Immuonoendocrine Interactions During Chronic Cysticercosis Determine Male Mouse Feminization: Role of IL-6


*J Immunol* 2001; 167:4527-4533; doi: 10.4049/jimmunol.167.8.4527
http://www.jimmunol.org/content/167/8/4527

This information is current as of May 2, 2017.
Immunoendocrine Interactions During Chronic Cysticercosis Determine Male Mouse Feminization: Role of IL-6

Jorge Morales-Montor,*2† S. Baig,* R. Mitchell,* K. Deway,* C. Hallal-Calleros,† and R. T. Damian*

Taenia crassiceps cysticercosis results in an impressive feminization in male mice during chronic infection, characterized by increased serum estradiol levels 100 times their normal values, while those of testosterone and dihydrotestosterone are decreased by 85 and 95% respectively. Concomitantly, the levels of follicle-stimulating hormone and IL-6 are increased 70 and 90 times their normal values in the infected male mice. Since a specific Th1/Th2 shift of the immune response has been previously reported during the chronic infection, and this shift may be associated with the feminization process, we proposed that this shift is induced by immunoendocrine interactions during the disease, and this gives way to a change in the initial resistance to the infection in the male mice, which become as susceptible as female mice. To confirm this hypothesis, we depleted immune system activity in two different ways: total body irradiation and neonatal thymectomy. Our results show that when immune system activity is depleted using either strategy, the male mice do not feminize, and the levels of follicle-stimulating hormone and IL-6 are inhibited. Depletion of IL-6 using IL-6 knockout mice does not produce the feminization process stated above, while restitution of the IL-6 knockout, irradiated, and thymectomized mice with murine recombinant IL-6 restores the feminization process. Expression of the IL-6 gene was found only in the testes and spleen of infected animals. Our results illustrate the importance of immunoendocrine interactions during a parasitic disease and show a possible new mechanism of parasite establishment in an initially resistant host. The Journal of Immunology, 2001, 167: 4527–4533.

Cysticercosis caused by Taenia crassiceps (1–3) is well known as a source of cross-reacting Ags useful in the immunodiagnosis of human cestode disease (4–6), as well as a practical model for testing candidate vaccines against porcine Taenia solium cysticercosis (7–9). It is also a manageable experimental system designed to explore the roles of biological factors involved in host susceptibility (10–12).

A sexual dimorphism exists in the normal immune responses and in many autoimmune diseases, suggesting a linkage between the immune and reproductive endocrine systems (13, 14). It has been demonstrated that the cellular immune response in females is less effective than that in males, while the humoral response is enhanced (15). This fact has been explained by the differences in estrogen levels, which normally are higher in females than in males (16).

Endocrinological, reciprocal interactions between host and parasite are receiving increased attention as being important in parasite success (17). Particularly, during T. crassiceps cysticercosis, females of all strains of mice studied sustain larger intensities of infection than males (18), but during chronic infection (>4 wk) these differences disappear, and the males of BALB/c AnN strain show a feminization process characterized by high serum estradiol levels (200 times their normal values), while those of testosterone are 90% decreased (19). Gonads are required for the increased estradiol synthesis, because gonadectomized and infected male mice had no detectable changes in blood levels of these hormones (19). Concomitantly, infected male mice progressively decrease their normal sexual behavior, loosing first the ejaculation response, then the intromission response, and finally, at 16 wk of infection, the mount response is totally abolished (20). Because sexual behavior is completely restored after testosterone or dihydrotestosterone restitution of parasitized mice, the disturbances in sexual behavior observed have been related to the high estradiol and low androgens levels detected (19, 20). The changes in steroid production and sexual behavior are also associated with tissue damage in the reproductive system (21) together with a specific change in mRNA levels for the enzymes involved in normal male steroid metabolism: a decrease in the expression of 5α-reductase (the enzyme in charge of the conversion from testosterone to dihydrotestosterone (DHT)) and an increase in the expression of aromatase (which is responsible for the conversion from testosterone to estradiol) (22).

On the other hand, immunological experiments have led to the theory that estradiol positively regulates parasite reproduction in

---

Cite this article as: Jorge Morales-Montor,*2† S. Baig,* R. Mitchell,* K. Deway,* C. Hallal-Calleros,† and R. T. Damian*. Immunoendocrine Interactions During Chronic Cysticercosis Determine Male Mouse Feminization: Role of IL-6. The Journal of Immunology, 2001, 167: 4527–4533.

---

*Department of Cellular Biology and Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30606; and † Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico

Received for publication May 30, 2001. Accepted for publication August 9, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by a grant from United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases and a grant from the Department of Cellular Biology, University of Georgia. J.M.-M. was a Pan American Fellow under the U.S.-Mexico Cooperative Biomedical and Behavioral Sciences Program established by formal agreement between the U.S. National Institutes of Health and the National Council of Science and Technology of Mexico and was supported by the National Council of Science and Technology of Mexico and the University of Georgia Office of the Vice President for Research.

2 Address correspondence and reprint requests to Dr. Jorge Morales-Montor, Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, Mexico D.F. 04510, Mexico. E-mail address: jmontor66@hotmail.com

3 Abbreviations used in this paper: DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; murIL-6, murine rIL-6.
host of both genders, presumably by interfering with the thymus-dependent cellular immune mechanisms that obstruct parasite growth (Th1) and enhancing those that facilitate it (Th2) (11, 23).

IL-6 is a multifunctional cytokine that regulates various aspects of the immune response, acute phase reaction, and hemopoiesis (24). Normally, it is involved in regulation of the humoral immune response (Th2) and in viral and bacterial infections and is an important in vivo SOS signal that coordinates the activities of liver cells, macrophages, and lymphocytes (24). The endocrine effects of this cytokine have been extensively demonstrated. For instance, it has been shown to stimulate the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in cultured pituitary cells (25), to enhance the secretion of adrenocorticotropic hormone through the stimulation of hypothalamic corticotropin-releasing hormone production in freely moving rats (26), and to stimulate the release of prolactin from the anterior pituitary gland (27). On the other hand, IL-6 inhibits FSH-stimulated progesterone production by rat granulosa cells in vitro (28). This pleiotropic cytokine is produced by many endocrine tissues, such as the anterior pituitary gland (29) and the medial basal hypothalamus (30). It is also produced by ovarian cancer cell lines and primary ovarian tumor cultures (31). In some reports the IL-6 activity has been shown to be an important factor that affects the activity of P-450 aromatase, mainly in estrogen-dependent breast cancer cells (31, 32).

Previously, we have shown that blood levels of IL-6 and its production by splenocytes in vitro are augmented in chronically cysticercotic male mice (19). IL-6 has been suggested to be capable of performing those immunoenocrinological interactions that lead to the feminization process in male mice because of its effect on P-450 aromatase. Since a feminization process has been previously shown (22) together with a shift of the normal immune response that occurs during infection (33, 34), an interaction between the immune and endocrine systems was envisaged and, ultimately, IL-6 involvement in the abnormal production of sex steroids and in loss of the sex-associated susceptibility between male and female mice previously reported. This hypothesis was tested by studying the effect of the depletion of the immune system on parasite loads; estradiol, testosterone, and DHT serum levels; serum gonadotropins (FSH and LH); and humoral and cellular immunity. Also the possible role of IL-6 as an important factor controlling this feminization process was studied using IL-6−/− knockout mice.

Materials and Methods

Mice

Male BALB/c AnN, male C57BL/6 × SV 129 F1, hybrids, and IL-6−/− (C57BL/6 × SV129) mice were bred in our animal facilities starting with an original stock from The Jackson Laboratory (Bar Harbor, ME) and were used at 6 wk of age. All mice were fed Purina Diet 5015 and water ad libitum. Animal care and experimentation practices at the University of Georgia are frequently evaluated by the university animal care and use committee and by governmental agencies to ensure compliance with established federal regulations and guidelines.

Infections

The fast-growing ORF strain of T. crassiceps isolated by Freeman in 1962 (1) was used for mouse infection in all experiments. The parasites have been maintained in female BALB/c mice by i.p. sequential inoculation. Larvae for experimental infections were obtained from female donor mice infected 3–6 mo before. Ten small (−2 mm in diameter) nonbudding T. crassiceps larvae were suspended in 0.3 ml PBS (0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.2) and injected i.p. into each 42-day-old mouse using a 0.25-gauge needle. Mice were sacrificed at 8 wk of infection by cervical dislocation prior to ether anesthesia. A complete parasite count was visually performed in each mouse after sacrifice by collecting them after thoroughly rinsing the peritoneal cavity with PBS. Parasites were never found outside the peritoneal cavity.

Testosterone, DHT, and estradiol measurements

Blood for steroid determinations was collected by cardiac puncture performed in mice under ether anesthesia. After incubation for 5 h at room temperature and for 18 h at 4°C, the blood clots were centrifuged, and sera were obtained. Steroids were ether-extracted and solubilized in the buffer used for immunoneutralization. The serum concentrations of estradiol, testosterone, and DHT were determined using liquid phase kinetics enzyme immunoassay kits (ALPCO, Windham, NH), according to the manufacturer’s instructions. When the reactions were completed, the samples were read at 450 nm in an ELISA reader.

LH and FSH assays

LH and FSH were extracted and concentrated from mouse sera using a C18 Sep-Pak equilibrated column (Advanced ChemTech, Louisville, KY). After injection into the column, the samples were eluted using 3 ml HPLC grade 0.1% trifluoroacetic acid (Baker, Phillipsburg, NJ) diluted in HPLC grade water (Baker). A second elution was performed with 3 ml HPLC grade 60% of acetonitrile (Baker) in 0.1% trifluoroacetic acid. Eluates from the samples were evaporated to dryness in a centrifuge concentrator (Millipore, Bedford, MA). Once the serum samples were completely dried, they were reconstituted with 250 μl RIA buffer. The concentrations of LH and FSH were determined using human RIA kits with hormone-specific antisera, according to the manufacturer’s instructions (Advanced ChemTech); each sample was determined in duplicate.

Ab determination

Levels of anti-cysticerci Abs in serum were assessed by ELISA. Briefly, the plates were sensitized with 100 μg total extract of T. crassiceps cysticerci in carbonate buffer. After overnight incubation at 4°C, the plates were washed three times with PBS-0.3% Tween and blocked with 0.1% BSA (Sigma, St. Louis, MO) for 1 h at 37°C. The serum samples were added at a final dilution of 1/1000, and the reactions were revealed using goat anti-mouse peroxidase Ab (Sigma). The plates were read at 490 nm after stopping the reaction with 4 N H2SO4.

Spleen lymphocyte proliferation assays

The spleen was surgically dissected in sterile conditions, and the cells were extracted by perfusion of the organ with RPMI-supplemented culture medium (10% FBS, 0.05% essential amino acids, 1% l-glutamine, and 1% antibiotics; Life Technologies, Grand Island, NY) using a 0.25-gauge needle. The lymphocytes were obtained after lysing the RBC by incubating the cell suspension for 3 min at 4°C in a lysis buffer (0.1 M NH4Cl and 2.5% Trizma base). The lymphocytes were then incubated in 96-well culture plates (Costar, Cambridge, MA), adjusting to 105 cells/well. The cells were stimulated by adding of Con A to a final concentration of 10 μg/ml or specific parasite Ag extract (10 μg/ml) and were incubated for 54 h in an atmosphere of 5% CO2 at 37°C. After this time, a pulse of 1 μCi [3H]thymidine/well was added, and the cells were cultured for another 18 h. Finally, lymphocytes were harvested in an automatic cell harvester (model 11028, Skatron Instruments, Sterling, VA) and counted in Betaplate system (model 1205, Wallac, Gaithersburg, MD). A separate group of stimulated cells was used for cytokine determination.

IL-2, IL-4, IL-6, and IFN-γ assays

After centrifugation of the stimulated cells as described above (10 min at 1500 rpm), the supernatants obtained were used for cytokine (IL-2, IL-4, IL-6, and IFN-γ) determination. Cytokines were measured by an ELISA sandwich method, according to the manufacturer’s instructions (PharMingen, San Diego, CA).

IL-6 gene expression

Total RNA from spleen, testes, seminal vesicles, and epididymis from uninfected and 8-wk postinfected mice were reverse transcribed, followed by specific PCR amplification of IL-6 and β-actin genes. The nucleotide sequence of the primers used for amplification flanked the mouse IL-6 cDNA sequence in 258 nt (5′-GAGGACACAGAGTGGACGACATG-3′) to +505 nt (5′-GAATGGTGAATGCAAGAAGGAG-3′). The β-actin primers sequences were: sense primer, 5′-CTACATAGCTTGCGTGTGG-3′; and antisense primer, 5′-AGAAGCTGGAAGTGCGTG-3′. Briefly, 2 μg total RNA was incubated at 37°C for 1 h with 400 U Moloney murine leukemia virus reverse transcriptase (PerkinElmer, Norwalk, CT) in a 20-μl reaction volume containing 400 μM of each dNTP. The reverse transcriptase activity was stopped by heating the samples to 95°C for 5 min. Ten microliters of the cDNA reaction was subjected to PCR to amplify a specific sequence of the specified genes. The 50-μl PCR reaction included 10 μl synthesized CDNA, 5 μl 10× PCR buffer (PerkinElmer), 1 mM
MgCl$_2$, 0.2 mM of each dNTP, 0.05 μM of each primer, and 2.5 U Taq DNA polymerase (PerkinElmer). After an initial denaturation step at 94°C for 4 min, temperature cycling was initiated as follows: 94°C for 55 s, 53°C for 55 s, and 72°C for 45 s for 30 cycles. An extra primer extension at 72°C for 10 min was performed for every gene. Twenty-five microliters of the total RT-PCR reaction products of each sample were electrophoresed in 2% agarose gel. The PCR products obtained were visualized by staining them with ethidium bromide, and m.w. was determined using a 100-bp ladder as a m.w. marker (Life Technologies). A single band was detected in each case. After the PCR products where electrophoresed, they were transferred for 2 h to Hybond N$^+$ membranes (Amerham, Arlington Heights, IL) using a vacuum blotter (model 785, Bio-Rad, Hercules, CA) and cross-linked using the Stratalinker 1800 (Stratagene, La Jolla, CA).

Membranes were then hybridized at 42°C for 2 h to overnight as described in the ECL gene detection kit (Amerham) with IL-6 and β-actin probes end-labeled with fluorescein and identified by chemiluminescent detection system, as recommended by the manufacturer (Amerham). After hybridization, membranes were washed twice with 2× SSC at room temperature for 30 min each time, followed by 0.1× SSC-0.1% SDS at 50°C for 30 min and were exposed for 1–30 min using ECL Hyperfilm (Amerham).

Densitometric analysis

Hybridization signals were quantified by densitometric scanning of multiple autoradiograms of various exposures and were represented as the ratio of the signal from the IL-6 gene relative to the expression of β-actin, a constitutively expressed gene used as an an internal control (relative expression).

Total body irradiation

A group of 30 5-wk-old mice was irradiated with 600 rad of γ radiation for 3 min. Gonads and heads were protected using lead bells, with the thickness sufficient to completely protect these organs against any irradiation effect. The effectiveness of irradiation was confirmed by total white blood cell count in every mouse. Mice that had white blood cell counts similar to those of the nonirradiated controls were excluded from the study. The animals were supplied with antibiotics (penicillin-streptomycin, 1 mg/kg body weight) in their drinking water. After 1 wk of recovery, mice were infected as described above.

Neonatal thymectomy

Thymectomy was performed in male mice 72 h after birth, as previously described (35). All mice were necropsied after assessment of experiments to confirm that they were thymus free. Mice showing any reminiscence of thymus were excluded from the study. A group of mice was used for sham surgery, and they were treated under the same conditions as thymectomized mice. The animals were supplied with antibiotics in their drinking water (penicillin-streptomycin, 1 mg/kg body weight) and infected as described above when they reached 6 wk of age.

IL-6 restitution experiments

Murine rIL-6 (R&D Systems, Minneapolis, MN) was injected i.p. into each irradiated, thymectomized, and IL-6−/− mouse after 4 wk of infection every other day for 4 wk. The IL-6 dose (120 pg/ml) was chosen based on the previous values obtained from normal infected and feminized animals at 8 wk of infection. A group of mice was injected with the vehicle (PBS) in which IL-6 was prepared and was treated in the same manner as the experimental groups.

Statistical analysis

Two experiments were performed (n = 10 each), and the data were analyzed using one-way ANOVA followed by Student’s t test of individual differences between means. The Epistat statistics program was used for calculating probability values.

Results

Fig. 1 illustrates the effect of total body irradiation, neonatal thymectomy, and of IL-6 gene (IL-6−/− mice), which result in a 3 times increased parasite load (1200 ± 275) recovered from the peritoneal cavity of 8-wk infected male mice compared with the control and sham groups, which had similar number of parasites (380 ± 98; p < 0.01).

The effects of different treatments on serum testosterone, DHT, and estradiol levels in male mice are shown in Fig. 2. Serum testosterone levels in infected mice are significantly decreased (85%) compared with those in normal and sham mice (p < 0.01); however, irradiated, thymectomized, and IL-6−/− infected male mice had no change in serum testosterone levels, which remained as high as those in control and sham mice. The same pattern was observed when treated with estrogenestradiol (p < 0.01) compared with control mice (p < 0.05). KO, Knockout; Thx, thymectomized.
observed for serum DHT levels; only the infected mice showed an important decrease (95%) in DHT levels compared with noninfected (control) and sham groups. Irradiated, thymectomized, and IL-6−/− infected male mice had DHT values similar to those of control uninfected mice (Fig. 2). On the other hand, after 8 wk of infection with *T. crassiceps* cysticerci, infected male mice increased serum estradiol levels by 90% over their normal levels (*p* < 0.01). In contrast, estradiol levels in irradiated, thymectomized, and IL-6−/− infected male mice remained unaltered, showing values similar to those in control uninfected mice (Fig. 2).

In Fig. 3 are shown the effects of different treatments on levels of gonadotropins (LH and FSH) during the 8-wk infection period in male mice. LH levels did not change in the control, infected, control irradiated, irradiated infected, and sham groups. However, thymectomized and IL-6−/− infected mice showed a slight decrease (25%) in their LH levels. On the other hand, levels of FSH, the normal stimulus for aromatase function, were 8 times increased in the infected mice (*p* < 0.05) compared with the normal mice. Irradiation, thymectomy, and IL-6−/− have no effect on serum FSH levels in infected male mice (Fig. 3).

The cellular immune response, evaluated as splenocyte proliferation (specific to parasite Ag), was 50% decreased in control infected male mice (*p* < 0.05) compared with that in control and sham mice. Irradiation, thymectomy, and lack of the IL-6 gene decreased this response even more in infected mice by 90% (Fig. 4A). There was a marked reduction in IL-2 production of 70% (*p* < 0.01) in infected mice compared with normal and sham IL-2 production, while IL-2 in irradiated, thymectomized, and IL-6−/− infected mice was 98% decreased (Fig. 4B). Also, IFN-γ production by stimulated splenocytes was decreased by 70% (*p* < 0.01) in infected male mice compared with control and sham male mice, while irradiation, thymectomy, and IL-6−/− infected mice showed a complete depletion of IFN-γ production by splenocytes (Fig. 4C).

Fig. 5 shows the course of the humoral immune response in the different treatments in male mice. There was a strong Ab response to infection with *T. crassiceps* cysticerci, which reached an OD of 2.0 at 8 wk of infection compared with noninfected animals (*p* < 0.01). However, this decreased to an undetectable level in irradiated, thymectomized, and IL-6−/− infected male mice (Fig. 5A). Levels of IL-4 and IL-6, two cytokines involved in the control of
humoral immune response, were also assessed. There was a 2-fold increase in IL-4 production in infected mice (p < 0.01) compared with the control and sham groups (Fig. 5B). On the other hand, production of IL-4 by the splenocytes of irradiated, thymecto-
mized, and IL-6−/− infected mice was not detectable (p < 0.01).

IL-6 mRNA was markedly induced in the testes and spleen of the infected mice during the 8-wk period (12- and 15-fold, respectively) compared with that in control mice. There was no expression of this cytokine in any other reproductive tissue of the control or infected mice tested or in any tissue of the IL-6−/− mice (Fig. 6).

Table I shows the effects of murine rIL-6 (murIL-6) restitution on estradiol, testosterone, DHT, and FSH serum levels. It is evident from the Table I that IL-6−/− mice treated with murIL-6 had 40-fold increased estradiol levels (p < 0.01) compared with respective infected control mice, while IL-6-restituted thymecto-
mized and irradiated infected mice had an increase of 20% (p < 0.01) compared with their respective controls. The levels of testo-
estosterone and DHT decreased 50% (p < 0.01), while FSH levels increased 3-fold (p < 0.01) in the murIL-6-restituted mice com-
pared with their respective controls (infected and untreated mice).

The vehicle in which IL-6 was diluted did not have an effect on the hormonal parameters measured (not shown).

**Discussion**

In this study we show an important immunoendocrine interaction in male mice infected with *T. crassiceps* cysticerci, which deter-
mines the previously reported feminization process (19–22). When the infected male mice have an intact immune system, there is an increase in serum estradiol levels and a decrease in those of testosterone and DHT; however, when the immune system is

knocked down by total irradiation or neonatal thymectomy, there is no change in the levels of serum steroids in chronically infected male mice, and these levels remain nearly at the level of the non-

infected male mice. Moreover, IL-6 was shown to be critical, since IL-6−/− infected mice do not develop the feminization process stated above, while the restitution with murIL-6 of the IL-6 knock-

out, thymectomized, or irradiated and infected mice again pro-

duces the feminization process observed.
The hormonal changes induced by cysticercosis have an important functional impact in the male host, which progressively loosens sexual activities in the course of chronic infection (20). The changes in steroid production and sexual behavior are also associated with tissue damage in the reproductive system. Chronically infected animals had extensive damage in the testes, seminal vesicles, prostate, and epididymus, characterized by infiltration of inflammatory cells and a change in the morphology of the male reproductive system (21). Moreover, it has been shown that the specific expression of 5α-reductase type II, the enzyme in charge of the normal metabolism of testosterone to DHT, is markedly decreased in cysticercotic male mice reproductive tissues, while the expression of enzyme P-450 is highly increased in those animals. Parasitism does not affect other steroidogenic enzymes earlier in the metabolic pathway of sexual steroids (22). Those results pointed to the aromatase enzyme as a key factor during the feminization process. Our results support and extend the idea of cysticerci driving the hormonal environment of the male host, decreasing testosterone and DHT levels and increasing estradiol levels, possibly involving P-450 aromatase, the enzyme in charge of the metabolism of testosterone to estradiol.

The intriguing question is how the cysticerci are able to drive the hormonal environment of the host, limiting testosterone-associated mechanisms that are restrictive for their establishment and growth toward an estrogen-enriched medium that is highly permissive. The simultaneous down-regulation of the Th2 response and up-regulation of Th1 are perhaps the cause of the arrest of parasite growth (23), since it is well known that the cellular immune response is critical in the control of this parasite infection (11). Immune system shutdown returns the hormonal environment to a dominance of testosterone, an androgen known to favor the immune cellular response against cysticerci (19), and thus the hormonal changes induced by blocking immune system function closes a circle that is conducive to a restriction of parasite growth.

The expression of the IL-6 gene in the testes of parasitized mice was enhanced, a fact that can explain the primordial role of the testes in the feminization process produced by cysticercosis. Thus, IL-6 could activate aromatase expression in the testes of cysticercotic mice and produce an active aromatization from androgens to estrogens. Our finding of IL-6 expression in the testes of mice is also supported by the fact that IL-6 has been shown to be produced by several types of cells in the rat testis. Moreover, IL-6 expression is involved in the paracrine control of testicular function (35, 36). Another important factor associated with aromatase activation is the pituitary hormone FSH, the natural activator of its expression in female mice (24). FSH secretion is influenced by IL-6 (25, 26). The fact that we found increased serum FSH in chronically infected mice supports this statement, and the interaction with IL-6 directly in the testes could be the key for explaining the feminization process observed in the male mice. The enhanced production of estradiol observed in the testes of chronically infected mice could be the result of increased testicular aromatization produced by IL-6 and FSH interaction. IL-6 has been demonstrated to stimulate aromatase in breast cancer cells (27), and we found increased levels of IL-6 in mice chronically infected with cysticerci together with an enhanced expression in the testes. In addition, spleen lymphocytes from infected animals produced higher levels of IL-6 in vitro. The fact that FSH could be involved in the induction of IL-6 expression in the testes of the infected male mice is supported by previous studies in human Sertoli cells, in which FSH only stimulated IL-6 production by Sertoli cell-enriched preparations, but increased the release of both IL-1 and IL-6 in germ cell-depleted Sertoli cell cultures (37). In addition, LPS and latex beads enhance the production of IL-6 by Sertoli cell cultures, whereas human chorionic gonadotropin and LPS enhance the release of IL-6 by Leydig cells (37). The demonstration that the testes of infected male mice produce IL-6 under the control of FSH and exogenous

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Estradiol (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>DHT (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfected</td>
<td>112 ± 65</td>
<td>1350 ±</td>
<td>987 ± 120</td>
<td>121 ± 121</td>
</tr>
<tr>
<td>Infected</td>
<td>997 ± 215*</td>
<td>250 ± 76*</td>
<td>85 ± 29*</td>
<td>829 ± 42*</td>
</tr>
<tr>
<td>IL-6 -infected</td>
<td>ND</td>
<td>1189 ± 211</td>
<td>599 ± 43</td>
<td>75 ± 10</td>
</tr>
<tr>
<td>IL-6 + IL-6</td>
<td>460 ± 158**</td>
<td>650 ± 98*</td>
<td>220 ± 58**</td>
<td>312 ± 69**</td>
</tr>
<tr>
<td>Thx-infected</td>
<td>77 ± 45</td>
<td>850 ± 76</td>
<td>485 ± 29</td>
<td>69 ± 21</td>
</tr>
<tr>
<td>Thx-infected + IL-6</td>
<td>374 ± 58*</td>
<td>550 ± 98*</td>
<td>120 ± 58**</td>
<td>128 ± 69**</td>
</tr>
<tr>
<td>Idx-infected</td>
<td>27 ± 21</td>
<td>730 ± 29</td>
<td>485 ± 41</td>
<td>91 ± 42</td>
</tr>
<tr>
<td>Idx-infected + IL-6</td>
<td>397 ± 158**</td>
<td>428 ± 98*</td>
<td>98 ± 32**</td>
<td>129 ± 23**</td>
</tr>
</tbody>
</table>

* Data represent the mean ± SD of one experiment done in quintuplicate. Thx, Neonatally thymectomized; Idx, irradiated; ND, nondetectable. Vehicle in which IL-6 was diluted had no effect and behaved in the same way as in control infected groups. *p < 0.05 compared with noninfected group; **p < 0.01 compared with IL-6 -infected and vehicle groups. Dose of IL-6 was 160 μg/ml.
The Journal of Immunology

4533

factors (cysticerci) opens the possibility of studying the involvement of these cytokines in the control of testis function in normal and pathological conditions.

The molecular mechanisms of testicular aromatase activation induced by IL-6 are not clear, but could involve its regulation by a distal promoter, namely promoter I.4, as previously shown for induction by IL-6. Sex hormone changes induced by the parasite lead to feminization of the male host in murine Taenia crassiceps cysticercosis. Steroid Biochem. Mol. Biol. 52:575.


The Journal of Immunology

4533

factors (cysticerci) opens the possibility of studying the involvement of these cytokines in the control of testis function in normal and pathological conditions.

The molecular mechanisms of testicular aromatase activation induced by IL-6 are not clear, but could involve its regulation by a distal promoter, namely promoter I.4, as previously shown for other steroidogenic tissues (38). The stimulation of expression in adiopose stromal cells by IL-6 is mediated via the Janus kinase-STAT3 signaling pathway and a GAS (IFN-γ activation site) element upstream of promoter I.4 (38). Future perspectives include molecular analysis of the precise mechanism by which IL-6 activates aromatase transcription in the testes of infected mice.

The decreased expression of steroid 5α-reductase type II previously found (28) may be the consequence of the decreased levels of DHT in parasitized animals, since the expression of this enzyme is down-regulated by testosterone levels (39). The low testosterone levels would result from increased aromatase activity (22). This dehydrogenation would also contribute to weaken the Th1-dependent cellular immune response (33).

Succinctly, based on the endocrinologic (19–21, 23) and immunologic (Th1/Th2 shift) (11, 12, 33, 34) events associated with chronic infection female mice may involve the joint action of the immune system and the uptake and incorporation of 19C-leucine. The low testosterone levels would result from increased aromatase activity (22). This is down-regulated by testosterone levels (39). The low testosterone levels would result from increased aromatase activity (22).

Thus, striking differences in susceptibility to cysticercosis between male and female mice may involve the joint action of the immune system and the gonads, both driven by a parasite that is able to change the normative male hormonal milieu during chronic infection to a more permissive female environment (Fig. 7).

Acknowledgments

We thank Dr. Pavel Petrossian for correcting the English version of the manuscript.

References


