

Anaphylactic Response to Parasite Antigens: IgE and IgG1 Independently Induce Death in *Trichinella*-Infected Mice

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Key Words

Anaphylaxis · *Trichinella* · IgE · IgG1 · Mice

Abstract

The response of animals infected with different *Trichinella* species (*T. spiralis*, *T. britovi*, *T. pseudospiralis*) to antigens of different *Trichinella* species was evaluated in outbred mice (CD1) and inbred mice (BALB/c, C3H, SJL, C57BL/6). In mice deficient for IgE or IgG1, *T. spiralis* only was used. In homologous conditions (i.e., same *Trichinella* species for antigens and infectious agent) mortality was very high (up to 100% in SJL and C57BL/6). The anaphylactic response in heterologous conditions was much lower and death was observed only in C57BL/6 and SJL mice. Experiments with deficient mice have shown that both specific IgE and IgG1 independently induce a fatal anaphylactic response.

Introduction

IgE-dependent mast cell activation has long been regarded as an essential factor in active anaphylaxis [1], yet recent reports have shown that active anaphylaxis can be induced in IgE-deficient mice, suggesting the existence of an IgE-

independent anaphylaxis [2]. Furthermore, a type of antigen-specific hypersensitivity that occurs within 1 h of intravenous antigen challenge has been described. This reaction, which has been defined as “early-type hypersensitivity” [3], can be transferred by either immune sera or T-cell-derived low-molecular-weight factors, thus differing from IgE-mediated hypersensitivity and from delayed-type hypersensitivity.

Most studies on the host immune response to *Trichinella* infection have focused on *Trichinella spiralis* [4]. Briggs and De Giusti [5] used a challenge with antigen to study the anaphylactic response. However, they only used a homologous challenge (i.e., the same *Trichinella* species was used as both antigen and infectious agent). As recently described by Wakelin and Goyal [6], a number of studies evaluated host immunity in terms of antibody response, T cell response, cytokine profile, and inflammatory response versus different *Trichinella* species. These studies have revealed differences in responses in relation to the specific isolate or species of the parasite studied.

In helminthic infections, hypersensitivity reactions are usually referred to as “IgE-dependent mast-cell activation processes” (type I hypersensitivity), though it is not known whether other mechanisms can be involved in these reactions. To clarify this issue, we carried out a study on the effects of intravenous injections of *Trichinella* antigens in mice previously infected with this parasite, in homologous

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Table 1. Details of the experiments in immunocompetent mice

Exp.	Crude antigens	Dose, µg	Mice ¹	Infection	Results shown in
1	Ts/Tb/Tp	75	CD1	Ts/Tb/Tp	fig. 2
2	Ts/Tb/Tp	75	CD1	Ts/Tb/Tp	fig. 2
3	Ts/Tb/Tp	50	C3H/He	Ts/Tb/Tp	table 2
4	Ts/Tb/Tp	50	BALB/c	Ts/Tb/Tp	table 2
5	Ts/Tp	50	C57BL/6	Ts/Tp	table 2
6	Ts/Tp	50	SJL	Ts/Tp	table 2

Ts = *T. spiralis*; Tb = *T. britovi*; Tp = *T. pseudospiralis*.

¹ All mice were injected 28 d.p.i., except in experiment 1, where mice were injected 21 d.p.i.

and heterologous conditions. We also studied the role of different antibody classes involved in anaphylactic processes in mice selectively deficient for IgE or IgG1.

Materials and Methods

Mice

Eight-week-old female mice were used. Swiss CD1 outbred mice and BALB/c (H-2^d), C3H/He (H-2^k) and C57BL/6 (H-2^b) inbred mice were obtained from Charles River, Italy. SJL (H-2^s) inbred mice were obtained from Jackson Laboratories, USA. These mouse strains were chosen for the different levels of infectivity with *T. spiralis* [7]. Mice were maintained at 18–20°C in polycarbonate cages, housed 10 per cage and allowed food and water ad libitum.

In experiments with deficient mice, we used SJL/J and SJA/9 mice, which were purchased from Ohmura Laboratory Animals (Kanagawa, Japan). MRL/n and MRL/gg1d mice were bred at the Central Institute for Experimental Animals (Kawasaki, Japan). The SJA/9 mouse is a selectively IgE-deficient and congenic strain of the SJL/J mouse [8]. The MRL/gg1d mouse is a selectively IgG1-deficient mutant of the MRL/n mouse [9].

Wistar rats weighing 500–600 g were used for passive cutaneous anaphylaxis (PCA) reactions; these rats were purchased from Ishikawa Laboratory Animals (Saitama, Japan).

Parasites

Trichinella isolates, belonging to *T. spiralis* (code ISS3), *T. britovi* (code ISS2), and *T. pseudospiralis* (code ISS13), were used for experimental infections. All isolates were maintained in Swiss CD1 female mice with serial passages every 2 or 3 months. Parasites were collected after artificial digestion, following the standard technique, as previously described [10]. Mice were infected per os with 500 L₁ infecting larvae (ML). The same inoculum dosage was used for each parasite species.

Antigens

The crude ML antigen (CA) was prepared according to the standard method [11]. The protein concentration was tested according to different methods [12, 13].

SDS-PAGE

The same amount (10 µg) of crude antigens of 6 isolates belonging to *T. spiralis*, *T. nativa* (code ISS71), *T. britovi*, *T. pseudospiralis*,

Trichinella T5 and *T. nelsoni* (code ISS29) was analyzed by SDS-PAGE in 7.5 and 12% slab gels [14] under reducing conditions. After running, the gels were stained with Coomassie blue or silver stain to observe the proteins.

Antibody Determination

Mice were infected with 500 muscle larvae of *T. spiralis*. The infected mice were bled from the retro-orbital sinus one day before antigen challenge. The blood (0.2 ml) was diluted 1:5 with heparin-saline, and the plasma was used for antibody determination.

Anti-*T. spiralis* IgE Antibodies

Anti-*T. spiralis* IgE antibodies were detected by PCA in rats [15]. Serial dilutions of plasma samples (0.1 ml) were intradermally injected into the shaved dorsal skin of normal Wistar rats. One day after injection, animals were challenged (i.v.) with 1 ml of 0.5% Evans Blue containing 1 mg *T. spiralis* antigen. Since no mouse IgG subclass can react on rat mast cells, at this time, only specific IgE can be detected. Titers were expressed as the highest dilution that induced blue reaction.

Anti-*T. spiralis* IgG1 Antibodies

Anti-*T. spiralis* IgG1 antibodies were detected by an ELISA. Microtiter plates (Immulon 2) were coated with 50 µg/ml of *T. spiralis* antigen. After blocking with bovine serum albumin (BSA), plasma samples (1:50 dilution) were applied. Anti-mouse IgG1-peroxidase (affinity-purified horse anti-mouse IgG1, Zymed Lab., Inc., USA) was then added. Azino-bis (ethylbenz-thiazoline-sulfonic acid) and H₂O₂ were used as substrate. In the reaction, normal mouse plasma showed an optical density of less than 0.25. Data were expressed as optical density of ELISA.

Total IgE

Total IgE levels were measured by an ELISA [15]. The plates were coated with 10 µg/ml anti-mouse IgE monoclonal antibody (6HD5). After blocking with BSA, plasma samples (1:20 dilution) were applied. The secondary antibody was a biotinylated anti-mouse IgE monoclonal antibody (HMK-12), which recognizes a different epitope of IgE molecule from that recognized by the clone 6HD5 [16]. Peroxidase-streptavidin (Zymed Lab., Inc.) was then added. The successive steps were the same as those for IgG1 ELISA. Total IgE levels in normal mice were less than 400 ng/ml. Data were obtained referring to a standard curve realized with known concentrations of mouse IgE (100 up to 1,200 ng/ml).

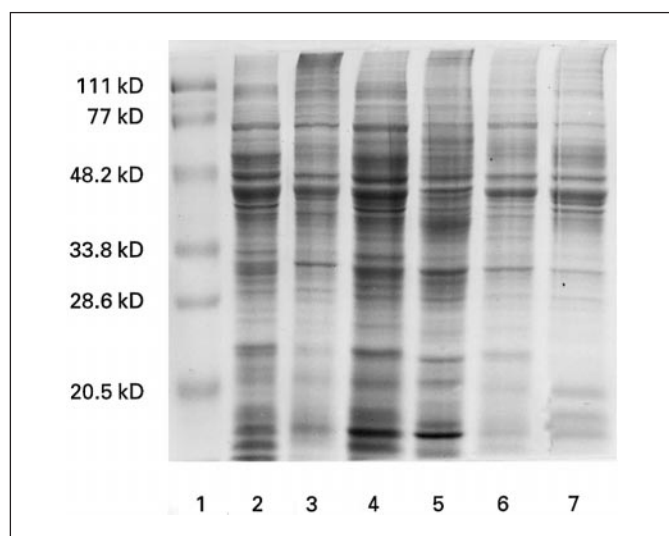


Fig. 1. SDS-PAGE of crude antigens from *T. spiralis* (lane 2), *T. nativa* (lane 3), *T. britovi* (lane 4), *T. pseudospiralis* (lane 5), *Trichinella* T5 (lane 6) and *T. nelsoni* (lane 7). Lane 1: markers of molecular weight. After running, proteins were visualized with Coomassie blue.

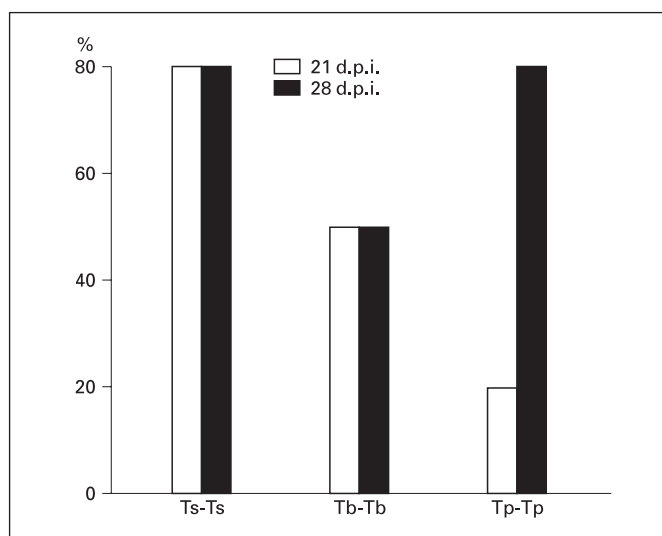


Fig. 2. CD1 mice infected with *T. spiralis* (Ts), *T. britovi* (Tb) and *T. pseudospiralis* (Tp) dying following the injection of crude antigens at 21 or 28 d.p.i. (experiments No. 1 and 2). Species used for the infection-species used for the antigen challenge. Standard deviations (not shown) were never greater than 10%.

Injection of Antigen in *Trichinella*-Infected Mice

Infected mice were injected via the caudal vein with CA at different dosages (table 1). Each injection was carried out in groups of 10 mice. Each mouse was observed for 1 h after injection for anaphylactic response (see Results, where the response is described as: “no symptom”, “shock”, or “death”). Five uninfected mice were injected with antigen, and 5 infected mice with saline, as control groups for each experiment.

Results

Protein profiles of different *Trichinella* species

When comparing crude extracts among the five *Trichinella* species, the specific protein profile of each species was more evident in the 12% slab gel, compared to the 7.5% slab gel (fig. 1). The 45-kD protein was present in all of the species evaluated. With silver staining, between 98 and 116 kD, a sharp band was observed only in *T. nelsoni* and *T. nativa* (data not shown).

Experiments in immunocompetent mice

After antigen injection, infected animals showed a slowing down of motor activity and respiratory difficulties, followed in some cases by death. This occurred as early as 21 days after infection at different times (ranging from 3 to 25 min) after antigen injection. The degree of symptomatology was quantified but this did not provide further information

compared to mortality rates (data not shown). None of the mice in control groups (uninfected mice injected with antigens or infected mice injected with saline) underwent shock or died. Preliminary experiments indicated that the maximum anaphylactic response was obtained with a challenge dose of 50 µg of antigen; 75 µg produced the same level of response. In preliminary experiments, no significant differences in anaphylaxis, either for specificity or degree of response, were observed in mice infected with 5 different *T. spiralis* isolates, 4 *T. britovi* isolates, and 2 *T. pseudospiralis* isolates. Thus, only data on one isolate for each species are presented in this study.

Outbred CD1 mice

The injection of the *T. spiralis* homologous antigen caused death in 80% of mice, both at 21 and 28 days post injection (d.p.i.) (fig. 2). Death rates higher than 40% were also obtained when using homologous antigen injections with other *Trichinella* species, with the exception of *T. pseudospiralis* at 21 d.p.i., when only 20% of mice died; this rate increased to 80% at 28 d.p.i. (fig. 2). Differences between 21 and 28 d.p.i. were more relevant in heterologous antigen injections; at 28 days, an increase in mortality was observed in most cases (data not shown). In an experiment performed at 10 weeks of infection, 50% of *T. spiralis*-infected mice died following homologous antigen injection.

Table 2. Percentage of inbred mice, infected with *T. spiralis* (Ts), *T. britovi* (Tb) and *T. pseudospiralis* (Tp), which died following the injection of homologous or heterologous crude antigens (experiments No. 4, 5, 6, 7)

Mice Infected with											Uninfected mice
Crude antigen challenge	Ts				Tb		Tp				
	BALB	C3H	C57	SJL	BALB	C3H	BALB	C3H	C57	SJL	
Ts	60	10	100	100	0	0	0	0	50	70	0
Tb	0	0	n.d.	n.d.	30	0	0	0	n.d.	n.d.	0
Tp	0	0	0	10	0	0	40	0	100	100	0
Saline	0	0	0	0	0	0	0	0	0	0	n.d.

Ten mice were used in each group; n.d. = not done; BALB = BALB/c; C57 = C57BL/6.

Table 3. Plasma total IgE, specific IgE and IgG1 in different mouse strains at 28 d.p.i. with *T. spiralis* (Ts); (in parentheses the number of animals tested)

Mouse strain	Anaphylaxis ¹ %	Anti-Ts IgE ²	Anti-Ts IgG1 ³	Total IgE ⁴ , ng/ml
SJL/J (14)	100	23±7 (14)	1.15±0.18	1,151±184
SJA/9 (14)	100	0 (14)	1.21±0.21	<100
MRL/n (15)	26	53±27 (14)	1.19±0.20	4,540±611
MRL/ggld (15)	26	47±18 (15)	0.18±0.004	4,712±1,025

¹ Percentage of mice exhibiting anaphylactic response (symptoms or death) among the total tested animals.

² Mean ± SD of PCA titers.

³ Mean ± SD of optical densities.

⁴ Values were obtained referring to a standard curve.

Inbred Mice

C57BL/6 and SJL mice were more susceptible to a homologous antigen injection in *T. spiralis* and *T. pseudospiralis* experiments than BALB/c and C3H mice. In the latter strain, only 10% of mice died after homologous injection (table 2). In the homologous antigen injection, BALB/c mice responded similarly to outbred CD1 mice, with mortality ranging from 30 to 60%. The injection of *T. pseudospiralis* antigen, which caused a significantly higher mortality in CD1 mice compared to BALB/c mice (90 versus 40%), is an exception.

The injection of heterologous antigens, compared to homologous antigens, induced lower levels of response. In C3H mice, there was no response at all, whereas in SJL mice the injection of *T. spiralis* antigen in *T. pseudospiralis*-infected mice resulted in a mortality rate of 70%.

Experiments in IgE-Deficient and IgG1-Deficient Mice

IgE-deficient SJA/9 mice and IgE-producing SJL/J mice (control) infected with *T. spiralis* were challenged with *T. spiralis* antigen at 28 d.p.i. Both of these strains exhibited anaphylactic symptoms (table 3). Anaphylactic death was

observed in 9 of the 14 mice in each strain. Levels of anti-*T. spiralis* IgG1 were similar when comparing the two strains, though only SJL/J mice are capable of producing specific IgE (table 3), suggesting that anaphylactic response can be induced also in mice without IgE. Similar experiments were carried out in IgG1-deficient MRL/ggld mice and IgG1-producing MRL/n mice (control) at 28 d.p.i. Only 4 of the 15 mice in each strain showed anaphylactic response and none of the mice died. Both MRL/ggld and MRL/n mice produced similar amounts of total IgE and anti-*T. spiralis* IgE antibodies. Even with a greater amount of antigen (200 µg) used for challenge, anaphylactic response was observed only in 2 of 5 the MRL/ggld mice and in 3 of the 5 MRL/n mice. None of the mice died in this experiment.

To evaluate the susceptibility to anaphylactic reactions, uninfected mice (5 mice/strain) of the SJL/J, SJA/9, MRL/ggld, and MRL/n strains were injected (i.v.) with an anti-mouse IgE monoclonal antibody (6HD5, 50 µg/mouse). All SJL/J mice died, whereas none of the SJA/9 mice died. By contrast, both MRL/ggld and MRL/n mice showed anaphylactic symptoms without death. These re-

Table 4. Anaphylaxis in SJA/9 mice transfused with serum after heat treatment (2 h at 56°C)

Mouse	Transferred serum from Ts-infected (4 weeks) MRL/ggld mice			
	non heated		heated	
	anti-Ts IgE titer	anaphylaxis	anti-Ts IgE titer	anaphylaxis
1	1:40	D	0	N
2	1:40	D	0	N
3	1:40	D	0	N

Three SJA/9 recipients were transfused with heated or non heated serum (0.6 ml) from *T. spiralis* (Ts)-infected MRL/gg 1d mice (4 weeks) and challenged with *T. spiralis* antigen (100 µg/mouse) 48 h after transfer. Anti-*T. spiralis* IgE antibody was detected by PCA reactions. D = Death; N = no response.

sults indicate a genetic difference in susceptibility to anaphylactic reactions. Further experiments were performed to clarify the anaphylaxis-inducing activity of IgE antibody. The serum from MRL/gg1d mice infected with *T. spiralis* 4 weeks earlier was transferred to SJA/9 mice. In other words, anti-*T. spiralis* IgE antibody without specific IgG1 was transferred to mice with normal IgE receptors and without IgE. The recipients died after challenge with *T. spiralis* antigen (table 4). The role of IgE antibody was confirmed by experiments using the heat-inactivated serum (table 4).

Discussion

In mice, anaphylactic antibodies belong to two different classes of immunoglobulins: IgE and IgG1. Both immunoglobulins are capable of inducing anaphylactic shock due to hypersensitivity and they probably act independently. In rats, IgE and IgG2a (the other anaphylactic antibody in rats) have been shown to act independently [17].

We observed an anaphylactic response leading to death within 1 h of antigen injection, and the IgE antibody was not the only isotype responsible for this response. The fact that 100% of IgE-deficient SJA/9 mice underwent an anaphylactic response could lead to the conclusion that IgG1, and not IgE, was responsible for anaphylactic shock under our experimental conditions. However, the transfer of serum from infected MRL/gg1d mice to normal SJA/9 mice shows that IgE can induce shock, since heating of the transferred serum is able to suppress this effect. Consequently, both specific IgE and IgG1 are capable of independently mediating fatal anaphylactic shock.

The injection of anti-mouse IgE in MRL strains did not cause death, verifying that these strains have a low response to antigen challenge, due to their low susceptibility to anaphylactic shock.

It can be hypothesized that the MRL strain is particularly refractory to shock for reasons other than the anaphylactic antibody serum concentrations. Antibodies are certainly essential in the first phase of the anaphylactic response, but other humoral and cellular factors play a role in the complex mechanism leading to the expression phase of the anaphylactic reaction (not only mediator release but also sensitivity to mediators). It has recently been shown that *Trichinella* antigens can induce the activation of mast cells through an IgE-independent mechanism [18], yet this effect seems to have played no role in our model, since anaphylactic response was absent in normal mice injected with parasite antigens. In both MRL mouse strains, the responsiveness of mast cells could be 'down-regulated'.

The level of host infection is the result of a balance between the programmed infectivity of the etiological agent and the host's response to the infection. This means that genetic variability in both the host and the parasites [19] has to be taken into account when analyzing the host-parasite relationship. In the last 20 years, authors have debated the relative importance of the immune response in controlling the infectivity of different *Trichinella* isolates [20, 21] and the role of a programmed infectivity, irrespective of the host's immune response [22].

Our data show that mice infected with different *Trichinella* species undergo different anaphylactic responses to parasite antigens injected intravenously. In fact, while a homologous antigen produced death in a high percentage of recipients, a heterologous antigen did not result in death, at least in inbred mice. *T. pseudospiralis* antigens induced an anaphylactic response in *T. pseudospiralis* infected animals, and *T. britovi* antigens in *T. britovi*-infected mice. Stewart et al. [23] obtained similar results with *T. pseudospiralis* in mice injected with the homologous antigen, but they did not observe an anaphylactic response with *T. spiralis* or *T. pseudospiralis* antigen injected in *T. spi-*

ralis-infected mice or with *T. spiralis* antigens injected in *T. pseudospiralis*-infected mice. However, in another experiment, *T. spiralis* excreted/secreted antigen produced results similar to ours when it was injected into *T. spiralis* infected mice [Stewart, pers. commun.]. Other studies have observed a stronger immune response to homologous *Trichinella* antigens with respect to heterologous *Trichinella* antigens [24, 25].

C3H mice, which have the highest parasite burden among the different inbred strains [26], had the lowest response to both homologous and heterologous antigens, clearly showing a lack of correlation between parasite burden and anaphylactic response.

These results suggest that the anaphylactic response to *Trichinella* antigens is related both to the genetic variability

of the host and to the antigenic composition of the parasite species, which are characterized by biological differences [27].

Further studies are needed to characterize possible species-specific antigens responsible for the anaphylactic response.

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