21). The bones were removed from the suspension through a steel mesh. The larvae were collected by sedimentation in standing tubes. After several washings with saline, the larvae were suspended in 5 ml of saline. The number of larvae was counted under a microscope in five samples from each suspension.

RESULTS

Nb Infection. Mice were injected subcutaneously with 750 third-stage larvae of Nb. In SJL/J mice, no circulating IgE could be detected (<10 ng/ml) before Nb infection. However, following Nb infection the level of total IgE reached a peak after 2 wk but decreased thereafter (Fig. 1a). In contrast, IgE was not detected at any time following Nb
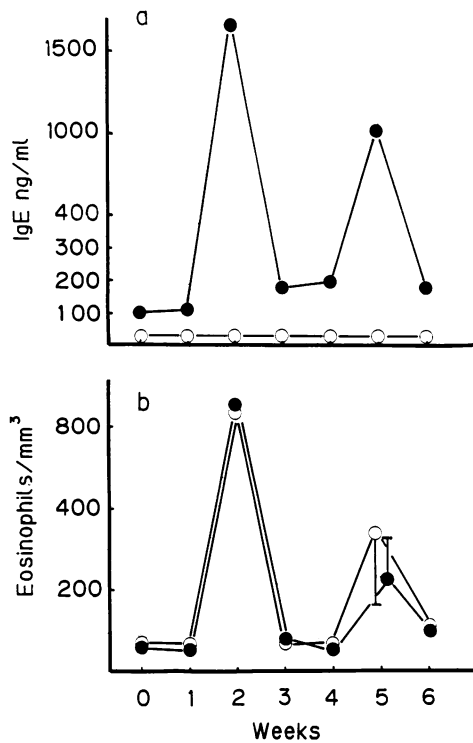
Abbreviations: Nb, *Nippostrongylus brasiliensis*; PCA, passive cutaneous anaphylaxis; Ts, *Trichinella spiralis*.
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FIG. 1.   (*a*) Total IgE levels in the serum from Nb-infected SJA/9 (○) and SJL/J (●) mice. Seven hundred fifty third-stage larvae of Nb were subcutaneously injected at 0 and 4 wk. (*b*) Kinetics of eosinophils in peripheral blood of Nb-infected SJA/9 (○) and SJL/J (●) mice. Vertical bars indicate SD.

infection in SJA/9 mice. Moreover, anti-Nb IgE antibody, determined by PCA reactions, was not detected in either SJA/9 or SJL/J mice following Nb infection. This suggests that the IgE produced in infected SJL/J mice is not parasite specific but is the result of polyclonal IgE B-cell activation.

The number of eosinophils in the peripheral blood of the SJA/9 mice increased 2 wk after Nb infection but reached normal levels by 3 wk (Fig. 1*b*). The time course of blood eosinophilia in SJL/J mice was similar to that in SJA/9 mice. When the mice were reinfected with 750 third-stage larvae of Nb 4 wk after primary infection, blood eosinophils increased 1 wk after the secondary infection but decreased by 2 wk in both strains.

Numbers of adult worms in the small intestine were determined. Seven days after the primary infection, the mean (± SD) worm burden was 188 ± 33 in SJL/J and 201 ± 46 in SJA/9 mice. Fourteen days after primary infection, no adult worms were recovered in both strains. Seven days after the secondary infection, few adult worms were recovered from both strains. In SJA/9 mice, five of six mice were negative and one mouse had 6 worms. In SJL/J mice, three of six mice were negative and three mice had 1–3 worms. Thus, in the absence of detectable IgE, worm expulsion and protective immunity in Nb infection were normal.

Ts Infection. In Ts-infected SJL/J mice, anti-Ts IgE antibody, detected by PCA reaction using antigen extracted from muscle larvae, was detected 2 wk after infection and persisted for >8 wk (Fig. 2*a*). Anti-Ts IgE antibody production in SJL/J mice was slightly enhanced after secondary infection with 100 muscle larvae. On the other hand, no anti-Ts IgE antibody was detected in SJA/9 mice after primary or secondary infection.

Peripheral blood eosinophils increased slightly 2 wk after Ts infection and reached a peak by 3 wk in SJA/9 and SJL/mice (Fig. 2*b*). An increase of eosinophils was observed 2 wk after secondary infection in both strains. The number of
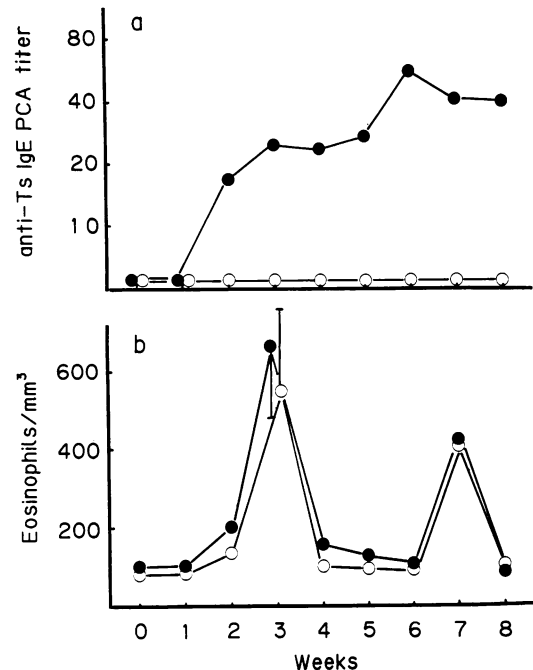

FIG. 2.   (*a*) Anti-Ts IgE antibody in Ts-infected SJA/9 (○) and SJL/J (●) mice. One hundred muscle larvae of Ts were administered orally at 0 and 5 wk. (*b*) Kinetics of eosinophils in peripheral blood of Ts-infected SJA/9 (○) and SJL/J (●) mice. Vertical bars indicate SD.

eosinophils in SJA/9 mice was not significantly different from that in SJL/J mice at any time during the experiment.

Effects of IgE on the protective immunity were examined in Ts-infected mice by counting muscle larvae after digesting the tissues with pepsin (Table 1). Half of the mice were reinfected with the same dose of larvae 5 wk after the primary infection (group 3). The other half of the mice did not receive a secondary infection (group 2). Other mice were infected at the same time as those given secondary infection (group 1). The number of Ts larvae in SJA/9 mice was not significantly different from that in SJL/J mice 4 wk (group 1) and also 9 wk (group 2) after primary infection. The number of Ts larvae recovered after secondary infection (group 3) was comparable to that from mice kept without secondary infection (group 2) in both strains. These results suggest that anti-Ts IgE antibody is not necessary for protective immunity in Ts-infected mice.

DISCUSSION

The aim of this study was to investigate the role(s) of IgE in the host with helminth infections. Our results indicate that IgE is not essential for the development of eosinophilia and protective immunity in Nb and Ts infections, as there was no difference between SJA/9 and SJL/J mice. These results are perhaps surprising because IgE-mediated hypersensitivity reactions are thought to attack parasites through antibody-dependent cellular cytotoxicity (5). IgE-dependent killing of

Table 1.   Worm burden in Ts-infected mice

Group	Time after infection, wk	Ts larvae, no. per mouse	
		SJL/J	SJA/9
1	Primary, 4	13,041 ± 2657	11,750 ± 3956
2	Primary, 9	14,650 ± 5703	12,667 ± 2189
3	Secondary, 4	14,063 ± 2904	11,950 ± 3156

Data are mean ± SD determined from six mice in each group.

helminths by eosinophils and macrophages has been reported (22).

In Nb infection, although Nb antigen has no eosinophil chemotactic activity, mesenteric lymph node cells spontaneously release a chemotactic factor (23). It is thought that expulsion of Nb apparently involves antibody and T lymphocytes. However, our results and those of others (24) suggest that it is unlikely that IgE is critical for worm expulsion. The worm burden in mice infected with Nb, but also with suppression of Ig formation (including IgE antibody) by injection of anti-μ heavy-chain antibody, was similar to that of IgE-producing controls (25).

Worm burden for Ts is said to be inversely related to anti-Ts anaphylactic antibody production (15), suggesting an important role for IgE in protective immunity. Critical evidence for IgE-dependent reactions to Ts has been reported by using rats with selective IgE deficiency induced by repeated injections of anti-rat IgE antibody. Such rats have an increased susceptibility to Ts (21). The reasons for the discrepancy between our experiments and these (21) are not understood, as infecting doses were similar and the amount of anti-Ts IgE antibody in SJL/J mice seems to be comparable to that in the rats. The differences observed might be due to studies done in mice vs. rats and also congenital vs. acquired IgE deficiency. In addition, injections of anti-IgE antibody might have other effects than simply depleting IgE antibody. It is known that anti-IgE is a potent mast-cell degranulator and is very effective in reverse PCA (17).

If the SJL/J and SJA/9 mice were more sensitive to the vascular effects of vasoactive amines than other strains such as BALB/c, it would be possible to assume that mediators responsible for worm eliminations could pass more freely through the tissue. In this case, it might be that in other strains (but not in SJL/J) vasoactive amines liberated as a consequence of anaphylactic reactions involving IgE antibodies could play an enhancing role. If this were the case, then a difference in vascular response to vasoactive amines between SJL/J (and SJA/9) mice, on one hand, and other strains such as BALB/c mice, on the other hand, should exist. However, we did not find any difference in SJL/J and BALB/c mice when the responses to intradermally injected histamine were compared (data not shown).

It was remarkable that no difference in the number of Ts larvae in SJA/9 and SJL/J mice was observed and especially that after secondary infection the number of Ts larvae was not significantly greater than after primary infection. This indicates that in both strains a comparable immunity developed after primary infection.

In IgE-deficient rats, the eosinophil response was depressed following IgE suppression (21). It has been suggested that eosinophils can kill Ts (26, 27). Some mechanisms of eosinophilia independent of IgE antibody have been reported. Eosinophilopoietin and eosinophil stimulation promoter can be released from T cells (28–31). Such IgE-independent mechanisms might be operative in our mouse system (SJA/9).

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