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Hormonal Modulation of Sex Differences in Resistance to *Leishmania major* Systemic Infections

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Differences in susceptibility to intraveneously inoculated *Leishmania major* were observed in male and female mice of the BALB/cAnPt, DBA/2N, and DBA/2J strains and (BALB/cAnPt × DBA/2N)F_1 hybrids. In all cases, males had significantly higher liver parasite burdens than females. Orchidectomy of BALB/c males resulted in a 20% decrease in the number of parasites in the liver compared with either normal or sham-gonadectomized controls. Additionally, testosterone treatment of female BALB/c mice resulted in an 88% increase in the number of liver amastigotes. These results suggest that the hormone testosterone can modulate systemic *L. major* infections in BALB/c mice.

Sex-associated differences in patterns of resistance to bacterial, viral, and parasitic infection (10, 12, 15, 20, 32, 36, 40), tumor development (4), autoimmune responses (1; A. H. W. Schuurs, Clin. Immunol. Newsl. **6**:177–180, 1985), and cellular and humoral immune responses in general (5, 9, 13, 39) are well documented. *Leishmania major* infections also vary between male and female mice (2, 3, 11, 16, 24, 26–28). Males of the CXS recombinant inbred strains exhibited more-variable phenotypic responses after subcutaneous infection with amastigotes of *L. major* NIH 173 than did females (28). When backcross progeny generated from a cross between BALB/c and (BALB/c × C57BL/6)F_1 mice were infected subcutaneously with *L. major* Neal promastigotes, they also segregated according to gender: more than 60% of male progeny were susceptible to this parasite strain compared with 37% of females (16). In BALB/c mice, the strain most susceptible to *L. major*, infection with stationary-phase promastigotes (*L. major* L137) produced larger cutaneous lesions earlier in male than female mice; by 90 days postinfection, however, lesion scores were equivalent (24). The enhanced susceptibility of male mice to cutaneous infections with *L. major* is, in fact, most easily demonstrated in mice of a BALB/c background. In contrast to the studies described above, the studies of DeTolla et al. (8) did not find male-female susceptibility differences in F_2 progeny between BALB/cJ and C57BL/6J mice. Further, Alexander (2) demonstrated that males of a DBA/2 subline are more resistant to subcutaneous inoculation of *L. major* amastigotes in the shaven rump than females. Also, Giannini (11) documented the relative resistance of male B10.129 (10M)/ScSn mice to intradermal infections with promastigotes of *L. major* WR300. This *H-11* congenic line is, however, quite susceptible to subcutaneous infection with *L. major* Neal (6). It is clear that both mouse strain and parasite strain influence the outcome of such studies.

In our earlier studies (26, 27) and in the present study, a marked difference in liver parasite burdens between the sexes was observed when *L. major* NIH 173 amastigotes were administered intravenously (i.v.). We took advantage of this system to examine sex differences in systemic susceptibility among three strains of mice. The relationship between altered host resistance and the androgenicity of sex hormones was determined by investigating the effect of gonadectomy on male liver parasite burdens and testosterone treatment on female liver parasite burdens in BALB/c mice.

For analysis of systemic infections of *L. major*, mice were injected i.v. with 10^7 viable amastigotes in the tail vein and sacrificed after either 2 or 4 weeks. *L. major* NIH 173 amastigotes were obtained from 3-week-old footpad lesions of infected BALB/c mice as previously described (25, 29). Viable parasites were quantified by fluorescein diacetate-ethidium bromide staining of amastigotes (19). Systemic disease, quantified by determination of the number of parasites in livers, was estimated by microscopic examination of organ impression smears and expressed in Leishman-Donovan units (LDU; the number of parasites per 1,000 nucleated cells × liver weight in milligrams) (35). BALB/cAnPt, DBA/2N, and (BALB/cAnPt × DBA/2N)F_1 hybrids were kindly provided by Michael Potter (National Cancer Institute contract N01-CB-71085 at Hazleton Laboratories, Rockville, Md.), and DBA/2J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were placed in specific-pathogen-free barrier rooms, where they were housed in microisolater cages (five mice per cage); cages were changed weekly in a laminar flow hood. The mice were fed autoclaved NIH 31 mouse chow and given apples as a source of water.

When 8-week-old inbred and hybrid mice were examined for their susceptibility to liver infections of *L. major*, males had consistently more liver amastigotes than females at 4 weeks of infection (Fig. 1). In addition, DBA/2J males had significantly lower liver parasite burdens than DBA/2N or BALB/cAnPt males. A two-way analysis of the variation (computed by the method of Sokal and Rohlf [34]) observed in liver infections of the mice revealed significant differences between the sexes ($F_{1,99} = 553$; $P < 0.001$) and among the strains ($F_{3,99} = 25$; $P < 0.001$). All data were normalized by $\log_{10}$ transformation before analyses of variance were done.

Three separate experiments were performed in which liver LDU values were determined 2 weeks after *L. major* infection. After 2 weeks, male BALB/cAnPt and DBA/2N mice had liver LDU values of 752 ± 10 (geometric mean ± standard error) ($n = 17$) and 577 ± 6 ($n = 14$), respectively.

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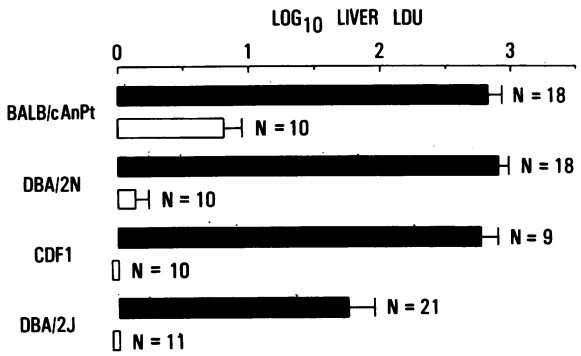

FIG. 1. Mean log liver LDUs for male (■) and female (□) mice 4 weeks after i.v. inoculation with *L. major* NIH 173 amastigotes. CDF_1 individuals were the result of a cross between BALB/cAnPt females and DBA/2N males. N represents the total number of mice examined in three separate experiments. Error bars represent one standard error.

In contrast, female BALB/cAnPt and DBA/2N mice had much lower liver LDU values of 62 ± 3 (n = 25) and 9 ± 1 (n = 15), respectively. A two-way analysis of the variation observed in 2-week liver infections revealed significant differences between sexes ($F_{1,67}$ = 219.47; $P < 0.001$) and strains ($F_{1,67}$ = 49.24; $P < 0.001$).

To assess the potential impact of sex hormones on the systemic susceptibility of male mice to systemic *L. major* infections, levels of infection in orchidectomized individuals were compared with levels in sham-gonadectomized mice. Gonadectomies were performed on male mice at 8 weeks of age under anesthesia. Both testes were excised after a ligation of the vas deferens and spermatic vessels through a midline incision of the lower abdomen (38). Sham-gonadectomized mice received a midline incision and had their testes manipulated. The skin incisions were closed by a wound clip. Infections with *L. major* were performed 21 days following the orchidectomies.

A 20% decrease in numbers of liver parasites was observed in orchidectomized male mice compared with normal and sham-gonadectomized controls (Fig. 2). An analysis of variance with treatment group (gonadectomized versus sham and normal) as a source of variation revealed that the decrease seen in orchidectomized mice was statistically significant ($F_{1,19}$ = 36; $P < 0.001$). Testosterone levels were not measured in these mice; however, the accessory glands of gonadectomized mice were visually smaller than those of sham-gonadectomized mice. This is well documented in

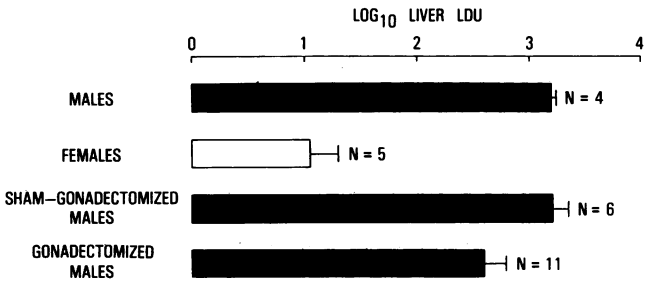

FIG. 2. Mean log liver LDUs of gonadectomized mice 4 weeks after i.v. inoculation with *L. major*. Gonadectomies were performed 3 weeks before infection. Error bars represent one standard error, and N represents the total number of mice examined in one experiment.

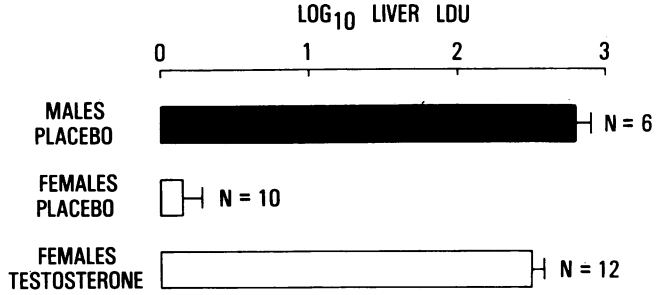

FIG. 3. Mean log liver LDUs of testosterone-treated mice 4 weeks after i.v. inoculation with *L. major*. Male and female control mice received subcutaneous implants of placebo pellets, and experimental female mice received testosterone pellets 16 days before infection. Error bars represent one standard error, and N represents the total number of mice examined.

castrated animals and has been shown to be reversible in animals receiving testosterone treatment (37). Gonadectomy does not reduce testosterone levels to zero; additional sources of testosterone may also have influenced the susceptibility of these animals.

To assess the effects of testosterone treatment on leishmania infections in female mice, 5-mg testosterone time-release pellets (Innovative Research of America, Rockville, Md.) were implanted into the subcutaneous tissue of 6-week-old female BALB/c mice. The pellets provide 3 weeks of controlled testosterone release. A total of two 5-mg pellets per mouse was administered over the course of the study. Six-week-old control mice received placebo pellets which contained the carrier binder (cholesterol:methyl cellulose: α-lactose) used in constructing the testosterone pellets. Mice were inoculated with *L. major* 16 days after the first testosterone implant.

Female mice receiving testosterone treatment showed an 88% increase in liver amastigote numbers compared with females given placebo pellets; the liver infection levels of testosterone-treated females were equivalent to those of males (Fig. 3). The infection levels of females were compared in an analysis of variance with treatment (testosterone versus placebo) as the source of variation. Testosterone-treated females had significantly higher infection levels than females receiving placebos ($F_{1,20}$ = 192; $P < 0.001$).

The variety of phenotypic responses observed in mice infected with *L. major* reflects the stage, dose, method of passage, and strain of the parasite; the route and site of inoculation; and the strain, age, and sex of the hosts. The relatively small differences in susceptibility between males and females to subcutaneous infection with *L. major* NIH 173 amastigotes were exaggerated in hosts which were inoculated i.v. Significantly lower levels of infection were observed in females than males in all strains of mice and F_1 progeny with systemic infections. This phenomenon was observed at both 2 and 4 weeks after infection. Sex differences have also been noted in human responses to parasitic diseases, including *Leishmania* disease, and have been recently discussed in the context of hormonal modulation (3, 7).

The sex differences observed in liver parasite numbers after i.v. inoculation of *L. major* may relate to differences in the liver microenvironments of males versus females. The liver is considered a sexually differentiated tissue, and a variety of liver functions have been shown to differ between the sexes (30). Many of these differences are regulated by androgens and estrogens (14, 33).

The results of our studies are consistent with the hypothesis that testosterone levels influence the susceptibility of BALB/c mice to *L. major*. Gonadectomized males experienced lower liver parasite burdens than did control males, and testosterone-treated females clearly had many more amastigotes in their livers than did females given placebo pellets. In addition, liver LDU values were indistinguishable between testosterone-treated females and control males.

Testosterone modulation of systemic *L. major* infections may be either a direct or indirect effect of the hormone. Even though estrogens and androgens have been documented to be both immunoinhibitory and immunostimulatory, they tend to act mainly to suppress the cell-mediated immune system (13). Cell-transfer studies have documented that once *L. major* infects macrophages, resolution of infection depends primarily on cell-mediated immune mechanisms (17, 18, 21–23, 31). It is possible that males and testosterone-treated females both have suppressed cell-mediated immune responses to *L. major* over and above the susceptible genetic phenotype of BALB/c mice that controls natural resistance. This further impairment of cellular events in immunity results in increased liver parasite burdens.

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