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<td>学術誌</td>
<td>熱帯医学  特集: 熱帯虫寄生虫のマウスに対する抵抗性の研究</td>
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A Comparative Study on the Resistance to Various Stages of *Brugia pahangi* between Male and Female Mice

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**Abstract:** Sex differences in susceptibilities of mice to various stages of *Brugia pahangi* worms inoculated were examined. Infective larvae (L3), the fourth-stage larvae (L4) and adult worms were intraperitoneally inoculated into naive BALB/c mice aged over 12 weeks. When the recovery rates of stages of worms were compared at 15 days post-inoculation between male and female mice, sex difference in the resistant capacity was induced only by L3 but not others. Microfilariae (mf) were reproduced from inoculated adult worms in the peritoneal cavities of both sexes of mice. There was no difference in the mf count between the sexes. Intravenous inoculation of mf into mice also showed no sex difference in the rate and period of clearance.

**Key words:** *Brugia pahangi*, Stages of worm, BALB/c mouse, Resistance, Sex difference

Sex difference in the susceptibility to the infection with *Brugia pahangi* in sexually maturated mice has been reported (Nakanishi, 1987; Nakanishi *et al*., 1989a). Female mice showed greater resistance to the infection with *B. pahangi* than males in association with higher response of macrophages or eosinophils (Nakanishi, 1987). Such weak resistant capacity in matured male mice is caused by a suppressive effect of testosterone (Nakanishi *et al*., 1989a). Recently, it has been proved that macrophages have key roles to alter the susceptibility to *B. pahangi* infection between both sexes not only as effector cells but also as immunoregulatory cells (Nakanishi *et al*., 1989b).
B. pahangi molt twice in the host and developed an advanced stage. Our earlier result indicated that infective larvae (L3) but not the fourth-stage larvae (L4) induced sex difference in susceptibility of mice (Nakanishi, 1987). However, it is still remained unclear that such difference is due to qualities (specificities of components) or quantities (amount of antigens) of parasites.

In this study, therefore, we compared the resistant capacities of male and female mice to the different doses of L3 and L4. And to confirm the stage specific induction of sex difference in susceptibility, we used other stages of B. pahangi (adult worms and microfilariae).

Inbred male and female BALB/c mice were raised in our laboratory parental stocks under conventional conditions. Mice, more than 12 weeks old, were used in all the experiments.

L3 of B. pahangi were obtained from the mosquitoes (Aedes aegypti) which had been fed on microfilaremic Wistar rats 2 weeks previously. Mice were inoculated intraperitoneally (i.p.) with 50 L3 suspended in 0.5 ml of Hanks’ balanced salt solution (HBSS).

L4 were obtained from the BALB/c mice which had been inoculated i.p. with 400–500 L3 of B. pahangi 20 days before. Mice were inoculated with 20 or 40 L4 in 0.5 ml of HBSS through an 18–guage needle into the peritoneal cavity.

Adult worms were collected from the jirds (Meriones unguiculatus) which had been inoculated i.p. with 300–400 L3 of B. pahangi 3 months before. Active 20 adult worms (10 males and 10 females) were surgically implanted into the peritoneal cavity of mice using a Pasteur pipette through the opening of the lateral abdomen.

Microfilariae (mf) were obtained from the jirds which had been infected i.p. with 300–400 L3 of B. pahangi 3 months before. The animals were anesthetized with ether and their peritoneal cavities were flushed with 10 ml of sterilized HBSS. The peritoneal effusion was placed into a plastic dish (Sumitomo Bakelite Corp., Tokyo), and kept at 37°C in an incubator for 30 minutes in order to remove peritoneal adherent cells (Ah et al., 1974). After centrifugation at 2,000 rpm at room temperature for 5 minutes, the active mf were resuspended in sterile HBSS at a concentration of $3 \times 10^5$ mf/ml. Mice were injected with 0.75 x $10^5$ mf in 0.25 ml of HBSS via the tail vein. To follow the density of mf after the inoculation, 20µl of blood samples were taken from the retro-orbital plexus at the indicated time points. Mf were counted and the data were expressed as the number of mf/20µl blood.

Mice were killed on day 15 post-inoculation (PI) by over-dose of ether anesthesia. The majority of worms were recovered from the peritoneal cavity by flushing with 5 ml of heparinized HBSS. A small number of residual worms were also recovered by incubating the carcass in HBSS at 37°C for 2 hours.

Statistical significance of differences in mean values were assessed by using Student’s $t$–test.

As shown in Fig. 1, female mice showed significantly higher ($P<0.01$) resistance than males when 50 or 500 L3 were inoculated into mice. On the contrary, female mice
Table 1. Reproduction of microfilariae in the peritoneal cavities of male and female mice 15 days after implantation of adult *B. pahangi*

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<tr>
<th>Sex of animals</th>
<th>No. of animals</th>
<th>No. of mf reproduced* (mean ± SD)</th>
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<tr>
<td>Male</td>
<td>10</td>
<td>11603 ± 6257</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>17260 ± 13720</td>
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*Adult worms were collected from the peritoneal cavities of the jirds which had been intraperitoneally inoculated with 300–400 L3 three months previously. Both 10 male and female adult worms were intraperitoneally implanted into mice.

**These data are based on the mean and standard deviation (SD) of total mf count/peritoneal cavity. There was no statistical significance between the means from male and female mice.

Fig. 1. Recovery rates of worms at 15 days PI of various stages of *B. pahangi*, i.e., L3, L4, male adult worms and female adult worms, from male (open columns) and female (dotted columns) mice. Numerals in the parentheses indicate numbers of inoculated worms. From left to right each column represents the mean from seven, seven, eight, five, five, five, five, eleven, thirteen, eleven and thirteen mice, respectively. Vertical bars indicate the standard deviation. *Recovery rate of worms from female mice was significantly less than that from males (P<0.01).

Fig. 2. Kinetics of mf counts in male (closed circles) and female (open circles) mice after intravenous implantation of mf taken from the peritoneal cavity of the infected jirds. Ten male and eleven female mice were injected with 0.75 × 10⁵ mf suspended in 0.25 ml of HBSS. Mf counts were performed on 20 μl of peripheral blood. Vertical bars indicate the standard deviation.
showed rather lower resistance than males when L4 or adult worms were inoculated.

On the other hand, many reproduced mf were recovered from the peritoneal cavities of mice which had been inoculated with male and female adult worms (Table 1). No significant difference was observed in mf count between male and female mice.

Peripheral blood mf level in inoculated male mice was almost the same as that in females. Maximum mf counts were reached by day 7, then decreased rapidly within 2 to 3 weeks and almost disappeared by week 9 PI. There were no significant difference in mf counts between host sexes throughout the experimental period (Fig. 2).

The results reported here clearly show that only L3 could induce a difference in the resistant capacity between male and female mice.

As to the mechanisms of host resistance to filarial worms are concerned, complement-(Haque et al., 1982; Chandrashekar et al., 1985; 1986) or antibody-dependent (Mehta et al., 1981) cell-mediated killing of larvae have been reported. Macrophages and eosinophils have important roles in killing larvae (Haque et al., 1982; Chandrashekar et al., 1986). Serum-mediated cytotoxicity of leukocytes to mf of various species of filariae have also been reported by many workers (Subrahmanyam et al., 1976; Weiss and Tanner, 1979; Johnson et al., 1981). Nakanishi (1987) reported that the onset of sex difference in the susceptibility of mice to B. pahangi infection is correlated with the time when differential responses of inflammatory cells including macrophages and eosinophils was observed between male and female mice.

As regards the mechanisms to express sex difference in the susceptibility of mice to infection with B. pahangi, significant difference was not found between sexes before sexual maturation of animals (Nakanishi, unpublished data). After puberty, male mice increased their susceptibility because of immunosuppressive effect by androgen (Nakanishi et al., 1989a; Ansar Ahmed et al., 1985; 1987). Testosterone treatment in female mice increased susceptibility and suppressed inflammatory cell response (Nakanishi et al., 1989a). Therefore, it is obvious to define that the expression of sex difference in the susceptibility to the infection with B. pahangi in mice is entirely due to an increased susceptibility of males by an immunosuppressive effect of androgen.

Our study showed that mice give damages to various stages of B. pahangi, however, the resistant capacity of male mice was significantly suppressed only when L3 were inoculated. Since this suppressed resistance was depending on differences of stages but not doses of parasite inoculated, testosterone-regulated suppressive mechanism(s) in male mice might be restricted to L3. Although underlying mechanism that changes host (male mice) immunological response against stages of B. pahangi, especially between L3 and others, still remained unclear, such change seems to correlate closely to macrophage functions (Nakanishi et al., 1989b). Recently, some kinds of cytokines such as colony-stimulating factors (CSFs) were proved to have important roles not only as hemopoietic factors but also as functional modifier of inflammatory cells (Reed et al., 1987).

Thus, differences of host inflammatory responses and production of cytokines between male and female mice elicited by the inoculation of L3 or other stages of B.
pahangi, and component(s) of L3 which might alter host response should be further clarified.

REFERENCES


*Brugia pahangi* 各発育期虫体に対するマウス抵抗性の雄・雌間における比較
中西弘有，堀井洋一郎，森 章夫，在津 誠，上田正勝，黒川憲次（長崎大学医学部医動物学教室）
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*Brugia pahangi* 各発育期虫体に対するマウス感染感受性の性差について調べた。各発育期虫体すなわち、感染幼虫（L3）、第4期幼虫（L4）、成虫をそれぞれ12週令以上の正常 BALB/c マウス腹腔に移入した。移入後15日目にその回収率を雄・雌マウス間に比較すると、宿主抵抗性の性差は L3 を移入したマウスにのみ認められた。成虫を移入したマウス腹腔内ではミクロフィラリア（mf）の産生が認められたが、雄・雌マウス間において産生数の差はみられなかった。また静脈内に移入した mf の排泄の率や期間とも同様に性差はみられなかった。

熱帯医学 第30巻 第4号 257－262頁，1988年12月