FULL PAPER *Parasitology*

Androgen- and Estrogen-Dependent Sex Differences in Host Resistance to *Strongyloides venezuelensis* Infection in Wistar Rats

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ABSTRACT. The effects of male and female sex hormones on the protective capacity of Wistar rats against infection with *Strongyloides venezuelensis* were investigated. Male rats were more susceptible than females in terms of worm recovery from the lungs. Orchidectomy of male animals significantly reduced the plasma testosterone concentration and increased host resistance to the migratory stages of *S. venezuelensis* larvae. In contrast, ovariectomy of female animals significantly decreased host resistance in association with a significant reduction of estrogen levels. To examine the direct effect of sex hormones, exogenous testosterone and estrogen were implanted into animals. Susceptibility significantly increased or decreased in ovariectomized females given testosterone or estrogen, respectively. These results suggest that male and female sex hormones are important in the down- and up-regulation of host resistance against *S. venezuelensis* in Wistar rats.

KEY WORDS: estrogen, sex difference, *Strongyloides venezuelensis*, testosterone, Wistar rat.

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Interest in host sex and host hormones, that influence parasitic infection, dates back to the early 1940's, and the influence of hormones was first widely reviewed by Solomon [27]. However, many of the hypotheses and theories proposed still remain to be moot. Sex differences are usually attributed to either ecological (sociological in humans) or physiological causes, the latter usually being hormonal in origin [37]. Testosterone has immunosuppressive properties [9, 10, 15, 37]. The prevalence and intensity of parasitic infections are greater in males of many mammalian species than in females [5, 14, 17, 19–21, 30, 36], especially in abnormal hosts with a primary infection, but not always in normal hosts.

Our most recent study [24] demonstrated a significant sex difference in the susceptibility of sexually mature Wistar rats (*Rattus norvegicus*) to infection with *S. venezuelensis*. As reported [23], the strain of *S. venezuelensis* used in our studies was originally isolated from wild *R. norvegicus* in Okinawa Prefecture, Japan [11], indicating that laboratory rats including the Wistar strain are within the same group of natural/normal hosts for *S. venezuelensis*.

Since sex differences in susceptibility to a parasite are rare in a normal host, the present study examines whether host sex hormones affect the susceptibility of Wistar rats to infection with *S. venezuelensis*.

MATERIALS AND METHODS

Animals: Wistar rats (*R. norvegicus*) at 4 weeks of age were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan). These rats were housed in clean metal cages and fed with a standard diet and tap water *ad libitum* in an air-conditioned room (23 ± 1°C), under conventional conditions with a 12:12 hr light/dark cycle. They received human care as outlined in the "Guide for the Care and Use of Laboratory Animals" by The Faculty of Agriculture, Miyazaki University.

Parasitological techniques: A strain of *S. venezuelensis* was originally isolated from wild brown rats (*R. norvegicus*) in Okinawa, Japan in 1988 [11] and maintained in our laboratory by serial passage in Wistar rats and/or Mongolian gerbils (*Meriones unguiculatus*). Third stage infective larvae (L_3) were prepared from the cultures of infected feces using filter paper methods [25, 26].

Rats were infected with 3000 or 4000 L_3 of *S. venezuelensis* by subcutaneous inoculation into the groin. The protective capacity of each rat was measured by larval recovery from the lungs 3 days after infection according to the method described previously [13] with a slight modification. In short, infected animals were killed and both lungs removed, cut into small pieces using a motor driven disperser, and incubated on a stainless mesh (100 mesh) in petri dishes with saline at 37°C for 3 hr. The number of larvae that emerged was counted under a dissecting microscope.

Gonadectomy and hormone replacement: We examined whether or not sex and host sex hormones in Wistar rats are important in controlling the susceptibility to *S. venezuelensis* infection.

In one experiment, 4-week-old males (n=6) and females (n=6) were orchidectomized and ovariectomized, respectively and an equal number of males and females were sham operated. Surgical operations were performed under ether anesthesia. In males, the vas deferens and spermatic vessels were ligated, then both testes and epididymis were removed through a mid-line incision of the scrotal skin. Ovaries along with a portion of the uterus were excised through a

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small incision on the dorsal skin. The skin incisions were closed using a 9-mm wound clip (Clay Adams Brand, Mik-Ron Autoclip, Becton Dickinson and Co., MD, U.S.A.). Sham operations were performed using the same procedures except the gonads were left *in situ*. All animals were infected with *S. venezuelensis* at 10 weeks of age.

In another experiment, 4-week-old females (n=24) were assigned to four experimental groups. Each group (n=6) was either sham operated, ovariectomized, ovariectomized and implanted with testosterone or ovariectomized and implanted with β-estradiol. Gonadectomy was performed at 4 weeks of age as described above. Hormone replacement was applied 3 weeks after gonadectomy by subcutaneous implantation of a 15-mm Silastic tube containing 35 mg testosterone (Nacalai Tesque, Inc. Kyoto, Japan) or β-estradiol (Sigma Chemical Co., St. Louis, MO, U.S.A.). All animals were infected with *S. venezuelensis* at 10 weeks of age.

Measurement of sex hormones: To measure plasma estrogen and testosterone levels, rats were killed by decapitation and trunk blood collected into heparinized plastic tubes was centrifuged. Plasma was separated and stored frozen at –20°C. After diethyl ether extraction, concentrations of estrogen and testosterone in the plasma samples were measured using a specific RIA procedure as described by Kuroda *et al.* [16].

Briefly, standard and ^{3}[H] labeled steroids were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and NEN® Life Science Products (Perkin-Elmer Life Sciences, Boston, MA, U.S.A.), respectively. The upper and lower limits of the assay were 2,000 and 7.8 *pg*, respectively and the mean values of intra-assay and interassay variation were below 13% and 7.5%, respectively.

Statistical analyses: Results were statistically analyzed using Student's *t*-test and a *p* value below 0.05 was considered significant. All results are presented as means ± SEM.

RESULTS

Figure 1 shows a significant ($p<0.0001$) sex difference in the number of larvae recovered from the lungs of shams operated males (1198 ± 114) and females (25 ± 7).

The number of worms obtained from ovariectomized females (143 ± 38) and orchidectomized males (120 ± 36) did not significantly differ to each other ($p=0.2$). Ovariectomy caused a significant ($p<0.01$) increase in the susceptibility of females; about six-fold more larvae were recovered from the lungs of ovariectomized than from sham-operated females. In contrast, orchidectomy significantly increased the host resistance of male rats about ten times ($p<0.0001$).

The mean levels of testosterone in the plasma of sham-operated males (1.15 ± 0.09 *ng/ml*) were highest among the groups and significantly higher ($p<0.005$) than those of sham operated female rats (0.63 ± 0.029 *ng/ml*) and orchidectomized males (0.36 ± 0.02 *ng/ml*) (Fig. 2). The mean estrogen (estradiol) level of sham operated female rats (836.7 ± 109.3 *pg/ml*) was nineteen- and seven-fold higher than those of ovariectomized females (45.5 ± 3.0; $p<0.01$) and of sham operated male rats (117.0 ± 28.59 *pg/ml*; $p<0.05$), respectively (Fig. 3).

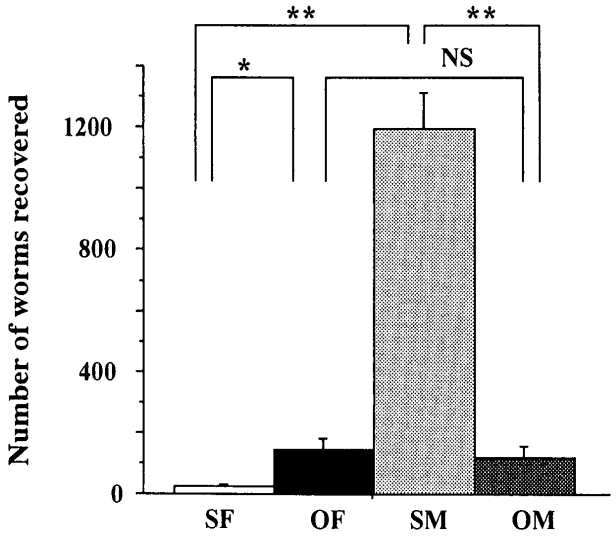

Fig. 1. Recovery of migrating larvae from lungs of Wistar rats 3 days after infection with 3000 L_3 of *S. venezuelensis*. From left to right, columns represent sham operated females (SF), ovariectomized females (OF), sham operated males (SM) and orchidectomized males (OM). Each value represents mean and SEM of 6 rats. NS, not significant; * $p<0.05$, ** $p<0.0001$.

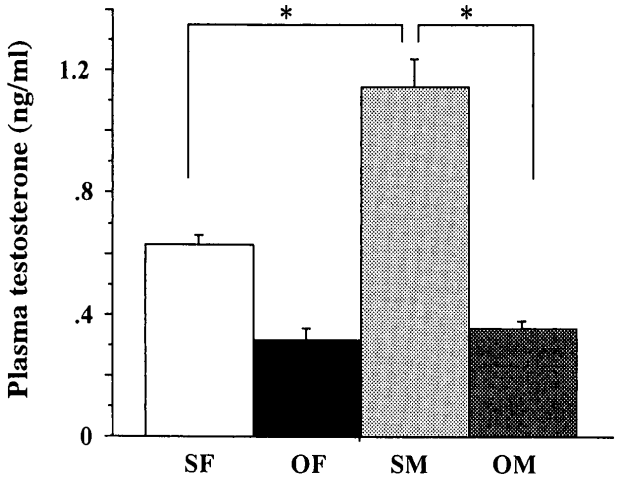

Fig. 2. Plasma testosterone levels of Wistar rats 3 days after infection with 3000 L_3 of *S. venezuelensis*. From left to right, columns represent sham-operated females (SF), ovariectomized females (OF), sham operated males (SM), and orchidectomized males (OM). Each value represents mean and SEM of 4 rats. * $p<0.005$.

To determine the direct effects of testosterone and estrogen on the protective capacity of Wistar rats, ovariectomized female rats were implanted with testosterone and β-estradiol. Figure 4 shows that twenty-fold more larvae were recovered from the lungs of ovariectomized (991 ± 85) than those of sham operated females (50 ± 6; $p<0.0001$). The number of worms recovered from

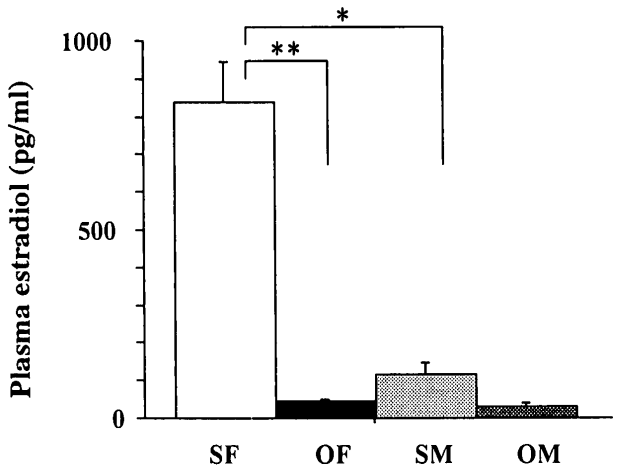

Fig. 3. Plasma estradiol levels of Wistar rats 3 days after infection with 3000 L_3 of *S. venezuelensis*. From left to right, columns represent sham-operated females (SF), ovariectomized females (OF), sham operated males (SM), and orchidectomized males (OM). Each value represents mean and SEM of 4 rats. * $p<0.05$, ** $p<0.01$.

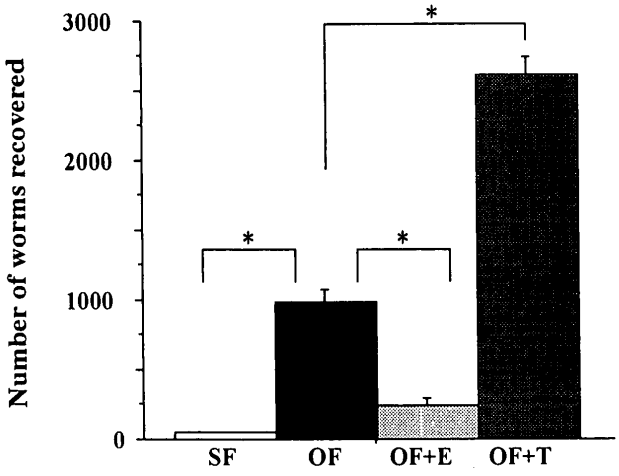

Fig. 4. Recovery of migrating larvae from lungs of Wistar rats 3 days after infection with 4000 L_3 of *S. venezuelensis*. From left to right, columns represent sham-operated females (SF), ovariectomized females (OF), ovariectomized and treated with estradiol (OF+E), and ovariectomized and treated with testosterone (OF+T). Each value represents mean and SEM of 6 rats. * $p<0.0001$.

ovariectomized females implanted with β-estradiol decreased to 243 ± 46 ($p<0.0001$). On the contrary, when ovariectomized females were implanted with testosterone, worm recovery significantly increased (2617 ± 128), compared with that from ovariectomized and estrogen-treated females ($p<0.0001$; 243 ± 46).

Figure 5 shows that the testosterone concentration in the animals implanted with testosterone was significantly higher (1.22 ± 0.13 *ng/ml*) than that from sham-operated females (0.63 ± 0.03 *ng/ml*), ovariectomized (0.32 ± 0.04 *ng/ml*) and ovariectomized and β-estradiol-treated females

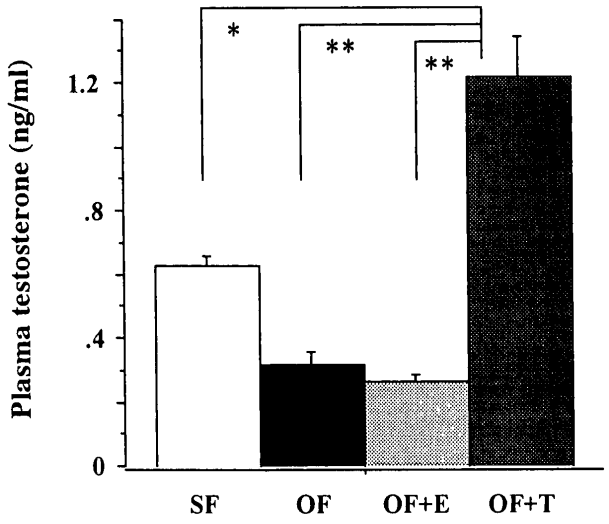

Fig. 5. Plasma testosterone levels of Wistar rats 3 days after infection with 4000 L_3 of *S. venezuelensis*. From left to right, columns represent sham-operated females (SF), ovariectomized females (OF), ovariectomized and treated with estradiol (OF+E), and ovariectomized and treated with testosterone (OF+T). Each value represents mean and SEM of 4 rats. * $p<0.05$, ** $p<0.01$.

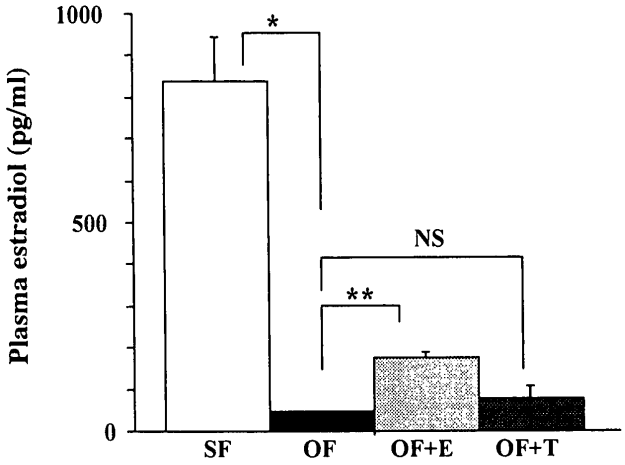

Fig. 6. Plasma estradiol levels of Wistar rats 3 days after infection with 4000 L_3 of *S. venezuelensis*. From left to right, each column represents sham-operated females (SF), ovariectomized females (OF), ovariectomized and treated with estradiol (OF+E), and ovariectomized and treated with testosterone (OF+T). Each value represents mean and SEM of 4 rats. NS, not significant; * $p<0.01$, ** $p<0.001$.

(0.27 ± 0.02 *ng/ml*; $p<0.05$, <0.01 and <0.005, respectively). No apparent differences ($p=0.2$) in plasma testosterone concentrations were found between ovariectomized only and ovariectomized, estradiol-treated females (Fig. 5).

Figure 6 shows that the plasma estradiol concentration in sham operated females (836.7 ± 109.3 *pg/ml*) was the highest among those in ovariectomized (45.5 ± 3.0), ovariectomized plus estradiol-treated (174.5 ± 14.6), and

ovariectomized plus testosterone-treated females (76.5 ± 29.7). However, the plasma concentration of estradiol-treated animals was significantly higher ($p<0.05$) than that of ovariectomized and testosterone-treated animals. Plasma estradiol concentrations between ovariectomized females and testosterone-treated animals did not significantly differ ($p=0.4$).

DISCUSSION

The present study shows that testosterone and estrogen both significantly affect the host resistance to the tissue migratory phase of *S. venezuelensis* infection in Wistar rats. Testosterone inhibited host resistance, whereas β-estradiol enhanced it. This contribution of male and female sex hormones to the down- and/or up-regulation of host resistance to parasitic infection explains the age-related sex difference in the susceptibility of Wistar rats to *S. venezuelensis* that we recently identified [24].

The early resistance of rats or mice expressed during the tissue migrating phase of parasitic larvae is probably caused by the activated mononuclear phagocyte system [1, 28, 31, 33]. Intravenous injections of carbon particles can reduce either phagocyte activity or the size of the mobilizable pool of the mononuclear phagocyte system. Thus, a macrophage blockade in mice caused by an injection of carbon particles completely abolished age- and sex-related differences [18, 20, 22, 33].

Individual immune cells including macrophages may behave as steroid-sensitive cells, in that they may represent a target for sex steroids, and sex hormones modulate the inflammatory mediators produced by macrophages [7, 8].

Several clinical and experimental studies have shown that the immune system is subject to sex dimorphism, that sex hormones contribute to this sex-specific immune response, and that testosterone seems to be the cause of immunodepression in males, whereas estradiol seems to be responsible for the enhanced immune response in females [2–4, 12, 34]. Steroid hormones act on target cells through their cognate receptors belonging to the intracellular steroid receptor superfamily and testosterone acts on cells through intracellular transcription-regulating androgen receptors that are present in macrophages [6]. In fact, the susceptibility of female C57BL/6 mice to *S. ratti* infection is increased by exogenous testosterone to the level of male animals or more [14].

Macrophages produce pro-inflammatory cytokines that may play a key regulatory role in defining the subsequent adaptive immune response. Nitric oxide (NO) synthesized by IFN-γ activated macrophages is a major effector against parasitic [29] and viral infections and it may play a similar role in bacterial infections in part by regulating macrophage phagocytic and microbicidal activity [32].

On the other hand, the expression of a sex difference in susceptibility to parasites in the normal host is unusual. The strain of *S. venezuelensis* used in this study was isolated from wild *R. norvegicus* [11]. However, Wistar rats (*R. norvegicus*) showed obvious sex differences to infection with this strain. Many of the sex differences in susceptibility to the early migratory stages of parasite infection in abnormal hosts have been explained by the inhibitory effect of testosterone on host resistance. Therefore, the role of estrogen in the up-regulation of host resistance to *S. venezuelensis*, a nematode parasite, may be unique to nematode infection in normal hosts. However, genetic variations in *R. norvegicus* have been reported [35], casting doubt upon whether or not the strain of Wistar rats used in this study is really a normal host for *S. venezuelensis*.

In conclusion, our results provide evidence that both male and female sex hormones regulate vertebrate host resistance to the nematode parasite, *S. venezuelensis*. The actual roles of effector cells and the exact mechanism(s) of protection occurring in host tissues requires further clarification.

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