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Regulatory Role of Testosterone on the Natural Defence Mechanism against Infection with *Strongyloides ratti* in C57BL/6 Mice

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Key words: *Strongyloides ratti*, migratory phase, natural defence, testosterone, C57BL/6 mice

Introduction

Sex steroid hormones are known to have regulatory effects on the immune (Dougherty, 1952) and/or hemopoietic (Miale, 1977) systems. Such effects seem to be responsible, at least in part, to the sex differences observed in a variety of infectious diseases (rev. by Solomon, 1969). For example, Haley (1958) reported sex difference in the resistance of hamstars to infection with *Nippostrongylus brasiliensis* and Solomon (1966) suggested that this difference was under the regulation of male gonadal hormones. Conversely, Dobson (1961) suggested that sex difference in the susceptibility of mice to infection with *Nematospiroides dubius* is regulated by estrogen. Recently it has been reported that the susceptibility of male C57BL/6 mice to infection with *Strongyloides ratti* is greater than that of the female animals (Dawkins *et al.*, 1980). We have found that this sex difference can be observed at the tissue migratory phase of the infection as early as 24h after a subcutaneous inoculation with the infective larvae (Kiyota *et al.*, submitted). Furthermore, orchiectomized male mice became highly resistant to the infection similarly to normal female mice, whereas ovariectomy did not affect the susceptibility, suggesting the regulatory role of androgen on the host's defence mechanism (Kiyota *et al.*, submitted). The present study was, therefore, designed to clarify the mode of action of testosterone on the host's defence mechanism against infection with *S. ratti*.

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Materials and Methods

Mice Inbred C57BL/6 mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu.

Castration and hormone treatment Orchiectomy was performed under ether anesthesia. Both testes and epididymis were, after a ligation of the vas deferens and spermatic vessels, excised through a mid-line incision of scrotal skin. The skin incision was closed by a wound clip (Clay-Adams, Parsippany, N.J.). To achieve physiologically maintained hormone replacement therapy in castrated animals, a 2 cm Silastic tube (I.D. 0.058 inch, O.D. 0.077 inch ; Dow Corning Co., Midland, Mich.) containing 6-8 mg of testosterone powder (Sigma) was implanted subcutaneously immediately after the castration procedure (Roubinian *et al.*, 1978). Testosterone treatment at the pharmacological dose was performed by subcutaneous implantation of 10 mg testosterone pellet through a mid-line incision of back skin.

Since preliminary experiments revealed that the susceptibility of sham-orchiectomized mice was, when tested more than 1 w after the operation, same as that of unoperated mice, unoperated mice were used as controls throughout this series of experiments.

Parasitological techniques The strain of *S. ratti* used in this series of experiments had been maintained in the Department of Parasitic Diseases, Kumamoto University Me-

dical School by serial passage in Wistar rats for more than 5 years. Filariform larvae (L_3) were collected from filter paper cultures after 4 day-incubation at 27 C and were washed several times with physiological saline (Tada *et al.*, 1979). Mice were infected subcutaneously in the flank with 3,000 L_3 suspended in 0.2 ml saline. The degree of the susceptibility was routinely monitored by daily fecal larval output. The number of adult worms in the small intestine or of tissue migrating larvae in the cranial cavity was examined by the method of Tada *et al.* (1979).

Results

Effect of castration on the susceptibility of male mice

Since levels of circulating androgen are known to fall below the range of detection by radio-immunoassay within 2 w of castration (Bartke, 1974), susceptibility of male mice to the infection with *S. ratti* was examined various time after castration. Three groups (5 mice per group) of male C57BL/6 mice were castrated 1, 3, or 5 w before infection. One group of five intact mice served as a control. All mice were infected with 3,000 *S. ratti* L_3. They were sacrificed 7 days after infection for counting intestinal worm burden. As shown in Fig. 1, the susceptibility decreased significantly at 1 w after castration. When the interval between castration and infection was longer than 3 w, mice became more resistant to infection.

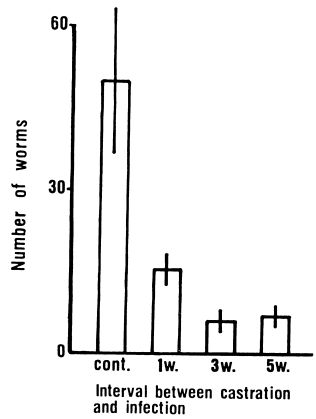

Fig. 1 Intestinal worm burden of 5 castrated male C57BL/6 mice 7 days after infection with 3,000 *S. ratti* larvae.
Cont: control group
Vertical bar indicates standard error of mean.

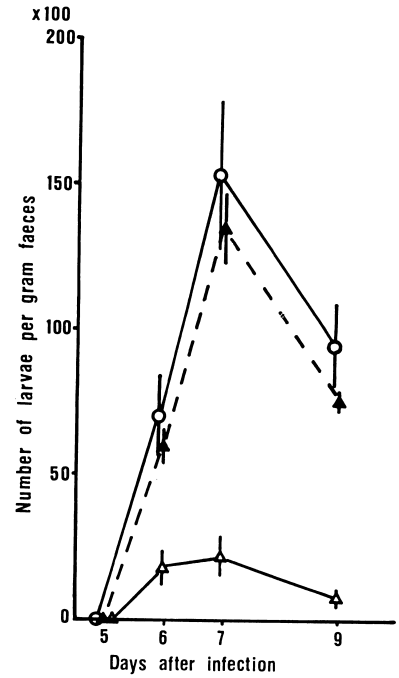

Fig. 2a Kinetics of daily larval output in feces in normal, castrated, or castrated and testosterone-treated male C 57 BL/6 mice after infection with 3,000 *S. ratti* larvae.
○——○ : control
△——△ : castrated
▲····▲ : castrated testosterone- treated
Vertical bar indicates standard error of mean.

Effect of testosterone on the reduced susceptibility of castrated male mice

To determine whether decreased susceptibility after castration is due to a lack of androgenic steroid hormone, the susceptibility of castrated male mice reconstituted with the physiological dose of testosterone was examined. Fourty-five male C57BL/6 mice, 4 w old, were divided into three groups (15 mice per group). Two groups were castrated. One group was given testosterone in a Silastic tube and the other was given empty Silastic tube. The third group served as a control.

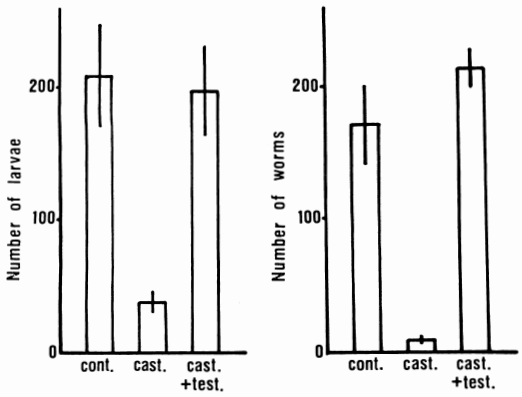

Fig. 2b Number of tissue migrating larvae in the cranial cavity (left) or intestinal worm burden (right) of nomal, castrated, or castrated and testosterone-treated male C 57 BL/6 mice after infection with 3,000 *S. ratti* larvae. Number of larvae in the cranial cavity was examined 36 h post-infection, whereas intestinal worm burden was examined on day 7.

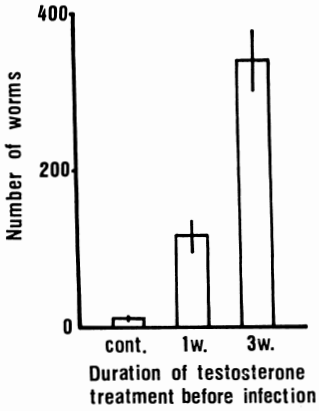

Fig. 3 Intestinal worm burden of testosterone-treated female C57BL/6 mice 7 days after infection with 3,000 *S. ratti* larvae.
Cont: Worm burden of control group.
Vertical bar indicates standard error of mean.

All mice were infected with 3,000 *S. ratti* L_3 5 w after surgical operation. The daily larval output in feces was monitored in 5 mice from each group up to day 9 post-infection. Five mice from each group were sacrificed 36 hr after infection for counting the number of migrating larvae in the cranial cavity. Remaining 5 mice from each group were sacrificed on day 7 for counting intestinal worm burden. The results were summarized in Figs. 2a and 2b. When the susceptibility was assessed by daily larval output in feces (Fig. 2a), normal male mice were much more susceptible than castrated males, and this reduced susceptibility of castrated males could be restored by testosterone treatment at the physiological dose. The number of tissue migrating larvae in the castrated males was significantly lower than that of control, whereas the number of larvae in testosterone-restored mice was comparable to that of control (Fig. 2b). Essentially identical results were observed in the number of adult worms recovered from the intestine of each group (Fig. 2b).

Effect of pharmacological dose of testosterone on the susceptibility of female mice

Female C57BL/6 mice are resistant against *S. ratti* infection (Dawkins *et al.*, 1980). This natural resistance was not affected by ovariectomy (Kiyota *et al.*, submitted). In this experiment, whether testosterone could modulate the natural resistance of female mice was studied. Two groups (5 mice per group) of female C57BL/6 mice, 8–10 w old, were subcutaneously inoculated with 10 mg of testosterone pellet 1 or 3 w before infection. Five untreated mice served as a control group. All mice were infected with 3,000 *S. ratti* L_3, and were sacrificed on day 7 for counting intestinal worm burden. As shown in Fig. 3, susceptibility of female mice markedly increased by testosterone-treatment. This effect was greater at 3 w than at 1 w.

Discussion

The results reported here clearly indicate that androgenic steroid hormone plays an important regulatory role on the host's defence mechanism against *S. ratti*.

S. ratti is naturally a parasite of rats, but host specificity in this genus is not absolute (Galliard, 1967). There is considerable difference in the susceptibility of inbred strains of mice to infection with *S. ratti* and no apparent

relationship between susceptibility and the H-2 haplotype of the various mouse strain (Dawkins *et al.*, 1980). Such a strain difference may be, at least in part, explained by the different sensitibity of mouse strains to androgen. Strain difference in sensitibity to androgen in relation to the immune reactivity has been already reported (Cohn, 1979).

In the present study, modulating effect of both physiological and pharmacological dose of testosterone was expressed at the tissue migratory phase as early as 36 h post-infection. Tada *et al.* (1979) reported that, after a subcutaneous inoculation, the vast majority of *S. ratti* larvae migrated through loose subcutis to the cranial cavity 15–50 h aftr the infection. Thus, it is conceivable to posturate that cells and/or tissues of the loose connective tissue of the skin act as natural defence mechanism against tissue migrating larvae and are under the regulation of androgen. Since the blockade of phagocytic activity by carbon injection was effective to enhance the susceptibility of female mice to *S. ratti* infection (Kiyota *et al.*, submitted), connective tissue macrophages (histiocytes) seem most likely to be the effector cells of the natural defence mechanism. Although humoral-and cell-mediated immune responses were generated by *S. ratti* infection in mice (Dawkins *et al.*, 1982) or in rats (Genta *et al.*, 1983), it is rather unlikely that specific antibody or primed lymphocytes are involved in the defence mechanism against early stage of tissue migratory phase of a primary infection. In fact, these specific effectors seem to be generated at relatively late stage of a primary infection (Dawkins *et al.*, 1982; Genta *et al.*, 1983). Furthermore, Moqbel and Wakelin (1981) reported that adoptive transfer of immune mesenteric lymph node cells, which was effective in conferring hastened expulsion of adult worms, appeared to have no effect upon worm establishment.

At present how androgen regulates the natural defence system remains uncertain. In the present study, susceptibility of castrated males gradually decreased and reached a steady state by 3 w after the operation. Conversely, when female mice were treated with testosterone, their susceptibility increased with time. Whether this relatively slow reaction to androgen is directly related to serum testosterone level or is related to the life-span of the target cells in subcutaneous connective tissue should be further clarified. Androgenic steroid hormones have regulatory effects on the hemopoietic system (Fried and Gurney, 1968; Udupa and Reissmann, 1975). Recently Honma *et al.* (1980) reported that the sex difference in the total number of peritoneal free cells was caused primarily by the testis. Thus, the number and/or functions of macrophages in the loose subcutaneous connective tissue may be regulated by androgen. Identification and characterization of the effector cells involved in the natural defence is necessary for futher clarification of this point.

Summary

The susceptibility to infection with *Strongyloides ratti* was examined various time after castration of C57BL/6 male mice, which are known to be more susceptible to infection than females. The susceptibility measured by intestinal worm burden became about 1/3 of control one week after castration. Three or five weeks after castration, the susceptibility further decreased by about 1/7 of normal level. Such an effect of castration was reversible, since reduced susceptibility could be restored by the treatment with physiological dose of testosterone. Female C57BL/6 mice, which is naturally resistant against infection, became susceptible after treatment with the pharmacological dose of testosterone (approximately 10 fold increase at 1 w and 30 fold increase at 3 w after the treatment). Modurating effect of testosterone was expressed at the early tissue migratory phase of infection, suggesting that testosterone-sensitive cells and/or tissues involved in the host's defence system are naturally existing ones rather than antigen-specifically induced.

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男性ホルモンによる C57BL/6 マウスの *Strongyloides ratti* に対する感染抵抗性の調節

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C57BL/6 マウスの *Strongyloides ratti* に対する感受性は，雄のほうが雌よりも高いことが知られている．そこで雄マウスを去勢し，時間を変えて *S. ratti* を感染させてみたところ，小腸内成虫数は，去勢後1週目で既に正常雄の約 1/3 となり，去勢後3，及び5週目では正常雄の約 1/7 となっていた．このような雄マウスの去勢による感受性の抵下が，生理量のテストステロン投与により消失したことから，去勢の効果は，可逆的であることが示された．

次に雌 C57BL/6 マウスに見られる強い感染抵抗性が，男性ホルモンによって影響されるかどうかを見るために，雌マウスの背部皮下に薬理量のテストステロンのペレットを埋没し，時間を変えて *S. ratti* を感染させたところ，小腸内成虫数は，ホルモン投与後1週目で正常雌の約10倍となり，3週目では約30倍となつていた．

テストステロンの感染抵抗性に及ぼす効果は，感染初期の体内移行期で既に発現されていたことから，テストステロンによって支配されている宿主の感染防禦機構は，抗原特異的なものではなく，非特異的なものであることが示された．