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ORIGINAL PAPER

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Taenia solium cysticercosis: lymphocytes in the inflammatory reaction in naturally infected pigs

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Abstract Immunohistochemistry was used to examine the type of lymphocytes in muscle taeniosis-cysticercosis in naturally infected pigs. The inflammatory response studied was classified into lesions of grades 1, 3, and 5. In grade 1, with a minimal inflammatory infiltrate consisting of eosinophils and a few mononuclear cells, the immunostaining showed more CD4+ cells than CD8+ cells and IgM cells. In grade 3, when the granulomatous reaction was not yet well developed and the destruction of the parasite began, CD4+ and Ig M+ were the predominant cells, although CD8+ cells showed a notable increase. In grade 5, with a few parasitic structures surrounded by an extensive granulomatous infiltrate, lymphocyte subsets were decreased in number and did not show differences from grade 1 except for IgM+ cells, which remained increased. The organization of an active inflammatory response against the metacestode of *Taenia solium* in pigs includes the sequential participation of CD4+, CD8+ and IgM+ lymphocytes.

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Introduction

Human and porcine taeniosis-cysticercosis is still an important public health problem in Mexico and other developing countries where pork is consumed, standards of hygiene are low and meat inspection is neglected (Acevedo 1982; Aluja 1982). In human beings the disease is most commonly localized in the brain and is often accompanied by severe neurological problems (Del Bruto and Sotelo 1988), high hospital costs and great suffering of the affected persons as well as their families. In addition, porcine cysticercosis causes losses for the producer because the affected carcass must be destroyed (Acevedo 1982).

The metacestode of *Taenia solium* in muscle, brain or other organs leads to the development of an inflammatory response when the immune system recognizes it. The inflammatory reaction observed in pigs inoculated with eggs of *T. solium* has been described (Aluja and Vargas 1988). The inflammatory response is characterized by neutrophils, eosinophils, macrophages, giant cells, lymphocytes and monocytes. Lymphocytes appear to play an important role in the destruction of the parasite, being numerous in a granulomatous response and forming structures comparable to lymphoid follicles (Aluja and Vargas 1988).

The objective of this study was to identify lymphocyte subsets in muscles infected naturally with the metacestode of *T. solium* using immunohistochemical techniques, and correlating these with the different histological grades of infection (Aluja and Vargas 1988).

Materials and methods

Skeletal muscles were obtained from ten adult pigs with heavy, natural infections of the metacestode of *T. solium*. The pigs were humanely killed and cysticercosis was confirmed by visual diagnosis. Tissue specimens were obtained immediately and embedded in O.C.T. compound (Tissue-Tek, Sakura Finetek, Torrance, Calif.) and stored at –70°C. Cryosections cut at 8 μm were air-dried, fixed in acetone for 20 min at room temperature. Thereafter

sections were processed with a conventional immunohistochemistry technique using a 1:500 dilution of primary monoclonal antibodies specific to pig CD4, CD8 and IgM (VMRD, Pullman, Wash.). A biotinylated rabbit anti-mouse IgG (Zymed, San Francisco, Calif.) and a horseradish peroxidase-conjugated avidin-biotin complex (Dako, Carpinteria, Calif.) were used according to the directions of the suppliers and the chromogen was 3,3′diaminobenzidine tetrachloride (Sigma, St. Louis, Mo.). Tissue sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. Negative controls consisted of sections incubated without primary antibody.

Lymphocyte subset quantification

Sections were examined with a 40× objective lens. A total of 15 microscopic fields were selected for each grade (Aluja and Vargas 1988) and an independent evaluation by two of us (M. Ustarroz and A. Pérez-Torres) was made. Quantification was not entirely random, because the fields were selected to avoid the repeated counting of the same cells. Only cells showing a dark brown immunostaining and a visible nucleus were counted as positive.

Statistical analysis

Cell counts were expressed as mean ± SEM of positive cells in each grade and were analyzed using a Student's *t*-test. A confidence level of $P < 0.01$ was considered significant.

Results

The lesions characterized by a minimal focal inflammatory infiltrate were classified as histopathological grade 1. The focal cellular response contained some CD8+ cells, more CD4+ cells and also a few IgM+ cells (Fig. 1). However, the majority of inflammatory cells were negative to the monoclonal antibodies used, and were identified as eosinophils and mononuclear cells.

The lesions formed by a cavity containing a metacestode and surrounded by a granulomatous reaction of variable degree were classified as histopathological grade 3. Here, CD8+ cells had an increased score, but CD4+ cells and IgM+ cells showed a clear predominance in all areas of the granulomatous reaction observed (Fig. 1). The histopathological grade 5, formed by a granulomatous reaction around a cavity containing necrotic debris practically without parasitic structures, had a similar distribution pattern of positive cells to grade 1 lesions, except that IgM+ cells remained significantly increased (Fig. 1). Clusters of IgM+ cells were observed in some tissue sections.

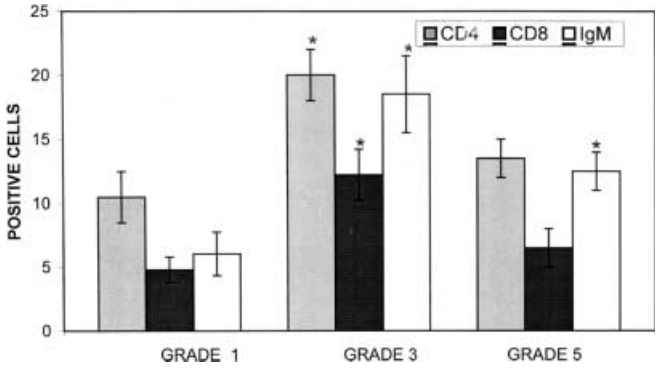

Fig. 1 Number of lymphocyte subsets in the inflammatory reaction in muscle of naturally infected pigs with the metacestode of *Taenia solium*. In grade 1 lesions (minimal inflammatory response with metacestode), CD4+ cells predominate over CD8+ cells. The three types of cells showed a significant increase in grade 3 lesions (granulomatous reaction and the destruction of the parasite begins). In grade 5 lesions (granulomatous reaction without parasitic structures) only IgM+ cells maintain a significant increase, whereas CD4+ and CD8+ cells were similar to grade 1. *Asterisks* denote statistically significant difference ($P < 0.01$)

Discussion

The present study has demonstrated the presence of T cells and B cells associated with a histopathological grade 1 inflammatory reaction, and intense granulomatous inflammation (histopathological grade 5) in muscle cysticercosis of *T. solium* in naturally infected pigs. CD4+ cells were the first type of lymphocytes that increased in number when the parasites were morphologically intact. In histopathological grade 3, CD4+ and IgM+ cells increased considerably; the capsular tegument of the parasites was degenerated, a change which demonstrates that the parasite is dying. This cellular response continued, although to a minor degree, in grade 5 lesions, where recognizable structures of the parasites were absent due to their complete degeneration, except for calcareous corpuscles and hooks. Other cells that were not immunostained in grade 1 lesions probably corresponded to neutrophils, eosinophils and other inflammatory cells. Unstained macrophages, fibroblasts and fibrocytes, appeared in the infiltrate along with CD4+, IgM+, and CD8+ cells in lesions with destroyed cysticerci as observed in histopathological grades 3 and 5.

Our results complete the histopathological findings described elsewhere (Aluja and Vargas 1988). These findings correlate with the immunohistological results found in muscular granuloma of pigs naturally infected with the metacestode of *T. solium*. It is interesting to note that the presence of circulating antibodies determined by enzyme immunotransfer blot in infected pigs seems to correlate with the findings reported here. A recent study found that when the majority of lesions caused by the metacestodes were histologically graded as 5 or 6, antibodies were either very low or no longer present (Aluja et al. 1999).

It has been proposed that a number of the parasite molecules produced in the early stages of cysticercus lesions induce immunosuppression and inhibit inflammation (Letonja and Hammerberg 1983; Leid et al. 1987; Laclette et al. 1992; Tato et al. 1995, 1996), and a granulomatous response occurs when the parasites are dying (Tato et al. 1995). However, it has been clearly observed that the host expresses not only a Th1 response when the parasite is in its vesicular form (histopathological grades 1 and 2), but that the response also

includes the presence of CD8+ cells, which increase in number when the granulomatous reaction is not yet completely developed (histopathological grade 3) and the parasite is being destroyed. This suggests that they have a cytotoxic role like the CD8+ lymphocytes of other mammalian species. This is also supported by the presence of CD8+ cells in different proportions, according to the inflammatory reaction to the metacestode of *T. solium* (Aluja and Vargas 1988).

Further studies, such as the double immunostaining of histopathological lesions and cytokine message by in situ hybridization, are needed to elucidate the role of T cells (and B cells) located in the close vicinity to the cysticerci of *T. solium*. We think that the correlation between the functional state of T cells within the granulomatous reaction and the generalized immune response could be relevant in the pathogenesis of cysticercosis in natural and experimentally infected pigs, and also in the murine model (Robinson et al. 1997).

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