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Suppression of host Th1-type granulomatous inflammation by *Taenia solium* metacestodes is related to down-regulation of osteopontin gene expression

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Abstract

Inflammation and granuloma formation in human neurocysticercosis has been attributed to Th1-type immune responses of the host. In the present murine model, over 94% of *Taenia solium* metacestodes were viable and elicited no granulomatous inflammation, whereas parasites killed by praziquantel treatment elicited rapid granuloma formation that calcified within 2 weeks. Osteopontin (OPN) is a Th1-related cytokine that is up-stream of IL-12 and which may play an essential role in granuloma formation and calcification. OPN mRNA expression was down-regulated in tissues surrounding viable cysticerci, but was up-regulated in inflammatory tissues surrounding degenerating cysticerci. Moreover, co-culture with a viable cysticercus or ES products from these metacestodes led to a decrease in OPN, IFN-γ and IL-12 expression, whereas co-culture with somatic proteins enhanced OPN expression by leukocytes. Addition of recombinant mouse OPN (rmOPN) counteracted the down-regulation of IL-12 and IFN-γ mRNA expression, but not OPN mRNA expression, in leukocyte cultures. Furthermore, injection of rmOPN into the tissues surrounding implanted cysticerci enhanced inflammatory responses while a similar injection of an anti-rmOPN antibody reduced inflammation. These findings suggest that the suppression of host Th1-type granulomatous inflammation by ES products from *T. solium* metacestodes is related to down-regulation of OPN gene expression.

Keywords: *Taenia solium*; OPN; Cysticercosis; Th1-type responses; IL-12; IFN-γ; Granulomatous inflammation

1. Introduction

Neurocysticercosis is a disease of the human central nervous system caused by infection with metacestodes of *Taenia solium*. In active cysticercosis, severe inflammatory changes often surround the degenerating cysticerci. However, there is an asymptomatic period that typically lasts 4–5 years between initial infection and the onset of neurological symptoms (White et al., 1997b). Moreover, viable cysts rarely cause apparent clinical manifestations; symptomatic infections generally follow degeneration of the cysticerci (White, 2000). The immune response in neurocysticercosis is predominantly of the Th1 type. Elevations in levels of IL-12, IL-18 and IFN-γ, as well as macrophage infiltration, have been demonstrated near the degenerating cysticerci. Granulomatous inflammation and tissue repair have been observed in areas surrounding the cysticerci. Furthermore, the inflammatory tissues are gradually replaced by collagenous structures that eventually lead to calcification (Restrepo et al., 1998, 2001; White, 2000). These findings suggest that granulomatous inflammation accompanies the Th1 response in active cysticercosis.

Metacestodes survive through the actions of ES products that modulate the immune response of the host. In mice infected with metacestodes of *Taenia crassiceps* or *Taenia taeniaformis*, these products may elevate the levels

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of IgG1, IL-4, IL-10 and IL-6, and suppress levels of IL-1 and Th1-related cytokines, such as IL-12, IL-2 and IFN-γ (Burger et al., 1986; White et al., 1997b; Terrazas et al., 1998; Rodriguez-Sosa et al., 2002). Moreover, a metacestode factor (MF) derived from viable cysticerci of *T. solium* suppressed lymphocyte proliferation, decreased the production of both IL-2 and IFN-γ in spleen cells from normal mice, and inhibited the production of TNF-α by macrophage cell lines in vitro (Molinari et al., 1990; Tato et al., 1995; Arechavaleta et al., 1998). However, the mechanism of these protective changes remains unclear.

Osteopontin (OPN), also known as early T lymphocyte activation-1 (Eta-1), is a secreted phosphoprotein originally isolated from the skeletal tissue and is associated with a wide range of pathological changes (Patarca et al., 1989; Denhardt and Guo, 1993). OPN is also produced by leukocytes (including macrophages, natural killer cells, T and B lymphocytes and neutrophils), epithelial cells, endothelial cells and fibroblasts in inflamed tissues (Nau et al., 1997; O'Regan and Berman, 2000; Denhardt et al., 2001; Ueno et al., 2001; Sodek et al., 2006) and plays a critical role in Th1-related inflammation, granuloma formation and calcification (Ashkar et al., 2000; O'Regan and Berman, 2000). Cell-surface glycoprotein CD44 and αVβ3 integrin are the target receptors for OPN on the membranes of inflammatory cells (Weber et al., 1996; Bennett et al., 1997; Katagiri et al., 1999). In symptomatic neurocysticercosis, up-regulation of CD44 was observed around inflammatory cysticerci and considered to play an important role for the recruitment of Th1 type inflammatory cells to the site of the lesion (Londono et al., 2002). Therefore, it seems reasonable to speculate that OPN may be related to Th1-type inflammation and granuloma formation in active cysticercosis.

In the present study, we employed a murine model to determine the relationship between OPN production and granulomatous inflammation during *T. solium* metacestode infection. The results showed that viable metacestodes are able to down-regulate OPN gene expression which, in turn, causes a reduction in the expression of Th1-related IL-12 and IFN-γ and inhibits the formation of inflammatory granulomas.

2. Materials and methods

2.1. Parasites and laboratory animals

Adult worms of *T. solium* were collected from taeniasis patients following informed consent of the patients in Jinshui Hospital, Henan Province, People's Republic of China after treatment with Atabrine. This study was approved by the National Yang-Ming University Ethical Committee and all procedures were carried out in accordance with institutional ethical guidelines. The worms were washed in normal saline, packed safely and transported to our laboratory in Taipei within 1 week. Eggs were isolated from the last 10 gravid proglottids of the worms and stored in sterile PBS with gentamycin (3 mg/L) at 4 °C and used within 1 month. Hatching of oncospheres was carried out by the sodium hypochlorite method (Lightowlers et al., 1984; Wang et al., 1997). In all experimental processes, strict safety regulations were followed to avoid contamination of the work area. Researchers wore rubber gloves and isolation gowns. After use, all utensils were soaked overnight in 0.5% sodium hypochlorite solution to inactivate remaining oncospheres.

Female C3H/HeN mice (4 weeks old, purchased from National Laboratory Animal Center, Taipei) were used in this study. Mice were housed in a specific pathogen-free (SPF) animal room at 25 °C, and provided with a commercial pelleted diet and water ad libitum. Animal experiments were conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals (DHHS publication No. NIH 85-23, revised 1996).

2.2. Preparation of tissue specimens

Each mouse received a s.c. injection in the abdominal region of 5000 oncospheres in 0.2 ml PBS with gentamycin (3 mg/L) (Wang et al., 1999). Five mice were sacrificed weekly from 1–10 weeks p.i. for metacestode recovery and status assessment.

For histopathological studies, sandwich-like specimens (1 cm × 1 cm square) consisting of host skin, a cysticercus and host s.c. tissue were collected and fixed immediately in 10% neutral formalin. The fixed specimens were embedded in paraffin and cut into 5 μm-thick sections. These sections were stained with H&E and examined microscopically. In the chemotherapy group, mice were treated with praziquantel (25 mg/kg/day) at 4 weeks p.i. for 3 days and sacrificed at 6 weeks p.i. Tissue specimens were collected and processed as above for examination.

In addition, specimens (0.1 cm^3) of tissue surrounding 6-week-old viable cysticerci or cysticerci degenerating due to praziquantel treatment were collected for gene expression analysis. Tissue specimens collected from similar locations of the body from uninfected mice similarly treated with praziquantel served as controls. All specimens were stored in liquid nitrogen until used.

The effects of exogenous OPN on host inflammatory responses were assessed by the following procedures. Mice were implanted s.c. with three viable 8-week-old cysticerci according to Molinari et al. (1998). Under anaesthesia, an 8–10 mm incision was made in the abdominal region of the mouse. Subcutaneous tissues around the incision were separated with forceps, three viable cysticerci were implanted and the surgical wound was sutured. Preparations of PBS (0.2 ml), recombinant mouse OPN (rmOPN) (R&D System, Minneapolis, MN, USA) (0.2 μg/3 days) or anti-rmOPN antibodies (R&D System) (0.2 μg/3 days) were injected s.c. into the vicinity of the inoculation sites from the day of implantation until the day of sacrifice at 4 weeks post-implantation. Sandwich specimens (1 cm × 1 cm square) were collected and processed as above for histopathological studies.