

Effect of Parasitosis on Allergic Sensitization in Rats Sensitized With Ovalbumin: Interaction Between Parasitosis and Allergic Sensitization

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ABSTRACT

Some helminthic infections, especially nematode infections, may behave as allergens and induce allergic sensitization. In this study, the investigators explored whether infections with *Syphacia muris* and *Aspicularis tetraptera* have any effect on the development of allergen-induced cytokine responses and serum total immunoglobulin E (IgE) levels in ovalbumin (OVA)-sensitized rats.

Four groups of male Wistar rats were studied. OVA sensitization was generated in 2 groups of rats; the rats in 1 group were infected and those in the other group were not. On day 21 after sensitization, tumor necrosis factor- α , interleukin-10, and total IgE levels in serum samples of rats were measured by enzyme-linked immunosorbent assay. The results showed that average concentrations of tumor necrosis factor- α and interleukin-10 were significantly greater in the group of rats infected with parasites and sensitized to OVA compared with the group uninfected with parasites and sensitized to OVA ($P=.043$ and $P=.046$, respectively). Upon comparison of total IgE concentrations, the group of rats infected with parasites and given saline solution showed higher levels compared with the group uninfected with parasites and given saline ($P=.004$). In conclusion, the investigators were unable to show a protective effect of an existing parasitic infection against the development of allergic sensitization upon exposure to OVA.

Keywords: | interleukin-10; tumor necrosis factor- α ; allergic sensitization; parasitosis; in vivo animal model

INTRODUCTION

The global prevalence of allergic disease has increased markedly over the past few decades.¹ Evidence of allergic sensitization to common environmental allergens is a consistently strong risk factor for allergic disease in epidemiologic studies conducted in industrialized countries.² Some helminthic infections, especially nematode infections, may behave as allergens and induce allergic sensitization.³⁻⁵ Similarities in the immune response observed in allergic sensitization and nematode infection settings raise obvious questions about the relationship between allergic disorders and helminthic infections; however, results of epidemiologic trials conducted to study this relationship are controversial.^{3,6,7} Therefore, experimental models in which the relationship between allergic sensitization and helminthic infections can be studied must be established.

Syphacia muris and *Aspicularis tetraaptera* are 2 helminths that belong to the nematode group and frequently cause intestinal parasitosis in rats.⁸ In this study, the investigators explored whether infection with *S muris* and *A tetraaptera* may have any effect on the development of allergen-induced cytokine responses and serum total immunoglobulin (Ig)E levels in ovalbumin (OVA)-sensitized rats.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats weighing 180 to 200 g were used in the experiment. All animals were fed laboratory chow and were given tap water to drink ad libitum. The experimental procedures followed were previously approved by the local animal ethics committee. All rats were examined for *S muris* and *A tetraaptera* by tape testing and feces microscopy. Rats that were infected by 1 of these parasites were isolated from the uninfected rats by being placed in separate cages and rooms. Eventually, 4 groups were formed; each group consisted of 5 rats, 2 of which (groups 1 and 2) were infected

with parasites and 2 of which were uninfected (groups 3 and 4). All rats were examined regularly for helminthic infection during the study. Study groups are shown in Table 1.

Table 1. Composition of Study Groups

Group 1 (n=5)	Rats infected with parasites and sensitized with OVA (infected and sensitized rats)
Group 2 (n=5)	Rats infected with parasites and given saline (infected and nonsensitized rats)
Group 3 (n=5)	Rats uninfected with parasites and sensitized with OVA (uninfected and sensitized rats)
Group 4 (n=5)	Rats uninfected with parasites and given saline (healthy rats)

Allergic Sensitization With OVA

Allergic sensitization was generated in 2 groups of rats, 1 infected (group 1) and the other, uninfected (group 3), through administration of OVA, grade II (Sigma, St. Louis, Mo). Initially, rats were actively sensitized with a subcutaneous injection of sterile physiologic saline (1 mL) containing 1 mg OVA and 200 mg aluminum hydroxide (EM Industries, Hawthorne, NY). *Bordetella pertussis* vaccine (50 μ L) containing 6×10^9 heat-killed bacilli was given intraperitoneally as an adjuvant. One week later, sterile saline (1 mL) containing 1 mg OVA and 200 mg aluminum hydroxide was injected subcutaneously as a booster.⁹

The same procedure was repeated in the other 2 groups of rats, 1 infected (group 2) and the other, uninfected (group 4), with the use of sterile saline instead of OVA. Detection of cytokines and IgE levels revealed that 21 d after initial injection of OVA, all rats in the 4 groups were anesthetized by ether inhalation, and blood samples were drawn directly from the heart. Levels of tumor necrosis factor- α (TNF- α) (BioSource Europe SA, Nivelles, Belgium), interleukin (IL)-10 (BioSource), and total IgE were measured by the enzyme-linked immunosorbent assay method.

Statistical Analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS), version 10.0 (SPSS Inc., Chicago, Ill). Appropriateness of data to normal ranges was controlled with the Shapiro-Wilk test. A comparison between groups was performed with the use of independent sample *t* tests. Data were expressed as mean $\pm$ standard deviation (SD). A *P* value $< .05$ was considered statistically significant.

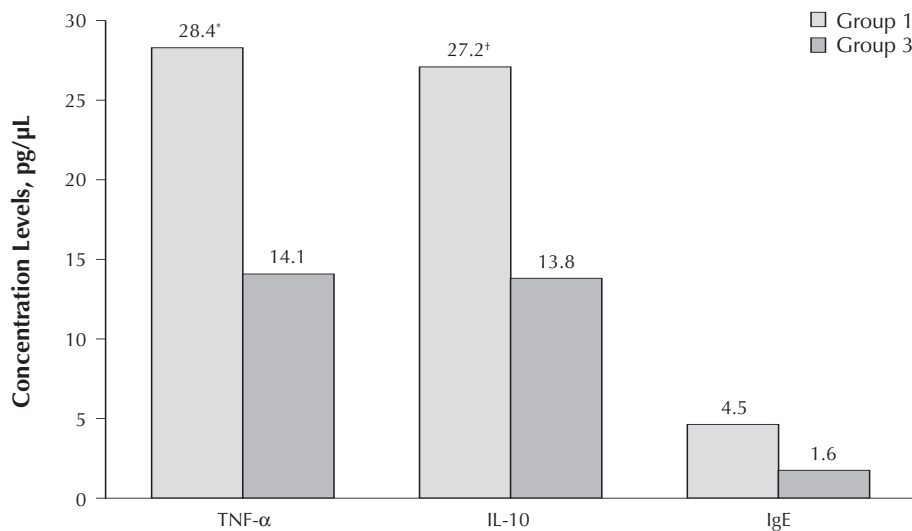
RESULTS

Mean TNF- α levels were 28.4 $\pm$ 10.4 pg/ μ L, 25.9 $\pm$ 9.3 pg/ μ L, 14.1 $\pm$ 8.3 pg/ μ L, and 2.2 $\pm$ 3.3 pg/ μ L in infected and sensitized rats (group 1), infected and nonsensitized rats (group 2), uninfected and sensitized rats (group 3), and healthy rats (group 4), respectively (Table 2). Mean IL-10 levels in these groups were 27.2 $\pm$ 11.0 pg/ μ L, 24.4 $\pm$ 11.0 pg/ μ L, 13.8 $\pm$ 7.2 pg/ μ L, and 1.6 $\pm$ 1.3 pg/ μ L, respectively (Table 3). The results showed that mean levels of TNF- α and IL-10 were significantly higher in infected and sensitized rats (group 1) compared with uninfected and sensitized rats (group 3) (P =.043 and P =.046, respectively). Levels were also significantly higher in groups 2 (P =.001 and P =.002, respectively) and 3 (P =.017 and P =.008, respectively) than in healthy rats (group 4) (Figs 1–3).

Table 2. TNF- α Concentrations				
Rats	Group 1	Group 2	Group 3	Group 4
1	23 pg/ μ L	18.5 pg/ μ L	1.5 pg/ μ L	1.5 pg/ μ L
2	25 pg/ μ L	18 pg/ μ L	17 pg/ μ L	8 pg/ μ L
3	18 pg/ μ L	27 pg/ μ L	24 pg/ μ L	0.5 pg/ μ L
4	45 pg/ μ L	25 pg/ μ L	12 pg/ μ L	0.2 pg/ μ L
5	31 pg/ μ L	41 pg/ μ L	16 pg/ μ L	0.8 pg/ μ L
Mean $\pm$ SD	28.4 $\pm$ 10.4 pg/ μ L	25.9 $\pm$ 9.3 pg/ μ L	14.1 $\pm$ 8.3 pg/ μ L	2.2 $\pm$ 3.3 pg/ μ L

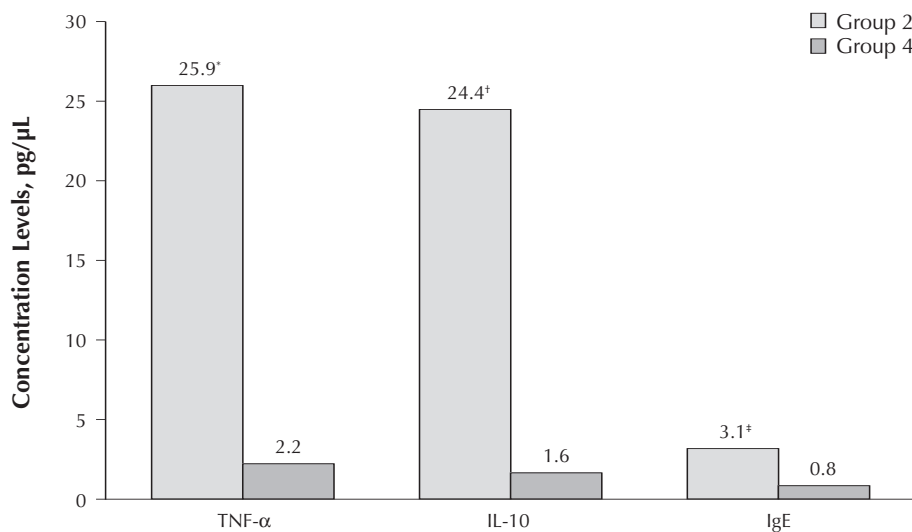
Table 3. IL-10 Concentrations				
Rats	Group 1	Group 2	Group 3	Group 4
1	20 pg/ μ L	18 pg/ μ L	5 pg/ μ L	2 pg/ μ L
2	22 pg/ μ L	17 pg/ μ L	14 pg/ μ L	6 pg/ μ L
3	19 pg/ μ L	21 pg/ μ L	22 pg/ μ L	0.5 pg/ μ L
4	44 pg/ μ L	22 pg/ μ L	10 pg/ μ L	0.3 pg/ μ L
5	31 pg/ μ L	44 pg/ μ L	19 pg/ μ L	1 pg/ μ L
Mean $\pm$ SD	27.2 $\pm$ 11.0 pg/ μ L	24.4 $\pm$ 11.0 pg/ μ L	13.8 $\pm$ 7.2 pg/ μ L	1.6 $\pm$ 1.3 pg/ μ L

Fig 1. Comparison of groups 1 and 3.



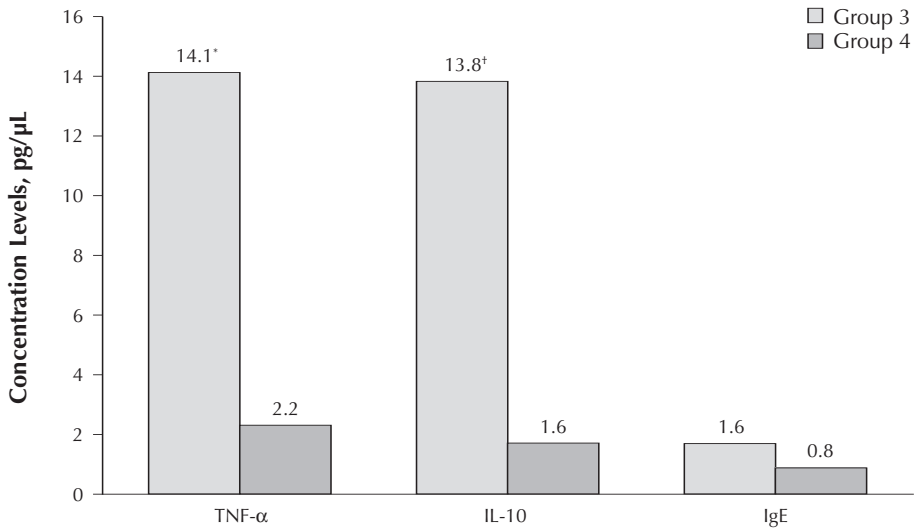
* $P=.043$; + $P=.046$.

Fig 2. Comparison of groups 2 and 4.



* $P=.001$; + $P=.002$; * $P=.004$.

Fig 3. Comparison of groups 3 and 4.



* $P=.017$; † $P=.008$.

The investigators found that mean IgE concentrations were 4.5 ± 4.3 pg/μL, 3.1 ± 0.9 pg/μL, 1.6 ± 2.3 pg/μL, and 0.8 ± 0.8 pg/μL in groups 1, 2, 3, and 4, respectively (Table 4). Upon comparison of average IgE concentrations, group 2 exhibited higher levels compared with healthy rats ($P=.004$) (Fig 2). When other groups were compared with each other (groups 1 and 3, groups 1 and 2, and groups 3 and 4), no significant differences were noted.

Table 4. Total IgE Concentrations

Rats	Group 1	Group 2	Group 3	Group 4
1	2.5 pg/μL	2 pg/μL	0 pg/μL	1 pg/μL
2	2 pg/μL	2.7 pg/μL	0 pg/μL	0 pg/μL
3	12 pg/μL	4 pg/μL	5 pg/μL	2 pg/μL
4	2 pg/μL	4 pg/μL	0 pg/μL	1 pg/μL
5	4 pg/μL	2.5 pg/μL	3 pg/μL	0 pg/μL
Mean±SD	4.5±4.3 pg/μL	3.1±0.9 pg/μL	1.6±2.3 pg/μL	0.8±0.8 pg/μL

DISCUSSION

Helminthic infections and “allergic diseases” are associated with predominant induction of a type 2 T helper (Th2)–type immune response.^{10–12} Studies of human populations have provided contradictory results regarding the relationship between helminthic infection and allergic disease. The data suggest that a strong Th2 response induced by parasitic infection would predispose to an allergic reaction; others have suggested that a high prevalence of helminthic infection in humans has a protective effect against allergic reactions.^{10,13} In the present study, levels of TNF- α and IL-10 in rats sensitized with OVA with parasitosis were significantly higher than those in rats sensitized with OVA but without parasitosis (Fig 1). This implies that parasitosis in rats facilitates the development of allergic sensitization. This result seems to be consistent with available data that suggest that Th2 immune responses evoked by parasitosis may predispose to an allergic reaction.¹⁴

TNF- α is a proinflammatory cytokine that is known to regulate the expression of adhesion molecules on vascular endothelium and leukocytes; it may be involved in the homing of Th2 cells to sites of allergic inflammation and in the regulation of Th2 cytokine-mediated immune response at mucosal sites. Its concentrations in serum increase in allergic disorders.^{12,15} The role of TNF- α in helminthic infection remains unclear; however, it has been shown that TNF- α concentrations are increased in parasite-infected animals, and that this cytokine is critical in mediating host protection to the parasite via IL-13-dependent and IL-14-independent mechanisms.¹⁵ Concordant with this suggestion, concentrations of TNF- α were significantly higher in rats with infection that were administered saline (group 2) compared with uninfected rats that were administered saline (group 4) (Fig 2). TNF- α concentrations in rats sensitized with OVA with infection (group 1) were significantly higher than those in rats sensitized with OVA but without infection (group 3) (Fig 1). Therefore, a protective effect of the Th2 immune response on the development of allergic sensitization as detected by an increase in TNF- α levels in infected rats could not be shown.

IL-10 is an anti-inflammatory cytokine produced by lymphocytes, monocytes-macrophages, and eosinophils. It has been characterized as having a wide variety of anti-inflammatory properties. It is considered an important immune regulatory cytokine because of its ability to reduce T-cell activation and inhibit cytokine synthesis of Th1 and Th2 cells. On the other hand, IL-10 may promote allergic responses *in vivo* through its effects on dendritic cells.¹⁶ Available data on IL-10 in atopic individuals are inadequate. It has been reported that allergic subjects have higher IL-10 levels than nonallergic subjects.^{3,10,17,18} In the present study, IL-10 levels in uninfected rats sensitized with OVA (group 3) were found to be higher than those in uninfected rats given saline (group 4) (Table 3, Fig 3). This result supports the suggestion that IL-10 levels increase during allergic sensitization. Production of IL-10 is a major feature of chronic helminthic infection. Proinflammatory stimuli produced by chronic helminthic infection also trigger the production of anti-inflammatory cytokines, such as IL-10, and an elevated IL-10 concentration regulates the immune system.¹³ In the present study, IL-10 levels that result from increased Th2 response in chronic helminthic infection and that support the immune system were found to be higher in rats with parasitosis (groups 1 and 2) than in rats with no parasitosis (groups 3 and 4) (Table 3). IL-10 levels were significantly higher in rats sensitized with OVA and with parasitosis (group 1) than in rats sensitized with OVA but with

no parasitosis (group 3). These results imply that elevated IL-10 levels caused by parasitosis do not show a protective effect against allergic sensitization in rats. This is consistent with existing data that suggest that parasitosis is not protective against allergic disease.^{14,19}

The general consensus is that IgE antibodies are an essential component of immune resistance to helminthiasis. Common environmental allergens stimulate IgE responses and produce allergic disease. In other words, IgE levels are high in parasitic infection and in allergic disorders.²⁰ In this study, IgE levels in rats given saline were significantly higher in the group with parasitosis (group 2) than in the group without parasitosis (group 4) (Table 4). IgE levels in rats sensitized with OVA and with parasitosis (group 1) were higher than those in rats with parasitosis that were given saline (group 2) and in rats sensitized with OVA without parasitosis (group 3). These results are consistent with the literature.^{10,20} Although statistically insignificant, higher levels of IgE in group 1 than in groups 2 and 3 suggest that existing parasitosis is not protective against allergic sensitization. The finding that IgE levels were parallel to other measured parameters (TNF- α and IL-10) in this study supports this result.

In the present study, an increased Th2 immune response in allergic sensitization generated by OVA was shown indirectly by increased concentrations of IL-10 and TNF- α , which are indicators of this response. IgE levels in the study groups were parallel to cytokine levels. None of the 3 parameters was higher in the group sensitized with OVA and with parasitosis (group 1) than in the group sensitized with OVA without parasitosis (group 3); this suggests a lack of protective effect against allergic sensitization by the Th2 response induced by parasitic infection (Fig 1).

In conclusion, the investigators were unable to show a protective effect with existing parasitosis against the development of allergic sensitization upon exposure to an antigen; however, larger-scale studies undertaken to investigate cytokines are required if a conclusive result is to be achieved.

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