Taenia crassiceps cysticercosis: A role for prostaglandin E2 in susceptibility

**Abstract** Several biological factors are involved in susceptibility and resistance to murine cysticercosis. A substantial body of evidence implicates prostaglandins as potent regulators of immune responses during parasitic diseases. Here we evaluated the role played by prostaglandin E2 in cysticercosis. Mice were treated in vivo with prostaglandin E2 or with indomethacin (a prostaglandin E2 synthesis inhibitor) before infection. Parasite growth was enhanced by prostaglandin treatment, which provoked poor Con-A responses, low Th1-type cytokines secretion, and high levels of IL-6 and IL-10. In contrast, mice receiving indomethacin showed a reduction in parasite load parallel to a strong Con-A response and high levels of IL-2 and IFN-γ, concomitantly with a decrease in IL-4, IL-6 and IL-10 production. Indirect in vitro studies suggest that an important source of prostaglandin E2 production could be related to host’s adherent cells. However, prostaglandin E2 from parasite origin cannot be discarded.

**Introduction**

It is well known that a number of diverse parasitic species such as Toxoplasma (Susuki and Kobayashi 1981), Plasmodium (Mshana et al. 1990), Trypanosoma (Tarleton 1988; Silva et al. 1992), and Leishmania (Proudfoot et al. 1995) exert an immunomodulatory activity causing potentially host-protective effector responses to be down-regulated. These anomalies are related to disturbances in the production and utilization of IL-2, activation of CD8+ suppressor T cells and the generation of indomethacin sensitive suppressor mechanisms.

Parasitic helminth infections which have similar features such as long-term persistence within the host and complex developmental cycles, are also frequently associated with specific or nonspecific immunosuppression (Maizels et al. 1993). Helminth parasites are known to express different mechanisms for immunological evasion in their hosts, such as immunoglobulin-cleaving proteases, protease inhibitors and antioxidants (Maizels et al. 1993), immunosuppressor factors (Arechavaleta et al. 1998), and other factors like paramyosin (Lalette et al. 1992), sulfated proteoglycans and PGE2 (Maizels et al. 1993; White et al. 1997), all aimed to maintain the infection.

Amongst those parasitic helminths that have been reported to directly produce PGE2, as a form to evade or modulate host’s responses are Brugia malayi, Wuchereria bancrofti (Liu et al. 1992), and Taenia tae-niformis (Maizels et al. 1993; White et al. 1997). In contrast, other helminths such as Schistosoma mansoni can induce PGE2 production by host’s cells mediated by parasite’s products (Velupillai and Harn 1994).

During the infection with T. crassiceps in a murine model of cysticercosis, the Th1-type immune response, which is at least partially protective (Robinson et al. 1997; Terrazas et al. 1999) is down-regulated; while Th2-type response is promoted (Villa and Khun 1996; Terrazas et al. 1998). However, the mechanisms that induce this immune polarization are unknown.

In recent years, a number of regulatory factors have been described as having the capacity to block macrophage functions. These molecules – also termed “macrophage-deactivating factors” – include prostaglandins of the E series, TGF-β, IL-4 and IL-10 (Strassmann et al. 1994). In cysticercosis by T. crassiceps and...
T. solium, the mechanisms employed to evade host immunity are still poorly understood (Flisser et al. 1991; Laclette et al. 1992; Arechavaleta et al. 1998). However, in the murine model, IL-10, but not IL-4, has been found to be associated with a decreased resistance to the parasite (Terrazas et al. 1999). The role of PGE2 has not been studied for this infection.

PGE2 is the major product of the arachidonic acid metabolism and it is secreted by antigen presenting cells such as macrophages (Edwards et al. 1986). In the immune system it is well documented that PGE2 acts as an immunomodulator promoting down-regulation of diverse cell’s functions such as IL-2 and IFN-γ production by T-cells (Hilkens et al. 1995), production of IL-12 and TNF-α by activated macrophages (Strassmann et al. 1994) and natural killer activity (Goto et al. 1983). Correspondingly it also promotes Th2-type responses (Parker et al. 1995).

Here we wanted to know whether PGE2 could be involved in susceptibility and polarization to Th2-type immune response during T. crassiceps infection. In vivo treatments with PGE2 and indomethacin were used in order to analyze their effects on susceptibility and cytokine profile during infection.

**Materials and methods**

**Mice**

Inbred BALB/c female mice, originally from Jackson Laboratories (Bar, Harbor Maine) in 1982, were used in this study; they have been maintained in controlled conditions at our animal facilities for more than 20 generations.

**Parasites**

Metacestodes of T. crassiceps (ORF strain) were used in this study. They were harvested from the peritoneal cavity of female BALB/c mice after 2-4 mo of infection. The cysticerci were processed as described elsewhere (Terrazas et al. 1998).

**Infections**

Experimental infections were achieved by intraperitoneal injection of each mouse with 10 small (ca. 2-mm diameter) non-budding cysticerci of T. crassiceps suspended in 0.3 ml PBS. Resulting individual intensities of infection were measured at 4 week after infection.

**Cell preparations and culture conditions**

Mice were killed by cervical dislocation. Spleens were removed from infected and control mice, cells were obtained, washed and resuspended in culture medium made of RPMI 1640 (Gibco), supplemented with 10% fetal bovine serum (Gibco), 10,000 units of penicillin/streptomycin, 2 mM glutamine, 25 mM HEPES buffer, 1% non essential aminoacids (ICN). Splenocytes were resuspended to 5 × 10^6 cells/ml in this medium. One hundred microliters of the cell suspensions were then placed into 96-well flat bottom culture plates (Costar, Cambridge, Ma.) and stimulated with 100 µl of concanavalin-A (Sigma, St Louis Missouri) mitogen solution (2 µg/ml) and plates were incubated at 37 °C and 5% CO2 during 72 h.

Eighteen hours prior to termination each well received 0.5 µCi of tritiated thymidine (methyl-3H TDR, sp. act. 247.9 GBq/mmol, NEN, Boston, Ma.). After further incubations for 18 h, cells were harvested onto glass filter papers and processed for liquid scintillation counting. Stimulation index was calculated as a ratio of cpm in mitogen-stimulated cells to cpm of cells cultured in medium alone (without stimulus). Individual values were obtained to determine the mean and SE of each group.

**Effect of indomethacin and prostaglandin E2 (PGE2) in vivo**

Mice received indomethacin, PGE2 or placebo in vivo two days prior to infection. They were delivered in the form of subcutaneous 3-weeks controlled dose-dependent release rate pellets (Innovative Research of America, Toledo, Oh). Doses of indomethacin were 0.5 mg/pellet, and PGE2 0.1 mg/pellet. Twenty-one days after the implantation of the pellets, all mice received a second pellet of the same dose according to treatment to maintain levels of indomethacin or PGE2 for another week. All animals of these experiments were killed at 4 weeks after infection, spleen cells were cultured and parasite load was determined as mentioned above.

In vitro effect of adherent cells and indomethacin onto proliferative response

Splenocytes from mice with 8 weeks of infection and from uninfected age-matched mice were obtained and treated as mentioned above. Adherent cells were depleted by adhering spleen cells onto polystyrene Petri culture dishes precoated with heat-inactive FBS for 2 h at 37 °C and 5% CO2. Non-adherent cells were carefully collected, washed and adjusted to 4 × 10^6 cells/ml. Adherent cells were recuperated and adjusted to 1 × 10^6 cells/ml. Splenocytes without adherent cells (100 µl) from parasitized mice were co-cultured with adherent cells from uninfected mice (100 µl) and viceversa (they were cross-matched), in both cases the response to Con-A was evaluated. In other sets of experiments, similar cultures were performed but each well received 20 µl of indomethacin (2 µg/ml). Nonspecific esterase staining was performed on adherent cells (> 85% of adherent cells were positive).

**Cytokines determination**

IL-2 activity was assayed with the use of CTLL-2 cells. Ten thousand cells were cultured in the presence of serially diluted supernatants and a neutralizing amount of anti-IL-4 (2 µg/ml; 11.B11, Pharmingen, San Diego, Ca.). During the last 8-h of the culture period of 48 h, cells were pulsed with 0.5 µCi [3H]thymidine. Recombinant murine IL-2 (Pharmingen, San Diego, Ca.) was used as a standard to calibration curves.

IFN-γ, IL-4, IL-6 and IL-10 were determined by ELISA, using paired monoclonal antibodies, each standard curve was performed with their respective murine recombinant cytokine. All antibodies and recombinant cytokines were purchased from Pharmingen (San Diego, Ca.).

**Statistical analysis**

The statistical significance of the effects of the experimental variables upon parasite intensities and immunological parameters were determined by nonparametric test Mann-Whitney U-Wilcoxon Rank.

**Results**

In order to analyze the early influence of PGE2 on susceptibility to T. crassiceps infection, mice were sub-