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Differential immunological responses induced by infection with female muscle larvae and newborn larvae of *Trichinella pseudospiralis*

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ABSTRACT

Trichinella pseudospiralis infection can modulate the immunological response of autoimmune and allergic diseases leading to the amelioration of these diseases. The present study was undertaken to compare immunity induced by adult worms and muscle larvae. Higher eosinophilia was observed from newborn larva (NBL) infection than from adult females while higher levels of IgE were observed in adult female infections over those induced by NBL. The IgG1 response to ES antigen was more prominent in infections with adult females. The IgG2 responses to larval crude antigen were prominent against NBL. The Th2 cytokine, IL-4 cytokine was elevated in adult female infection following re-stimulation with adult crude antigen and ES. Both infections induced strong IFN- γ responses. The present study demonstrates that adult female worms induced stronger Th2 responses (IgG1, IgE and IL-4 responses) than NBL. Further examination of the mechanisms involved in immune modulation may be helpful for identifying *Trichinella*-derived molecules responsible for regulating autoimmune and allergic diseases.

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1. Introduction

Parasitic worms of the genus *Trichinella* can establish, reproduce and survive for years in mammalian hosts. Reducing the inflammatory immune response may induce a tolerance to parasite antigens. *Trichinella*, as with other helminths, can ensure its survival by modulating host immunological response. *Trichinella* infection induces a Th2 response (IL-4, IL-5, IL-13) (Wakelin et al., 1994; Morales et al., 2002), and the production of immunoregulatory cytokines such as IL-10 and TGF- β (Beiting et al., 2007). *Trichinella* has been shown to be protective against autoimmune and allergic diseases such as EAE, type 1 diabetes and inflammatory bowel disease (Khan et al., 2002; Saunders et al., 2007; Gruden-Movsesijan et al., 2008; Wu

et al., 2010). The protective effects are presumed linked to a classic Th2 immune paradigm initiated by the infection.

Identification of *Trichinella*-derived molecules responsible for the protective effect of autoimmune and allergic diseases will be important in defining the mechanisms of immunoregulation and may provide new therapies for autoimmune and allergic diseases. Therefore, an understanding of the stage specific immunity to *Trichinella* and in particular *Trichinella pseudospiralis* induced by adult worms (Ad) in the intestine and larvae in muscle (ML) is required. So far, some studies have been carried out on the stage-specific immunity; however, typical infections induced by ML have generally only characterized overlapping immunological responses among Ad and ML.

Compared to *T. spiralis* infection, *T. pseudospiralis* is less pathogenic, causes considerably less inflammation in the intestine and muscle of host, tolerates higher ML infections, results in less weight loss, and induces higher total IgE and slower rejection of intestinal worms (Alford et al.,

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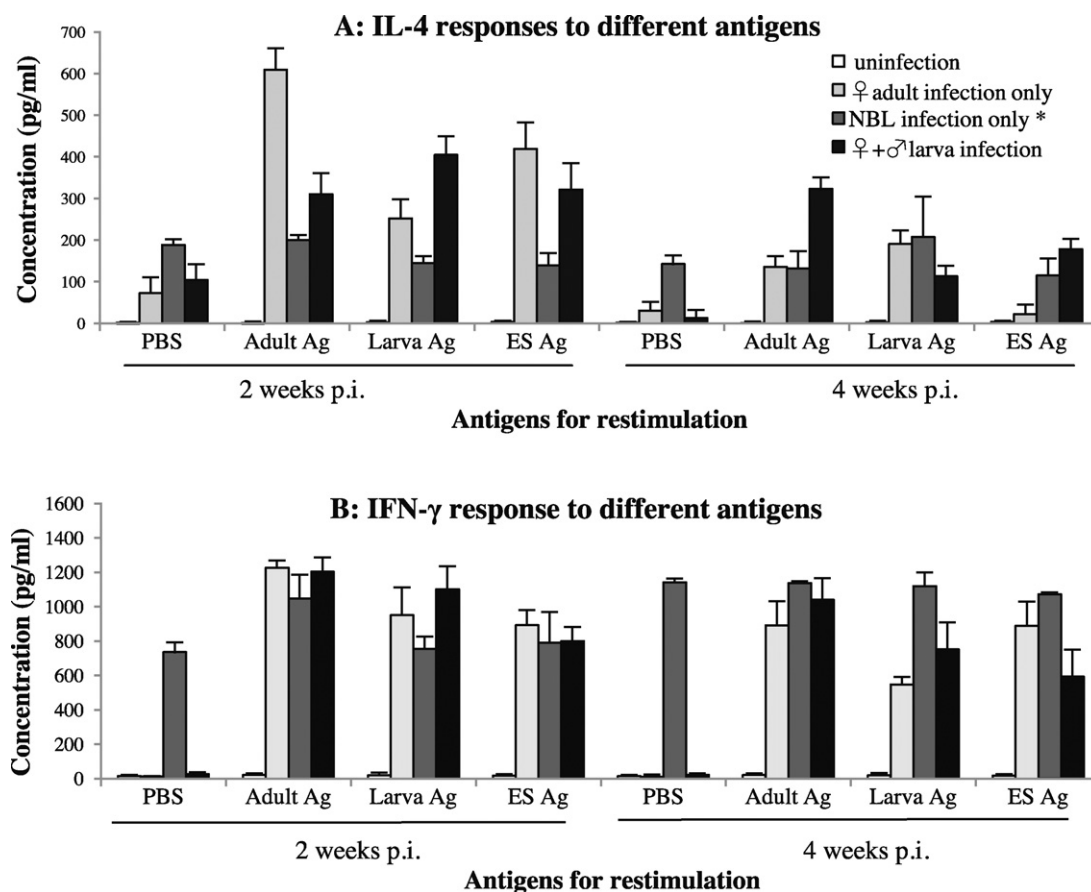


Fig. 1. IL-4 and IFN- γ responses of the splenocytes to the restimulation of *T. pseudospiralis* antigens (Ad, ML and ES antigens) among the 4 mouse infection groups: uninfected, ♀ Ad infected only, NBL-infected only, and ♀ + ♂ larvae infected.

1998; Lee and Ko, 2006). Therefore, we propose that *T. pseudospiralis* possesses stronger abilities to modulate the host during infection. As such, we compared immunological responses initiated by Ad and ML of *T. pseudospiralis*, by infecting mice with female ML which result in the immunological responses induced only by Ad, and secondarily by infecting a second group of mice with NBL which result in the immunological response induced only by NBL and ML. The comparisons included the Th2 (IL-4) and Th1 (IFN- γ) responses, antibody isotypes (IgG, IgG1, IgG2a and IgG2b), and eosinophilic responses (eosinophil count and total IgE).

2. Materials and methods

2.1. Parasite and infection

Female 6-week-old C57BL/6J mice and *T. pseudospiralis* (ISS13) were used in the present study. Mice were divided into 4 groups: (1) uninfected, (2) infected with 100 Ad females (which were identified based on the length of rectum), (3) infected with 10,000 NBL, and (4) infected with ML. Groups 1, 2, and 4 were observed at 2 and 4 weeks post infection (p.i.); group 3 was observed at 1 and 3 weeks p.i., which was considered comparable to 2 and 4 weeks p.i. relative to an oral infection.

Adult worms and ML, crude worm extracts of Ad and ML, and excretory–secretory (ES) products of ML were prepared by conventional methods (Wakelin et al., 1994). Endotoxin was removed with Detoxi-Gel™ Endotoxin Removing Columns (Takara Shuzo, Co., Ltd., Kyoto, Japan).

2.2. Cytokine and antibody detection using ELISA

To observe the cytokine responses, IL-4 and IFN- γ levels from splenocytes re-stimulated with PBS, Ad, ML and ES antigens among the four groups were measured by ELISA. Splenocytes were cultured in 24-well tissue culture plates at 5×10^6 cells/ml in the presence of Ad CWE, ML CWE, and ES (20 μ g/ml). After culture at 37 °C for 72 h, the supernatants were collected. Capture ELISA was used to quantify the levels of IFN- γ and IL-4 according to the manufacturer's instructions (Biolegend, CA, USA). Antibody levels, including total IgG, IgG1, IgG2a, IgG2b and total IgE, were determined with ELISA using conventional methods.

2.3. Eosinophil count

Blood smears were prepared from each mouse and stained with Wright–Giemsa. Differential cell counts were performed by counting 400 white blood cells.

2.4. Statistical analysis

Data were analyzed by using an unpaired or paired Student's *t*-test or two-way analysis of variance. A value of $p < 0.05$ and above was considered to be significant.

3. Results

3.1. IL-4 and IFN- γ responses induced by female Ad and NBL infections

As shown in Fig. 1A, the IL-4 production of splenocytes in the mice infected with female ML at 2 weeks p.i. was highest after re-stimulation with Ad CWE. Higher production was also observed after re-stimulation with ES antigen, but not with ML CWE. NBL infection induced IL-4; however, the levels were lower than those induced by female Ad or ML. As shown in Fig. 1B, high levels of IFN- γ were produced in all infection groups. At 2 weeks p.i., there IFN- γ was higher in mice infected with female Ad than in mice infected with NBL, while at 4 weeks p.i., IFN- γ levels were higher in NBL-infected mice. The production of IFN- γ in the splenocytes from NBL-infected mice can be seen even without antigen stimulation where the level increased by 4 weeks p.i.

3.2. IgG1, IgG2a and IgG2b responses induced by female Ad and NBL infections

As shown in Fig. 2B, at 2 and 4 weeks p.i. the IgG1 responses induced by female Ad were higher than those induced by NBL, including the responses to all other antigens. However, responses to ES antigen in NBL-infected mice were extremely low or near normal levels at both 2 and 4 weeks p.i. On the other hand, there were similar IgG1 responses between female Ad-infected mice and ML-infected mice. As shown in Fig. 2C, NBL infection induced higher IgG2a and IgG2b responses to Ad and ML antigens, but not to ES antigen. The IgG2a and IgG2b responses in NBL-infected mice were higher than those in ML-infected mice. Low or no responses were observed to Ad CWE at 2 weeks p.i.

3.3. Eosinophilic responses

Worm infections caused an increase in eosinophil counts in all infection groups examined (Fig. 3). At 2 weeks p.i., the eosinophil counts in NBL-infected mice (7.4%) was higher than in female Ad-infected mice (3.3%, $p < 0.05$). At 4 weeks p.i., eosinophil counts in both female Ad and NBL-infected mice (3.5 and 3.8%, $p > 0.05$) were lower than in ML-infected mice (10.2%, $p < 0.01$).

3.4. IgE responses induced by female Ad and NBL infections

At 2 weeks p.i., total IgE levels in mice infected with female Ad were significantly higher than that in NBL-infected mice ($p < 0.001$), and were similar to those levels in ML-infected mice ($p > 0.05$) (Fig. 4). Similar at 4 weeks p.i., IgE levels in female Ad-infected mice were higher than the

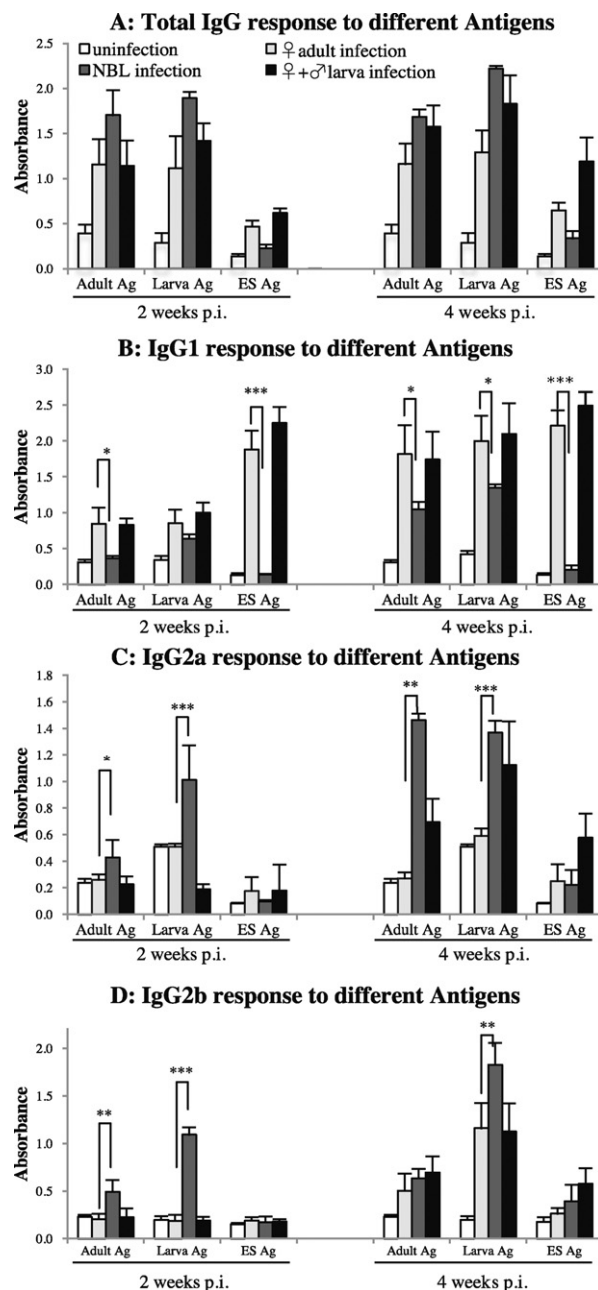


Fig. 2. Total IgG, IgG1, IgG2a and IgG2b antibody responses to the Ad CWE (adult Ag), ML antigen (larva Ag) and ES antigen (ES Ag) of *T. pseudospiralis* among the 4 mouse infection groups: uninfected, ♀ Ad-infected only, NBL-infected only, and ♀ + ♂ larvae infected. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

IgE levels in NBL-infected mice ($p < 0.05$), but lower than in ML-infected mice ($p < 0.01$).

4. Discussion

In the present study, we investigated and compared the immunological responses initiated by Ad and ML of *T. pseudospiralis*. Different responses were observed, including (1) greater levels of IL-4 and IgG1 antibody were

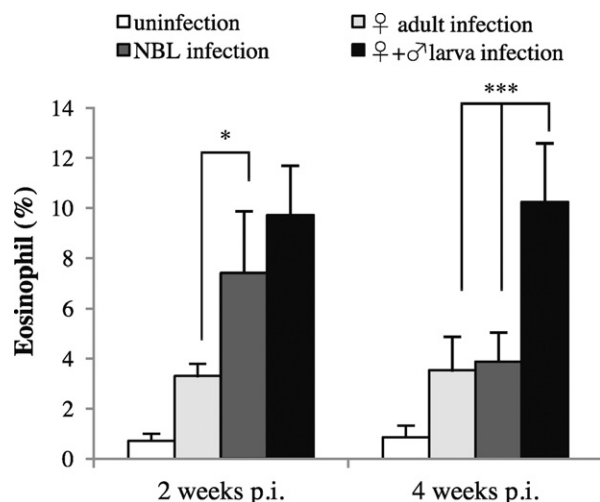


Fig. 3. Eosinophil counts among the 4 mouse infection groups: uninfected, ♀ Ad-infected only, NBL-infected only, and ♀ + ♂ larva infected. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

observed in Ad-infected mice, while higher IgG2 responses were observed in M-infected mice; (2) ML induced higher eosinophilic responses; and (3) Ad induced higher levels of IgE. It is well known that helminth infections induce strong Th2 responses. Similar to other helminth infection, *Trichinella* spp. induce a Th2 response as well in conjunction with immunoregulatory cytokines. This Th2 response has been proposed to ameliorate autoimmune diseases, EAE, T1D and inflammatory bowel disease (Khan et al., 2002; Saunders et al., 2007; Gruden-Movsesijan et al., 2008; Wu et al., 2010). The present study indicates that infection with Ad induce stronger Th2 responses than ML especially the response to the re-stimulation with Ad CWE, suggesting that Ad are a target for the study of immunoregulatory molecules. A high Th2 response to ES antigen was observed

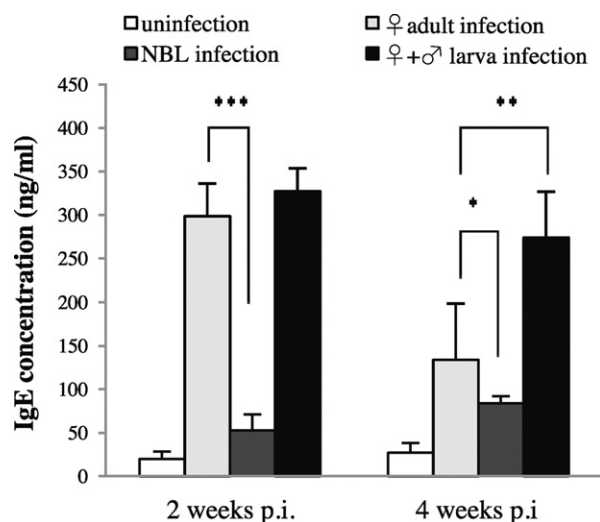


Fig. 4. Total IgE antibody among the 4 mouse infection groups: uninfected, ♀ Ad-infected only, NBL-infected only, and ♀ + ♂ larva infected. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

at 2 weeks p.i. suggesting that shared Ad and MLES antigens may play a role in initiating the Th2 response.

Th1 response to parasite infection is considered to be protective, and also partially responsible for the pathogenesis of autoimmune diseases. All 3 infecting agents i.e. female Ad, NBL and ML, induced strong Th1 responses. It was noticed that NBL induced high levels of IFN- γ in splenocytes even without antigen re-stimulation suggesting that NBL induce strong Th1 responses.

The production of IgG1-type antibodies is primarily induced by Th2-type cytokines, whereas production of IgG2a-type antibodies reflects the involvement of Th1-type cytokines (Hempel et al., 2001). The dominance of the specific IgG1 and IgE is associated with a strong Th2 response (Beiting et al., 2007). The present study indicates that Ad infections induced stronger IgG1 responses than NBL infections suggesting that Ad initiated a stronger Th2 response.

It was reported that helminth infections have protective effects on allergic diseases, although the mechanisms remain a matter of debate (Fallon and Mangan, 2007). The protective effect of helminth parasites against allergic disease may be explained by the IgE saturation hypothesis (Godfrey and Gradidge, 1976). This hypothesis was based on the observation that helminth infections stimulate extra high levels of polyclonal total IgE which in turn can saturate IgE receptors on the mast cell surface and prevent binding of allergen-specific IgE and mast cell degranulation (Fallon and Mangan, 2007). In the present study, much higher amounts of IgE were observed in mice infected with female Ad than in the NBL-infected mice at 2 weeks p.i. This may be linked to the protection of allergic diseases by *Trichinella* infection. Therefore, it is speculated that molecules from the *T. pseudospiralis* Ad could be suitable candidates in regulating allergic response.

In conclusion, the present study compared the immunological responses between Ad and ML-infected mice and demonstrated that Ad of *T. pseudospiralis* provoked Th2, IgG1 and IgE responses. The results provided some basic information for the study of the immunoregulation and parasite-derived molecules which may be linked to protective effects against autoimmune and allergic diseases.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

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