NOTE *Parasitology*

Age- and Sex-Related Changes in Susceptibility of Wistar Rats to *Strongyloides venezuelensis* Infection

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ABSTRACT. The effects of host age and sex on susceptibility to *Strongyloides venezuelensis* in Wistar rats were examined by counting larvae recovered from the lungs of animals 3 days after infection. The susceptibility of female rats to *S. venezuelensis* rapidly decreased with age and elevated estrogen. Resistance in female rats inoculated at 6 and 10 weeks of age was nine and twenty-fold higher, respectively than that in the youngest group (3 weeks). In contrast, the susceptibility of male animals was lowest in the youngest group, then increased with age and elevated testosterone. Sex differences in susceptibility were not evident in the youngest group, but became apparent with age.

KEY WORDS: *Strongyloides venezuelensis*, susceptibility, Wistar rat.

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Innate factors of host origin that influence helminth burdens include age, sex and genetic background. The influence of age and sex on helminth burdens appears to be largely hormonal [25]. *S. venezuelensis* is a good helminth model that has been used worldwide to investigate mucosal mast cell-dependent protection in the intestinal tract of host [18, 21].

We previously examined a strain of *S. venezuelensis* [18] that was originally isolated from wild brown rats in Naha, Okinawa prefecture, Japan [12]. During the maintenance of this strain using Wistar rats, female animals were resistant to infection with this strain of parasite and have seldom been used for the maintenance. However, this phenomenon and/or the underlying mechanisms have not yet been investigated.

The present study therefore examines the influence of host age and sex on the susceptibility to infection with *S. venezuelensis* and the relationship with sex hormones is discussed.

Wistar rats were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan) and housed in our laboratory under conventional conditions. The study included three groups of male (n=6) and female (n=6) rats aged 3 (40–60 g body weight), 6 (140–160 g for females and 160–180 g for males) and 10 weeks (220–250 g for females and 320–350 g for males).

The strain of *S. venezuelensis* used in this study was originally isolated from wild brown rats in Okinawa, Japan in 1988 [12] 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.

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Rats were infected with 3000 infective larvae (L_3) of *S. venezuelensis* each by subcutaneous inoculation into the groin. The protective capacities of male and female rats at various ages were measured by larval recovery from the lungs 3 days after infection. Infected animals were killed and both lungs removed, cut into small pieces using a motor driven disperser, and incubated in petri dishes with saline at 37°C for 3 hr. The number of larvae that emerged was determined by microscopy.

To measure plasma estrogen and testosterone levels, rats were killed by decapitation and trunk blood collected into heparinized plastic tubes and centrifuged. Plasma was collected 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.* [15]. 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 intra- and inter-assay variations were below 13% and 7.5 %, respectively.

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.

Table 1 shows significant sex- and age-related differences in the number of migrating larvae recovered from the lungs of rats infected with *S. venezuelensis*. Among female rats, the number was the greatest (742 ± 42) in the youngest group (3 weeks), then it decreased significantly with age (6 weeks; 83 ± 23, $p<0.0001$ and 10 weeks, 35 ± 16; $p<0.0001$). Among male rats, the number of worms recovered from the youngest group (3weeks) was the smallest (662 ± 42). However, the differences between males and females were not significant within the same age group (3

Table 1. Recovery of migrating larvae from lungs of Wistar rats 3 days after infection with 3000 L_3 of *S. venezuelensis*

	Number of worms recovered		
	3 weeks	6 weeks	10 weeks
Female	742 ± 42[a)]	83 ± 23[b)]	35 ± 16[b)]
Male	662 ± 93[a)]	1522 ± 91[c)]	1446 ± 146[c)]

Values are expressed as mean ± SEM (n=6). a) vs. b) $p<0.0001$; a) vs. c) $p<0.01$; b) vs. c) $p<0.001$. Values marked by the same letter are not significantly different.

weeks, $p=0.4$). The susceptibility of male rats increased with age and the sex differences in the number of worms were significant at 6 (males vs. females, $p<0.0001$) and 10 weeks (males vs. females, $p<0.001$).

The mean concentrations of testosterone and estrogen in plasma are indicated as the values corrected for the body weight of each animal (Fig. 1). Plasma testosterone ($p=0.3$) or estrogen ($p=0.3$) concentrations did not significantly differ between the youngest male and female rats, but thereafter, these concentrations increased with age and the differences between sexes became significant (Fig. 1A and B).

The present study shows that the susceptibility of Wistar rats to *S. venezuelensis* infection was affected by host age and sex. Male sex hormones seem to increase the host susceptibility, since the number of larvae recovered from the lungs increased with age in males as testosterone levels became elevated. In contrast, female sex hormones seem to decrease host susceptibility, because the number of larvae recovered from the lungs decreased with age in females correlated with elevated estrogen levels.

The immune system of rats, like some other species of rodents with a short gestation period may not be fully developed at birth, so protective functions are unlikely to operate at full adult levels for several weeks after [25]. After birth the ontogeny of immunity is influenced by several factors, of which the exposure to infection may be a key, because antigenic stimuli play a critical role in maturation of the immune system.

Similar age-related differences in susceptibility to the infection with forest rat filarias, *Breinlia booliati* have been reported [14]. In three inbred rat strains (Lewis, Wistar and Sprague Dawley), neonates (less than 24 hr of age) were significantly more susceptible than juveniles (4 weeks of age). Furthermore, Lewis and Wistar strains were more susceptible than Sprague Dawley, indicating that both genetic background and aging affect the susceptibility to infection with *B. booliati* in white rats.

The resistance of rats and/or mice expressed during the early tissue migrating stages of parasite infection is probably caused by the activated mononuclear phagocyte system [1, 23, 26, 28]. In neonatal animals, the chemotactic response and phagocytic activity of alveolar macrophages are insufficient [25]. Macrophages from neonatally thymectomized mice do not acquire virucidal activity, perhaps as a result of a deficiency of IFN-γ [25], which is essential for

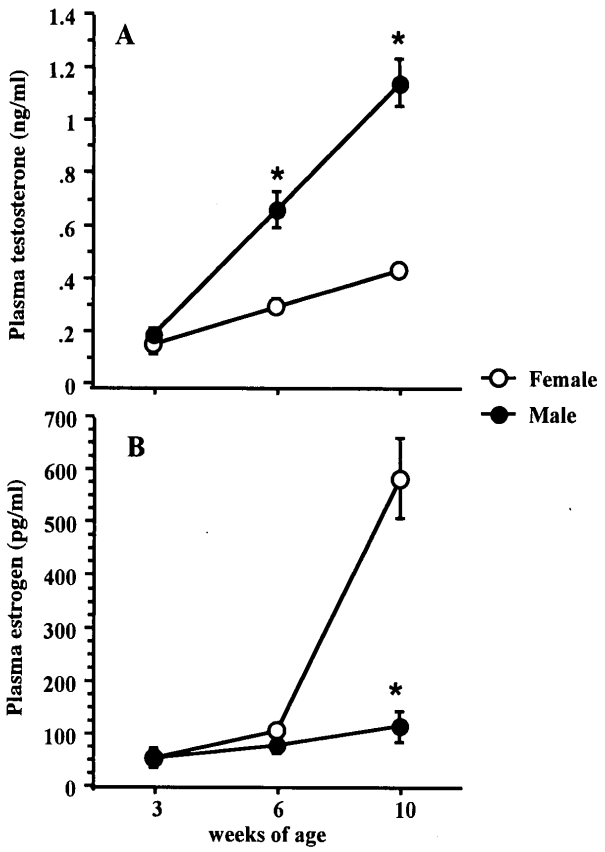

Fig. 1. Mean plasma levels of testosterone (A) and estrogen (B) in Wistar rats 3 days after infection with 3000 L_3 of *S. venezuelensis*. Data are corrected for body weight of each animal. Each value represents mean ± SEM of 6 rats. * $p<0.05$ (male vs. female).

the resistance to a variety of infections. The synthesis of nitric oxide (NO) by IFN-γ activated macrophages is also important. NO can act as a physiological messenger as well as a major effector of immune defense against parasitic infections [8–11, 13, 16, 19, 24, 27, 30].

Regarding the interaction between the host sex hormones and immune systems, androgens and female sex steroids participate in the down- and up-regulation of host immune systems [2, 3, 6, 7, 29]. The affinity of thymic androgen receptors for dihydrotestosterone did not differ with age, sex, nor species, but the concentration of androgen receptors was higher in males than females and in older compared with younger animals [20]. The thymus is androgen-responsive, so the immunoregulatory effects of androgens might be mediated in part through the thymus [4, 17, 22]. Furthermore, sex hormone receptors have been identified on various immune cells, suggesting a direct effect(s) of hormones [5].

In conclusion, the present study suggests that the susceptibility of Wistar rats to *S. venezuelensis* infection is regulated by both male and female sex hormones. However, the actual role and precise mechanisms of host sex hormones responsible for such an up- or down-regulation of host protective capacity against *S. venezuelensis* are not fully under-

stood and require clarification through further investigation.

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