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Inflammatory response during the muscle phase of *Trichinella spiralis* and *T. pseudospiralis* infections

Received: 27 February 2001 / Accepted: 13 March 2001 / Published online: 15 May 2001
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Abstract An attempt was undertaken to determine whether a concurrent infection of *Trichinella spiralis* and *T. pseudospiralis* can reduce cellular infiltrations against the former species during the muscle phase of worm development. BALB/c, nude and CBA/N mice were orally infected with either species or a mixture of both. New-born larvae (NBL) of either or both species were also injected subcutaneously into the right/left leg of BALB/c mice. In *T. spiralis* oral infection, myositis was strongest in BALB/c, intermediate in CBA/N and weakest in nude mice. In *T. pseudospiralis* oral infection, slight cellular infiltrations were observed around the worms in BALB/c but not in nude or CBA/N mice. However, in mixed oral infections of two species, infiltrations around the sites of *T. spiralis* were not reduced. In mice injected with *T. pseudospiralis* NBL, infiltrations around the infective-stage larvae were mostly absent. However, in mice injected with *T. spiralis* NBL, prominent granulomatous reactions were observed near the sites of worms. The tissue reaction was substantially stronger than that in oral infections. In mice injected with NBL of both species (into different legs), a heavy infiltration was also observed at the site of *T. spiralis*. A marked increase in levels of IL-4 and IL-6 was detected in the popliteal lymphocytes of BALB/c mice injected with either live or dead NBL of *T. spiralis* at days 15 and 20 post-injection. This indicated that the worms mainly elicited a Th2 response during the muscle phase of development. An indirect fluorescent antibody test and laser confocal microscopic studies demonstrated the presence of CD4 and CD8 cells in the cytoplasmic region of the nurse cell complex of *T. spiralis*.

Introduction

Most studies on the immunogenesis of trichinosis have largely been based on the inflammatory and cell-mediated responses against the adult *Trichinella spiralis* located in the host’s intestine (Wakelin and Grencis 1992; Wakelin et al. 1994). Earlier reports on the muscle phase of infection mainly described the general cellular infiltrations, encapsulation and myopathology (Gabryel et al. 1995; Stewart 1995). However, the relationship between immunocompetence and the intensity of parasite-induced myositis has not been examined in detail. Also, little is known about the role of Th1 cells and the cytokine response during the development of the new-born larvae (NBL) in muscles. Even the type of lymphocytes associated with the parasite complex in situ is uncertain.

It is well known that unlike *T. spiralis*, *T. pseudospiralis* elicits little inflammatory response in muscles (Kramer et al. 1981; Stewart 1995). Presumably, the latter species can undergo immune evasion by inducing an increase of corticosterone in host plasma and by expressing host-like antigens on its cuticle (Stewart et al. 1988; Stewart and Larsen 1989; Larsen et al. 1991). However, the hypothesis awaits further testing. The different patterns of immune response between *T. pseudospiralis* and *T. spiralis* infections have also been attributed to the differences in the antigens involved in initiating and controlling the outcome of immunity (Bolaz-Fernandez and Wakelin 1990; Kehayov et al. 1991). Such antigens, however, have not been identified.

To elucidate the above host–parasite relationship, the present study was undertaken to compare the myositis generated in hosts with different immunocompetence in single- or mixed-species infections. Attempts were also undertaken to determine the cytokine response during the development of the NBL of *T. spiralis* in muscles and to identify the lymphocytes associated with the nurse cell.

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Materials and methods

Myositis studies

Mice with different immunocompetence were infected orally with Trichinella spiralis or T. pseudospiralis, or with a mixture of both species. BALB/c mice were also injected subcutaneously with new-born larvae (NBL) of either or both species. The strain of T. spiralis used was originally isolated by R.C. Ko from a pig in Ontario, Canada, in 1967 and has since been maintained in the laboratory routinely by consecutive passages through Wistar rats and ICR mice. The strain of T. pseudospiralis was kindly provided by Prof. D.L. Lee, formerly of the University of Leeds, UK.

Oral infection

Infective-stage larvae were recovered from muscles of experimentally infected ICR mice by the standard pepsin digestion method using a Baermann’s funnel. Orally, 500 larvae were given to each of the following types of mice (males, 6–8 weeks old), using a Pasteur pipette: BALB/c (H-2k, Harlan, USA), CBA/N (H-2d, Olac, UK) and nu/nu (H-2d; Animal Research Centre, Australia). Each type of mouse was given the following different treatments: single infection with T. spiralis or T. pseudospiralis, or a mixed infection of two species. Four animals were used per treatment. Uninfected mice served as the negative control.

Subcutaneous injection

NBL obtained from gravid female worms were maintained for 24 h in RPMI 1640 medium. After concentration by centrifugation, approximately 1,000 NBL were injected subcutaneously into the thigh region of a BALB/c mouse, using a tuberculin syringe and 27-gauge needle. Four groups of mice (3 animals/group) were used. Mice in groups 1 and 2 were given either T. spiralis or T. pseudospiralis NBL. Group 3 mice were injected with a mixture of both species (1:1 ratio). In Group 4, each leg of the mouse was injected with a heterologous species. The animals were examined on day 23 post-injection. Mice injected with RPMI 1640 served as the negative control.

Scanning electron microscopy

Intact nurse cells were isolated from muscles by a series of enzyme digestions, following the method of Li et al. (1999). They were fixed in cold 2.5% glutaraldehyde (Sigma) in Hank’s balanced salt solution for 24 h at 4°C. After dehydration in a graded series of ethanol, the specimens were critical-dried in carbon dioxide and then coated with gold-palladium. The specimens were examined by a scanning electron microscope (Stereoscan S440, Leica).

Histopathology and immunocytolocalization

For light microscopy, the muscles were fixed in Bouin’s solution, embedded in paraffin and sectioned at 6 µm. The tissues were stained with haematoxylin and eosin. The indirect fluorescent antitbody test (IFAT) was used to determine the type of lymphocytes at the site of infection, mainly following the method of Lee et al. (1991). Monoclonal antibodies against mouse CD8α (Sigma F-7535) and CD4 (Sigma F-7400) were diluted 1:200 and employed as the developing antibodies. The sections were examined by a laser confocal system (MRC 600, Biorad) attached to an epifluorescent microscope (Optiphot 2, Nikon). Control sections were incubated for 2 h in normal mouse serum (diluted 1:100) at room temperature.

Cytokine study

BALB/c mice (180 males, 6–8 weeks old) were divided into three groups. Group 1 mice were each injected in the hind footpad with approximately 1,000 living NBL in 25 µl of RPMI 1640 medium. Group 2 mice were each injected with 1,000 dead NBL. Group 3 mice were each injected with RPMI 1640 medium only. At days 5, 10, 15 and 20 post-injection, popliteal lymph nodes draining the footpad were removed and pooled for lymphocyte culture.

Cytokine assay

Single-cell suspensions of lymphocytes were diluted in RPMI 1640 medium supplemented with 10% fetal calf serum to provide 5×10⁶ cells/ml. A 2-ml aliquot of the final suspension (10⁷ cells) was placed into each well of a 24-well plate (Costar) and incubated for 48 h at 37°C with 4 µg of Con-A/ml. The supernatant was removed and centrifuged in a microfuge for 1 min at 15,000 g to pellet the cells. The samples were divided into aliquots and stored at −70°C until use.

IFN-γ, IL-2, IL-4 and IL-6 were measured by sandwich enzyme-linked immunosorbent assay, using commercial cytokine assay kits (Intertest-γ Genzyme, Intertest-2X Genzyme, Intertest-4X Genzyme and Cytoscreen IL-6, Biosource). The level of cytokine was quantified using the standard curve of a known amount of recombinant cytokine. The sensitivities of these assays (picograms/millilitre) were: 125 for IFN-γ, 15 for IL-2, 5 for IL-4 and 8 for IL-6.

Statistics

The data were analysed by one-way analysis of variance and Student’s t-test. A P value of <0.05 was considered statistically significant.

Results

Myositis

Oral infection

In Trichinella spiralis infection, the cellular response in muscles was strongest in BALB/c mice, intermediate in CBA/N and weakest in nude mice. In BALB/c mice, at day 15 post-infection, infected myofibres were basophilic and contained hypertrophic nuclei. Mitosis was observed in some enlarged nuclei. A small number of polymorphs and mononuclear cells were observed near the sites of worms. At day 30, prominent cellular infiltrations and fibroplasia were observed outside the well developed collagenous capsule which circumscribed the larvae. In CBA/N mice, only slight cellular infiltrations were observed around the worms at days 15 and 30 post-infection. In nude mice, little cellular infiltrations or fibroplasia could be observed near the worms.

In T. pseudospiralis oral infection, cellular infiltrations and fibroplasia were virtually absent in the three strains of mice at both days 15 and 30 post-infection. However, in the muscles of BALB/c mice fed with a mixture of T. spiralis and T. pseudospiralis, the worm burden of the former species was heavier than that of the latter, in a ratio of about 4:1. The loss of myofibrils was more evident in muscles infected with the former species. The degree of