Difference in the Reaction of Peritoneal Cells to *Brugia pahangi* in Several Strain of Mice

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Abstract: The difference in the recovery rate of *Brugia pahangi* and reaction of peritoneal cells among the 5 strains of mice are described in the present study. When intraperitoneal infection was used, the recovery rates at 2 weeks after infection in male mice were 1.8, 9.1, 1.7, 17.0 and 2.3% in C3H/He, C57BL/6, BALB/c, ICR and ddy, respectively. The recovery rates in female mice were 6.8, 3.7, 4.8, 10.8 and 2.4% in C3H/He, C57BL/6, BALB/c, ICR and ddy, respectively. There was a significant difference between males and females in the recovery rates of C3H/He and C57BL/6 (P<0.05). A higher reaction to parasites was recognized in female C57BL/6 and ddy (54.5 and 55.6%). The scanning electron microscope could not reveal remarkable changes on the cuticular surface of worm. The transmission electron microscope showed eosinophils and lymphocytes on the worms surrounded by peritoneal cells.

Key words: *Brugia pahangi*, Peritoneal cells, Adhesion, Mice

INTRODUCTION

It is well known that large differences in susceptibility to filariae exist among laboratory animals. Also, a sex difference in susceptibility of the host to parasites has been reported (Ash, 1971; 1973; Sucharit and MacDonald, 1972). However, those authors state that the presence of infection was proven by the appearance of microfilariae in peripheral blood after the subcutaneous inoculation of infective larvae. Since the report by McCall et al. (1973) that adults and microfilariae were localized in the peritoneal cavity when the infective larvae were inoculated into the peritoneal cavity of jirds, there have been several reports describing the development of filarial worms in BALB/c (Mackenzie et al., 1985; Nakanishi, 1987) and ICR mice (Sakamoto et al., 1982). Intraperitoneal infection is an effective way to study the reaction of peritoneal cells to filarial worms in small laboratory animals, although the worms localized in the peritoneal cavity seem to follow an
aberrant mode of development. The present study describes the reaction of peritoneal cells to filarial worms in 5 strains of mice; C3H/He, C57BL/6, BALB/c, ICR, ddy.

**MATERIALS AND METHODS**

Inbred male and female C3H/He, and C57BL/6 BALB/c, were provided by Dr. Sachiko MATSUO of the Animal Research Center for Infectious Tropical Disease, Institute of Tropical Medicine, Nagasaki University. The other 2 strains of mice (ICR and ddy) were purchased. Mongolian jirds (Meriones unguiculatus) raised in our laboratory were used as the appropriate hosts of filarial infection. Mice and jirds, were used in the experiment over a period of 8 weeks. One hundred infective larvae of *B. pahangi* obtained from *Aedes aegypti* were inoculated into the peritoneal cavity. All of the mice were killed by ether at 2 weeks after the first inoculation of infective larvae, and then the peritoneal cavities were washed with Hanks’ solution to recover the filarial worms. The grade of adhesion of peritoneal cells to worms is classified as follows. Grade 0: No adhesion of peritoneal cells. Grade I: Half or less of body length was covered with peritoneal cells. Grade II: More than half of the body length was covered with peritoneal cells. The total number of recovered worms for each animal was counted, and the grade of adhesion of peritoneal cells was determined. A scanning electronmicroscopical study was carried out to examine changes on the surface of worms. The specimens were prepared as follows. The recovered worms were fixed with 5% glutaraldehyde, 0.1M phosphate buffer, pH 7.4. They were dehydrated in a graded series of amyl acetate through the mixture of ethanol and amyl acetate, dried in a critical-point dryer, mounted on stubs, and rotary-coated with gold in a vacuum evaporator. To examine the adherent cells, thin sections (60–90nm) were cut with glass knives, stained with uranyl acetate lead citrate and observed. The JEM 100CX (JEOL) electoron microscope was used for the examination of specimens by both scanning and transmission. Statistical analysis was performed where appropriate using Wilcoxon’s rank-sum test.

The animal experiment in this study was performed at The Animal Research Center for Infectious Tropical Diseases, The Institute of Tropical Medicine, Nagasaki University.

**RESULTS**

Table 1 shows the number of worms recovered from the peritoneal cavity of jirds and 5 strains of mice. The proportion of adhesion of peritoneal cells to *B. pahangi* also shown in Table 1. In jirds as the susceptible host, the total number of worms recovered was 342 (57.0%) and 485 (53.9%) in males and females respectively. Most of the worms were Grade 0 (95.3% in male and 94.2% in female). Inbred male mice C3H/He, C57BL/6 and BALB/c showed 1.8, 9.1 and 1.7% recovery rates, respectively. On the other hand, the recovery rates in female mice were 6.8, 3.7 and 4.8%, respectively. There is a significant difference between males and females in the recovery rates of C3H/He and C57BL/6 (P<0.05). ICR male mice show the highest recovery rate (17.0%) among the 5 strains of
mice. Both male and female ddy show a low percentage similar to that in inbred mice. The reaction of peritoneal cells to worms was observed as follows. Female C57BL/6 and male ddy showed a higher proportion of Grade II (54.5 and 55.6%). On the other hand, most worms recovered from the peritoneal cavity of male C3H/He, BALB/c, ICR and female C3H/He showed Grade 0. The scanning electron microscope was used to observe the adherent cells on the worms (Figure 1). The worms had already developed to 4th stage larvae at 2 weeks after the first inoculation of infective larvae. A remarkable change was not recognized on the cuticular surface with high magnification (Figure 2). A transmission electronmicroscopical examination revealed that eosinophils and lymphocytes were dominant in the worms surrounded by peritoneal cells (Figure 3).

Table 1. Reaction of peritoneal cells to Brugia pahangi

<table>
<thead>
<tr>
<th>Animal</th>
<th>SEX</th>
<th>No. of animals examined</th>
<th>Total No. of larvae recovered</th>
<th>No. of larvae recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade 0</td>
<td>Grade I</td>
</tr>
<tr>
<td>Jirds</td>
<td>M</td>
<td>6</td>
<td>342 (57.0)</td>
<td>326 (95.3)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>485 (5.39)</td>
<td>457 (94.2)</td>
</tr>
<tr>
<td>C3H/He</td>
<td>M</td>
<td>12</td>
<td>22 (1.8)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>81 (6.8)</td>
<td>58 (71.6)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>M</td>
<td>12</td>
<td>109 (9.1)</td>
<td>61 (56.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>44 (3.7)</td>
<td>18 (40.9)</td>
</tr>
<tr>
<td>Mice</td>
<td>BALB/c</td>
<td>M</td>
<td>12</td>
<td>20 (1.7)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>57 (4.8)</td>
<td>29 (50.9)</td>
</tr>
<tr>
<td>ICR</td>
<td>M</td>
<td>12</td>
<td>204 (17.0)</td>
<td>162 (79.4)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>129 (10.8)</td>
<td>69 (53.5)</td>
</tr>
<tr>
<td>ddy</td>
<td>M</td>
<td>12</td>
<td>27 (2.3)</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>29 (2.4)</td>
<td>15 (51.7)</td>
</tr>
</tbody>
</table>

( ) = %

Fig. 1. The adhesion of peritoneal cells is seen on the worm.

Fig. 2. Normal transverse striations of cuticular surface are seen with high magnification.
DISCUSSION

In this paper, the reactions of peritoneal cells to *B. pahangi* in 5 strain of mice infected by the intraperitoneal route are described. Male ICR mice showed a higher recovery rate at 2 weeks after the first inoculation of infective larvae. With intraperitoneal infection, a significant difference in the recovery rates was observed between male and female C3H/He and C57BL/6. Concerning the difference in the recovery rates of worms between male and female mice, Nakanishi (1987) reported that BALB/c mice showed a significant difference between the sexes to *B. pahangi* infection on day 10 and 15 after the first inoculation of infective larvae. On the other hand, Sakamoto *et al.* (1982) reported that a difference of recovery rates between male and female ICR mice was recognized when the subcutaneous infection was used but not when the intraperitoneal route was used. Ash (1971) described the preferential susceptibility of male jirds to infection with *B. pahangi* when the infective larvae were inoculated subcutaneously. However, there was no significant difference in the recovery rate at 15 days after infection between male and female jirds when intraperitoneal infection was used in the present study. Kiyota (1984) reported that male C57BL/6 mice showed a higher susceptibility than females in *Strongyloides ratti* infection, and suggested that testosterone (sex steroid hormone) was responsible for the difference of susceptibility between male and female mice. In the present study, C3H/He and C57BL/6 showed a significant difference in the recovery rate between male and female. However, there was no significant difference in the proportion of recovered worms showing a reaction of Grade II. On the other hand, there was no significant difference in the recovery rate between male and female BALB/c, ICR and dd, although a significant difference was recognized in the grade of the reaction to worms. It is unlikely that the number of worms recovered indicated the difference in susceptibility to *B. pahangi* between female and male mice at 2 weeks after the first inoculation of infective larvae. The cell adherence to parasites and the cytotoxic activity against worms have been identified.
as one of the major effector mechanisms to parasite worms (Butterworth et al., 1977; Mackenzie et al., 1977; Perrudet-Badoux et al., 1978; Weiss and Tanner, 1978; Butterworth, 1984). Recently, Nakanishi (1987) reported that the number of macrophages and eosinophils significantly increased in the peritoneal cavities of female BALB/c mice infected with *B. pahangi* intraperitoneally. Perrudet-Badoux et al. (1978) reported that the predominant adherent cells were eosinophils when *Trichinella spiralis* were incubated in medium containing immune antisera. In the present study, eosinophils and lymphocytes were shown to be dominant around the worms by electromicroscopical examination. It is likely that eosinophils were closely involved in the parasite-killing mechanism. However, the degree of adhesion of those cells to parasites was different among the strains of mice.

**References**


