Impairment of the inflammatory reaction on implanted *Taenia solium* metacestodes in mice by a *T. solium* RNA-peptide: a scanning electron microscopy study

**Abstract** Inhibition of inflammation by a *Taenia solium* RNA-peptide (metacestode factor, MF) was studied by scanning electron microscopy (SEM). Viable (96%) *T. solium* metacestodes obtained from a naturally infected pig were dissected and implanted in treated and control mice, removed at 6 and 12 days postimplantation (p.i.), and studied by SEM. At day 6, metacestodes in control mice showed vigorous inflammation, whereas in mice treated with MF they were apparently intact with exiguous inflammation. Mice immunized with *T. solium* metacestode antigens showed a moderate inflammation; those treated with both MF and *T. solium* antigens presented scanty inflammation. At day 12, metacestodes presented copious inflammation and severe damage to the sucker tissues in mice immunized with *T. solium*; in mice treated with either MF or MF and *T. solium* antigens there was only discrete inflammation. These observations illustrate the central role of MF in the inhibition of the early events leading to the parasite’s destruction by means of an inflammatory response.

**Introduction**

*Taenia solium* human neurocysticercosis follows a prolonged asymptomatic period (typically 4–5 years) before the onset of symptoms (Dixon and Lipscomb 1961). Rabiela et al. (1982), Ridaura (1987), and White et al. (1995) have reported that parasites without inflammation are more frequent in asymptomatic and immunodeficient patients. Another sign of immune depression in human neurocysticercosis is the negative antibody response that is often observed in asymptomatic patients and in those without inflammatory cells in their cerebrospinal fluid (Ridaura 1987; del Brutto and Sotelo 1988). In porcine cysticercosis, metacestodes without inflammatory reaction have also been reported (de Aluja and Vargas 1988). A close relationship between high metacestode viability (94–96%), a discrete inflammatory reaction around metacestodes, and an immunosuppressed state in naturally cysticercotic pigs has been reported (Molinari et al. 1993). Therefore, it appears that the clinical manifestations of human neurocysticercosis are closely related to the degree of the inflammatory reaction surrounding *T. solium* metacestodes.

In the early stage of illness, inflammation and humoral response seem to be two immunological functions inhibited by *T. solium* metacestodes. Research on molecules obtained from *T. solium* that inhibit the immune response has yielded some insight into this problem. A small RNA-peptide molecule (1,450 Da; metacestode factor, MF) obtained from cultures of viable *T. solium* metacestodes depresses the phytohemagglutinin- or concanavalin-A-stimulated incorporation of $[^{3}H]$-thymidine into human or murine lymphocytes (Molinari et al. 1990; Tato et al. 1995). In mice, MF inhibits the immune response to *Salmonella typhimurium* antigens (Molinari et al. 1989). In other studies we have observed that in mice, MF prevents the inflammatory reaction around implanted *T. solium* metacestodes and depresses humoral and cellular responses to homologous antigens; in addition, we have found that in comparison with the
natural host, naive and primarily immunized mice produce a faster and more vigorous inflammatory response against the implanted metacestodes (Tato et al. 1996). Since in human and pig cysticercosis the inflammatory reaction around the parasite is a slow, insidious, and progressive process that may last several months or years (Márquez 1971; Hernández-Jáuregui et al. 1973; Willms et al. 1980; Rabiela et al. 1982; Escobar 1983; Villagran and Olvera 1988; de Aluja and Vargas 1988), the characteristics of the inflammatory reaction induced by implanted cysticerci in the murine model could be used to study immune and inflammatory reactions in a short period. Therefore, we conducted the present study to determine by scanning electron microscopy some of the early events of the inhibition of the inflammatory reaction induced by the *T. solium* MF on implanted *T. solium* metacestodes in naive and immunized mice.

### Materials and methods

#### Metacestode factor

MF was obtained as described by Tato et al. (1995). Cysticercotic pigs aged 5–8 months were acquired from villages in an endemic area of porcine cysticercosis in Mexico. Metacestodes were carefully dissected from the parasitized tissues and incubated in 500 ml of distilled deionized water (DDW, 6 h, 23 °C). After centrifugation at 15,000 rpm for 15 min, the supernatant was lyophilized, suspended in 5 ml of DDW, and dialyzed in a Spectrapor 3 dialysis tube (Spectrum Med. Ind., molecular weight cut-off 3,500 Da) against 100 ml of sterile saline at 4 °C in autoclaved glassware. After 12 h the material outside the bag was lyophilized and stored at 4 °C. To determine the amount of RNA and protein contained in the lyophilized material (to be used in the experiments), we measured ribose and protein concentrations according to the methods of Ashwell (1957) and Lowry et al. (1951), respectively.

#### Viability test

We measured the viability of the metacestodes before and after obtaining the MF. The viability tests were carried out by evaluation of 50 metacestodes for scolex evagination according to Cañedo et al. (1982).

*Taenia solium* metacestode antigens

The antigens from *T. solium* were obtained as described previously (Molinari et al. 1993). In brief, dissected metacestodes from parasitized meat were blended for 6 s in a Sorvall Omni-mixer; the homogenized metacestodes were suspended in 0.02 M sodium phosphate buffer (pH 7.2) containing 0.4% sodium deoxycholate and 20 µg deoxyribonuclease/ml (Sigma) and then ground with glass sand in a mortar for 2 h at room temperature. The material was centrifuged at 1,500 rpm for 10 min, and the supernatant was dialyzed against 0.02 M sodium phosphate buffer (pH 7.2) for 6 days and subsequently centrifuged at 15,000 rpm for 30 min. The supernatant was removed and its protein concentration, determined (Lowry et al. 1951). Aliquots (3 ml) were filtered though Millipore (Bedford, Mass.) membranes (0.22 µm) into sterile 10-ml vials, lyophilized, and stored at −20 °C.

#### Experimental groups

Female BALB/c mice weighing 20 ± 2 g from our bioterium were used in all experiments. Treatments were designed as follows:

- (a) control group – 0.1 ml of sterile saline solution was inoculated by the s.c. route into eight naive mice;
- (b) MF group – 100 µg of MF (estimated as the ribose concentration) dissolved in 0.1 ml of sterile saline solution was inoculated s.c. at 4-day intervals, with a total of four doses being inoculated into four mice and five doses, into another four mice;
- (c) immunized group – a single dose of 100 µg of *T. solium* metacestode antigens dissolved in 0.1 ml of sterile saline was inoculated s.c. into eight mice; and
- (d) immunized and MF group – a single dose of 100 µg of *T. solium* metacestode antigens and a first dose of 100 µg of MF were separately inoculated s.c. into eight mice, with three additional doses of MF (100 µg each) being injected at intervals of 4 days into four of these mice and four doses, into the other four.

Metacestodes and implantation assays

A 6-month-old cysticercotic pig was selected and purchased in Sabana Grande, Iguala, Guerrero, Mexico, and brought to local porcine facilities. The pig was killed and metacestodes for viability assays were dissected from the parasitized meat. An assay for scoloc evagination was carried out according to the method described by Cañedo et al. (1982). Only when scolex evagination was above 94% were other metacestodes carefully dissected under sterile conditions and used for implantation; only unbroken metacestodes were implanted. Four mice were used per group. Implantation of metacestodes by the s.c. route was done 8 days after the beginning of treatment according to Tato et al. (1996). Mice were anesthetized with chloroform and an area of skin (4 cm²) in the back was shaved and cleaned with isopropanol. An incision of 8–10 mm was made and s.c. tissue around the incision was dissected with forceps; six intact metacestodes were implanted under the skin of each mouse and the surgical wound was sutured.

#### Scanning electron microscopy study

Implanted metacestodes were removed from mice at 6 and 12 days after implantation and were fixed in 3% buffered glutaraldehyde for 2 h; the samples were washed with 0.1 M phosphate buffer and dehydrated with increasing concentrations of ethanol (from 30% to 100%). Samples were dried to critical point in a Samdri-780 device (Tousims). Afterward they were covered with a fine coat of gold (approximately 300 Å) in a Jeol Sputter FC 1100 (Jeol) operating at 1.200 kV and 5 mA for 6 min. Samples were analyzed and micrographs were taken in a scanning electron microscope (Jeol, JSM-5410LV) at low vacuum (25 Pascal) and at a working distance of 10 mm.

### Results

#### Metacestode viability

A high degree of viability (96% and 94% scolex evagination) was observed in metacestodes that had been either freshly dissected from parasitized meat or kept in deionized water for 6 h.

#### Scanning electron microscopy studies

After 6 days of implantation the scanning electron microscopy (SEM) studies showed that s.c. implanted *Taenia solium* metacestodes in naive mice (control group) induced an intense inflammatory reaction to the implanted parasites; copious inflammatory host cells and cell debris were found adhering to the external
surface of the bladder wall tegments of broken and deteriorated parasites (Fig. 1); inflammatory cells could be seen on the exposed subtegumental tissue. Numerous white cells and cell debris had accumulated at the bladder wall entrance and in the spaces (spiral canal) formed between the folds that appear during scolex invagination (Fig. 2). In contrast, no alteration was observed in the external structures of implanted metacestodes removed from the MF group; the bladder wall tegumental tissue of these metacestodes was apparently intact, although some inflammatory cells were in contact with its surface (Fig. 3). The tegument folds and the spiral canal at the bladder wall entrance were intact and almost clean, except for the presence of a few inflammatory cells (Fig. 4).

In the immunized group, metacestodes showed a discrete inflammatory reaction on the scolex tegument, but the reaction was rather intense on the bladder wall tegument. A representative sample showed some cells disseminated in the double crown of hooks, on the suckers, and on the neck tegument, whereas the bladder wall tegument exhibited an intense inflammatory reaction that formed a dense net of fibrous material embedding numerous inflammatory cells (Fig. 5).

A comparison of this sample with those obtained from both the control and MF groups (Figs. 1 and 3) could be misleading; the images from the control and MF samples show only the bladder wall surface, whereas the one from the immunized group shows both the

Fig. 1 Scanning electron micrograph of a *Taenia solium* metacestode removed from a control mouse at day 6. The bladder wall is seriously damaged; the specimen has lost a large portion of its bladder wall tegument, whereas other regions of this structure are fragmented. Note that the exposed surfaces of both the tegumental (T) and the subtegumental (ST) bladder walls are deteriorated and covered by numerous inflammatory cells and debris. Fig. 2 Scanning electron micrograph from the tegument entrance region of a metacestode removed from a control mouse at day 6. The folds (F) and their spaces (S) exhibit many inflammatory cells and debris (arrows). Fig. 3 Scanning electron micrograph of a metacestode removed from an MF-treated mouse at day 6. The external tegument of the bladder wall is apparently intact; the folds and the spaces at the entrance also exhibit structural intactness. Fig. 4 Higher magnification of the region indicated in Fig. 3. The bladder wall entrance is almost clean, except for a few inflammatory cells and debris (arrows).
bladder wall and scolex teguments. In a comparison of these three samples' bladder wall tegument, the outermost structure of the cysticerci, a similar inflammatory reaction was noted in the control and immunized groups (Figs. 1 and 5), and a weaker reaction was seen in the MF group (Fig. 3). In the specimen from the immunized group, whose scolex had evaginated, the more internal scolex tegument structures showed a markedly weaker inflammatory reaction than did the external bladder wall tegument. In contrast, metacestodes removed from mice inoculated with *T. solium* metacestode antigens and MF exhibited few inflammatory cells on their bladder wall tegument. A sample from this group showed a slightly damaged bladder wall tegument without inflammatory cells (Fig. 6).

Metacestodes removed after 12 days from the control group were completely covered by an intense inflammatory reaction. A sample showed a dense collagen-like matrix embedding numerous cells and covering the whole bladder-wall-tegument tissue (Fig. 7); the density of this matrix did not allow a quantitative assessment of the cells participating in the inflammatory reaction. In comparison, metacestodes removed from the MF group at day 12 showed an inflammatory reaction slightly stronger than that observed at day 6. One of these specimens had partially evaginated its scolex during

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**Fig. 5** Scanning electron micrograph of a metacestode removed from an immunized mouse at day 6. The scolex is evaginated; inflammatory cells can be seen scattered among the hooks (*H*), on the suckers (*S*), and on the strobilar tegument (*ST*). At the lower left a portion of the bladder wall tegument (*BT*) can be seen covered by an intense inflammatory response formed by uncountable cells embedded in a fibrous matrix.

**Fig. 6** Scanning electron micrograph of a metacestode portion removed from an immunized and MF-treated mouse at day 6. Only a region of the bladder wall tegument is apparently damaged (*arrows*); the rest is intact and exhibits only scarce inflammatory debris.

**Fig. 7** Scanning electron micrograph of a metacestode portion removed from a control mouse at day 12. The bladder wall tegument cannot be seen since it is completely covered by an intricate and dense net of fibers embedding uncountable inflammatory cells (*C*).

**Fig. 8** Scanning electron micrograph of a partially evaginated scolex removed from a control mouse at day 12. Note the apparently intact scolex tegument, with scattered inflammatory cells adhering mainly to the tegument folds (*arrows*)
implantation (Fig. 8); the scolex tegument was apparently intact and displayed on its surface, especially in the folds of the neck tegument, a low number of inflammatory cells (probably eosinophils, E), several particles (probably eosinophil-like granules, EG), and fibers (presumably collagen, F) can be seen adhering to the microtriches. Fig. 10 Scanning electron micrograph of a completely evaginated scolex removed from an immunized mouse at day 12. An intense inflammatory reaction completely covers the scolex tegument. Note the large cell aggregates between and in the two deteriorated suckers and several ruptures of the sucker and rostellum teguments.

Fig. 11 Higher magnification of the right sucker illustrated in Fig. 10, showing various degrees of damage to its tissues. Note the sloughs from the outer layers on both the lower left and right sections of the sucker.

Fig. 12 Higher magnification of a region shown in Fig. 10. Note the uncountable inflammatory cells, eosinophil-like granules, debris, and dense fibrinous material covering many of these cells.

In contrast, at day 12, metacestodes from the immunized group exhibited a much stronger inflammatory reaction on the scolex tegument than did those removed at day 6, which demonstrates that there was a slow (as compared with the reaction against the bladder wall tegument) inflammatory reaction to the more internal scolex tissues. A representative specimen displayed an evaginated scolex, whose whole tegument was covered by a copious inflammatory process formed by numerous single or aggregated cells (Fig. 10). Areas of severe damage were observed primarily on the tegument and subtegumental sucker tissues; suckers showed ruptures of differing size and depth (Fig. 11). A higher-magnifi-
cation (×1,500) image of the scolex tegument of this specimen revealed an intense accumulation of different kinds of white cells, cell debris, and a dense layer of fibrinoid material (Fig. 12); all these structures completely covered the tegumental surface, making an exact quantitative analysis unachievable.

Finally, SEM analysis on metacestodes obtained from mice treated with both *T. solium* antigens and MF showed an inflammatory reaction that was less extensive on the bladder wall tegument than that observed on metacestodes from the immunized or control groups; a representative sample showed only a few white blood cells, cell debris, and fibrous material adhering to its apparently intact microtriches (Fig. 13).

Discussion

In this study, *Taenia solium* metacestodes implanted s.c. in mice triggered an intense inflammatory reaction. It is likely that the intensity of this inflammatory reaction was partially due to the observation that the mouse is not a naturally permissive host. In this context, the murine response to live implanted metacestodes may not be representative of the natural host’s usual response. However, the *T. solium* MF, (a small RNA-peptide molecule, 1,450 Da, liberated in vitro by viable *T. solium* metacestodes; Tato et al. 1995) induced inhibition of the inflammatory reaction to metacestodes implanted in both naive and immunized mice. The arrangement of the inflammatory processes in many of the samples studied, which included white blood cells, debris, fibrinoid material, collagen, and other structures, did not allow us to perform a quantitative analysis of the inflammatory reaction. Nevertheless, an evident qualitative difference in inflammatory intensity was noted between metacestodes removed from mice in the control group and those removed from the MF-treated group as well as between metacestodes obtained from the immunized group and those taken from mice that had been immunized and treated with MF. Related results obtained by light microscopy have shown that MF inhibits the inflammatory reaction surrounding implanted *T. solium* metacestodes in mice; it also suppresses humoral and cellular responses to metacestode antigens (Tato et al. 1996). In the present experiments the implanted metacestodes were larger than those used in the former report; therefore, they were not completely destroyed by the inflammatory reaction (as occurred with the metacestodes employed previously) within the observation period (12 days). This circumstance allowed us to carry out observations of the interactions between implanted *T. solium* metacestodes and the experimental host’s immune system at an earlier stage of the inflammatory process.

Several scolices had evaginated while they were located s.c. in mice, a parameter indicating that metacestodes survived for several days in a nonnatural host. Prominent inflammatory reactions were observed when metacestodes were implanted in both control and immunized mice, a situation that produced obvious and severe damage to the bladder wall tegument. In the case of the control group a uniform inflammatory reaction covered the outermost surface of the parasites, involving both tegumental and subtegumental tissues. The samples from the immunized group showed a difference in inflammatory intensity between the bladder wall and the scolex teguments. This situation may arise from the nature of the inflammatory process itself, since inflammatory cells first arrive at the external bladder wall tegument and, at a later stage, enter the parasite through the spiral canals leading to invaginated teguments (strobila and scolex). It should be pointed out that the *T. solium* antigen preparation used to immunize the animals did not contain any MF, since it had been dialyzed for 6 days (see Materials and methods), thus eliminating the possibility of the factor’s exerting an influence on the differential development of inflammation against the parasite’s external and internal structures.

In contrast, mice treated either with MF or with both *T. solium* antigens and MF produced only slight inflammatory responses against the implanted metacestodes, a situation suggesting that MF triggers an impairment of the immune response to *T. solium* metacestode antigens. Given that MF is produced by *T. solium* metacestodes, why do implanted parasites
induce inflammation in control animals? First, the mouse is not the natural host of *T. solium*, and this can lead to a disproportionately extreme and rapid inflammatory response. Second, the amount of MF possibly released by the six experimentally implanted metacestodes is minimal as compared with the purified and highly concentrated (100 μg/dose) MF used in the MF-treated group; hence, the ability of the control host to develop a vigorous inflammatory reaction.

The diversity of the cells constituting the inflammatory reaction to the metacestodes was evident, particularly in immunized mice; SEM, however, did not allow us to identify these cells. Experimental implants of *T. solium* in naive and immunized mice induced an intense inflammatory response formed mainly by eosinophils and neutrophils along with plasma cells, lymphocytes, and histiocytes (Tato et al. 1996). In the present study the inflammatory cells were not identified but, given the consistency of the results obtained in the present and the previous investigations (Tato et al. 1996), it would be reasonable to assume that eosinophils and neutrophils were the most abundant cell types involved in the inflammatory reaction and subsequent destruction of the parasite. However, the events described in this report occurred at an earlier stage than those previously communicated (Tato et al. 1996), a situation that sheds light on the development of inflammation against the parasite. We previously reported in this report occurred at an earlier stage than those previously communicated (Tato et al. 1996), a situation that sheds light on the development of inflammation against the parasite. We previously reported that an intense inflammatory reaction around *T. solium* metacestodes completely destroyed 40% of the parasites from pigs immunized with *T. solium* metacestode antigens and infected with *T. solium* eggs; on the other hand, only a discrete inflammatory reaction was detected around intact metacestodes from control pigs (Molinari et al. 1983a). Intense inflammation and destruction of metacestodes have been also described in naturally cysticercotic pigs immunized with *T. solium* antigens, contrasting with a discrete inflammatory reaction around metacestodes dissected from naturally cysticercotic controls (Molinari et al. 1983b). In both reports, eosinophils were considered to play a pivotal role in the destruction of metacestodes from immunized pigs.

Studies using MF in lymphocyte proliferation assays have revealed that *T. solium* MF suppresses either [3H]-thymidine incorporation or production of cytokines [interleukin 2 (IL-2), interferon-γ (IFN-γ), IL-4, and tumor necrosis factor-alpha (TNF-α)] (Arechavaleta et al. 1997). The in vitro suppressive effect of MF on T-lymphocytes and macrophages and on the production of anti-inflammatory cytokines suggests that in vivo, living metacestodes may release this anti-inflammatory product to create an environment favorable to its permanence in the host. Thus, *T. solium* MF would be another of the previously described immunomodulatory products secreted by taeniids, which include paramyosin that inhibits C1q (Laclette et al. 1992), sulfated polysaccharides that activate and consume complement (Hammerberg and Williams 1978), and taeniaestatin that inhibits both the classic and the alternative complement pathways (Suquet et al. 1984) and markedly impairs mitogen-stimulated proliferation and IL-2 production in mouse thymocytes (Leid et al. 1984, 1986). A *T. solium*-released proteinase that digests IgG has also been described (White et al. 1992), as have *Echinococcus multilocularis* glycosphingolipids that inhibit the human mononuclear proliferative response (Persat et al. 1996). The long incubation period observed in neurocysticercosis (Dixon and Lipscomb 1961) without an important inflammatory reaction around metacestodes or a clinical manifestation (Rabiala et al. 1982; Ridaura 1987; del Brutto and Sotelo 1988; White et al. 1995) may be due to the release by the parasite of substances (such as those described above) that modulate the host’s immune responses.

The present study confirms the inhibitory effect of MF on the host immune response and shows the early *T. solium* metacestode-antigen-induced destructive events on *T. solium* metacestode tegumental structures mediated by the cellular immune response in mice.

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