Enhanced Protection against the Migratory Phase, but Defective Protection against the Intestinal Phase of *Strongyloides venezuelensis* Infection in Cotton Rats, *Sigmodon hispidus*

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ABSTRACT. The protective capacity of the cotton rat, *Sigmodon hispidus*, against the migratory and intestinal phases of *Strongyloides venezuelensis* infection was examined. After subcutaneous infection with infective larvae (L₃), adult worm recovery rates from male and female animals on Day 71 were only 0.10% and 0.06% of initial dose, respectively. To determine whether this enhanced protection was expressed during the migratory phase or the intestinal phase, larval recovery from the lungs of cotton rat was evaluated 3 days after subcutaneous L₃ infection. The larval recovery rate was only 0.5% of initial dose and about 40-fold lower than that from control mice. Protection in the intestine was also evaluated after intraduodenal implantation of adult worms. About 30% of implanted worms became established and worm burden remained constant until Day 28. Despite a high worm burden on Day 28, EPG was about 25-fold lower than the peak count. To evaluate expulsive capacity and monitor the cellular responses in the intestine of cotton rats, adult *Nippostrongylus brasiliensis* worms were implanted in addition to *S. venezuelensis*. Cotton rats were unable to expel adult *S. venezuelensis* worms, even after 21 days of observation. Although the number of mucosal mast cells increased significantly, the intraepithelial migration of mast cells was not observed. In contrast, *N. brasiliensis* was expelled by Day 6 in association with goblet cell hyperplasia. These results suggest that in cotton rats, the defective intestinal protection against adult *S. venezuelensis* worms results from dysfunction of mucosal mast cells.

KEY WORDS: cotton rat, intestinal phase, migratory phase, protection, *Strongyloides venezuelensis*.

The intestinal nematodes *Nippostrongylus brasiliensis*, *Strongyloides ratti*, and *S. venezuelensis* are very common parasites of small rodents. Inoculation of laboratory mice and rats with these parasites has facilitated the study of immunological mechanisms during the migratory and intestinal phases of infection [20, 23]. In addition to these studies, novel immune responses against these parasites were observed in Mongolian gerbils [9–11, 13], several hamster species [24–26], and *Millardia meltada* [27–29]. For example, although Mongolian gerbils were unable to expel *S. ratti* and *S. venezuelensis* from their intestines, even after extended incubation periods, they routinely expelled *N. brasiliensis* adult worms [9, 10]. These gerbils exhibited enhanced protection against migratory stages but not intestinal stages of secondarily infected *S. venezuelensis* [13]. *M. meltada* showed sex differences in the expulsion of *N. brasiliensis* adult worms from the intestine due to suppression of goblet cell responses in male animals [27].

Cotton rats, *Sigmodon hispidus*, have been widely used as a model for a variety of viral or rickettsia infections that cause human diseases [15, 16, 21, 22] and for investigations of parasitic infections [7, 8, 17, 30]. In our most recent study, cotton rats showed enhanced protective capacity against the early migratory phase of *N. brasiliensis* infection, but were somewhat susceptible to infection with adult worms that were implanted intraduodenally [31]. These results suggest that the protective capacities of cotton rats differ based on the stage of *N. brasiliensis* infection. In the present study, we attempted to confirm these differences in the susceptibility of cotton rats to the segregated phases of parasite infection using *S. venezuelensis*, another common intestinal nematode of rodents.

MATERIALS AND METHODS

Animals: An inbred strain of cotton rat, *Sigmodon hispidus*, was raised in the Experimental Animal Center, Miyazaki Medical College, Japan. In the present experiments, we used sexually mature male animals (12–18 weeks old), unless stated otherwise. C57BL/6 mice of the same age were raised in our laboratory and used as controls. Male Wistar rats were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan) for the maintenance of *N. brasiliensis*. All animals were housed in clean metal cages and given a standard diet and tap water *ad libitum* in an air-conditioned room (23 ± 1°C), under conventional conditions with a 12:12 hr light/dark cycle. Care procedures followed the “Guide for Animal Experiments at the Faculty of Agriculture, Miyazaki University” and guidelines established by the.
Parasitological techniques: The strain of *S. venezuelensis* used in the present study was originally isolated from wild brown rats (*Rattus norvegicus*) in Okinawa, Japan [6] and maintained in our laboratory by serial passage in Wistar rats and/or Mongolian gerbils (*Meriones unguiculatus*). Third stage infective larvae (L₃) were prepared from cultures of infected feces using the filter paper method. Adult worms of *S. venezuelensis* were isolated from the intestine of Wistar rats at 7 days postinfection (pi) with 10,000 L₃ using a previously described method [10]. The strain of *N. brasiliensis* used in the present study has been maintained in our laboratory by serial passage in Wistar rats over 6 years by subcutaneous inoculation with 3,000–4,000 L₃ prepared by the charcoal culture method. Adult worms were obtained from the intestine of Wistar rats at 7 days pi with 3,000–4,000 L₃ using a previously described method [12]. To determine the protective capacity during the migratory and/or intestinal phases of the parasites, cotton rats were infected by subcutaneous inoculation with 6,000 L₃ or by intraduodenal implantation of 1,000 adult *S. venezuelensis* worms. Control mice received 3,000 L₃ subcutaneously. Adult *N. brasiliensis* worms were intraduodenally implanted into cotton rats as a control. The degree of parasite infection was monitored by daily counting of eggs in the feces (EPG, egg per gram of feces) and/or counting worms recovered from lungs or small intestine at autopsy. Animals were euthanized by an overdose of ether anesthesia on the designated days. For recovery of the migrating larval worm, both lungs were removed, cut into small pieces using a motor driven disperser, and incubated in petri dishes containing saline at 37°C for 3 hr. The number of larvae that emerged was determined under a dissecting microscope. For recovery of adult worms, the small intestine was cut open longitudinally and incubated in saline for 2 hr at 37°C, and the worms recovered were counted [12].

Histology: Small pieces (about 1.5 cm long) of jejunum taken about 5 cm distant from the pylorus, which is the major site of infection by both parasites, were fixed in either Carnoy’s fluid or buffered formalin. The samples were dehydrated, cleared in lemosol (Wako Pure Chemical Industries, Osaka, Japan), and embedded in paraffin wax. Sections (4 µm thickness) were stained with Alcian blue (pH 0.3) and Safranin-O (pH 0.1). The number of mast cells was counted for 50–80 villus crypt units (VCU: [18]). To count goblet cells, sections were stained with Alcian blue and periodic acid/schiff (PAS) [19].

Statistical analysis: Results were statistically analyzed using Student’s *t*-test, and a *p* value below 0.05 was considered significant. Data are present as means ± SD, unless stated otherwise.

RESULTS

After a primary infection with *S. venezuelensis* L₃, EPG kinetics of both male and female cotton rats were quite sim-
ilar, and no sex differences were observed in egg count during the period examined (Fig. 1). EPG rose rapidly from Day 5 to Day 10 and then remained primarily stable for 71 days pi, showing only a gradual decline. Maximal EPG was 2,000–3,000. The rate of adult worm recovery from male and female animals on Day 71 was only 0.10% (5.8 ± 5.3) and 0.06% (3.6 ± 39) of initial dose, respectively. The differences between host sexes were not statistically significant and thus, male animals were used in subsequent experiments.

To determine whether the susceptibility of cotton rats to S. venezuelensis infection was greater during the migratory phase or intestinal phase, cotton rats and control mice were challenged subcutaneously with 6,000 or 3,000 L3, respectively, and larval recovery from lungs was evaluated on Day 3. The larval recovery rate from the lungs of cotton rats was only 0.5% of initial dose or about 40-fold lower than that from control mice (Table 1). To evaluate intestinal protection, cotton rats were intraduodenally implanted with 1,000 adult S. venezuelensis worms, and EPG and worm burden were monitored on designated days (Fig. 2). Approximately 30% of implanted worms became established, and worm burden remained constant until 28 days and then gradually declined until 54 days pi. EPG rose immediately after implantation and peaked on Day 4, then declined quickly. Despite a high worm burden on Day 28, EPG was less than 100, or about 25-fold lower than the peak count (Fig. 2).

To evaluate expulsive capacity and monitor cellular responses in the intestine to other parasitic worms, adult N. brasiliensis worms were implanted in cotton rats as a control. Worm burden and EPG after intraduodenal implantation of 1,000 adult S. venezuelensis worms, and EPG and worm burden were monitored on designated days (Fig. 2). Approximately 30% of implanted worms became established, and worm burden remained constant until 28 days and then gradually declined until 54 days pi. EPG rose immediately after implantation and peaked on Day 4, then declined quickly. Despite a high worm burden on Day 28, EPG was less than 100, or about 25-fold lower than the peak count (Fig. 2).

Table 1. Larval recovery from lungs of cotton rats and control mice 3 days after infection with S. venezuelensis

<table>
<thead>
<tr>
<th>Animals</th>
<th>Infection dose</th>
<th>No. of larvae recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>3,000 L3</td>
<td>1269.3 ± 530.9 (42.3)</td>
</tr>
<tr>
<td>Cotton rats</td>
<td>6,000 L3</td>
<td>30.5 ± 16.4* (0.5)</td>
</tr>
</tbody>
</table>

* p<0.01. Data are the mean ± SD from 5 animals.

Table 2. Worm burden and EPG of cotton rats 2 and 21 days after intraduodenal implantation with 1,000 adult S. venezuelensis worms

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Worm burden</th>
<th>EPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>276.2 ± 120.0</td>
<td>20552.0 ± 12525.0</td>
</tr>
<tr>
<td>21</td>
<td>306.8 ± 99.5NS</td>
<td>773.0 ± 286.0*</td>
</tr>
</tbody>
</table>

NS: Not significant (p=0.15), * p<0.01. Data are the mean ± SD from 5 animals.

Table 3. Worm burden and EPG of cotton rats 2, 4 and 6 days after intraduodenal implantation with 300 adult N. brasiliensis worms

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Worm burden</th>
<th>EPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>241.8 ± 23.7</td>
<td>3860.0 ± 952.6</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>840.0 ± 297.3</td>
</tr>
<tr>
<td>6</td>
<td>5.0 ± 2.4</td>
<td>0</td>
</tr>
</tbody>
</table>

ND: Not done. Data are the mean ± SD from 5 animals.
DISCUSSION

The present study showed that cotton rats were highly resistant to the migratory stage of *S. venezuelensis*. In contrast, this animal could not expel intraduodenally-implanted adult *S. venezuelensis* worms, even after extended periods of observation. The mechanisms that protect the cotton rats from infection during the migratory stages of larval worms appear to involve complement-dependent cell-mediated cytotoxicity (CDCC), as is seen with *S. ratti* and other parasite infections [1–5]. The innate protection of cotton rats against migratory stages of *S. venezuelensis* larvae was as effective as the response to *N. brasiliensis* infection [31], indicating that this mechanism functions regardless of parasite species.

On the other hand, the expulsion of adult *S. venezuelensis* depends upon mastocytosis in the intestinal mucosa. For the expulsion of adult *S. venezuelensis* worms, not only the number of mast cells but also their distribution, particularly in the intraepithelial niche of the parasite, is important [20]. However, in the present study, we did not observe intraepithelial migration of mast cells. In contrast to *S. venezuelensis*, implanted *N. brasiliensis* adult worms were expelled from cotton rats following a kinetic profile similar to that in rats, the usual host for this parasite [12, 29]. Goblet cell hyperplasia was observed in the small intestine of cotton rats at the time of expulsion, as was previously reported for *N. brasiliensis* infected rats [12]. Thus, the data suggest that goblet cell mucin-mediated expulsion operates normally in the cotton rat intestine. Therefore, the inability of cotton rats to expel parasites is limited to *S. venezuelensis* but not to *N. brasiliensis*. This defect is likely due to a dysfunction of mast cell mobility in the intestinal mucosa of cotton rats, as is seen in hamsters and Mongolian gerbils [9, 11, 26].

In the cases of parasite expulsion from customary hosts, such as *S. venezuelensis* from mice [14] or *N. brasiliensis* from rats [12], the kinetics of adult worm expulsion paralleled those of egg counts in the feces. In the present study, the kinetics for *N. brasiliensis* expulsion from cotton rats corresponded to that of egg count. However, the kinetics in adult worm expulsion and egg count in *S. venezuelensis*-implanted cotton rats differed. Despite persistent parasitization by adult *S. venezuelensis* worms, the number of eggs in the feces quickly declined within two weeks of adult worm implantation. Similar dissociation between egg output and intestinal worm burden has been reported for *N. brasiliensis* infected male *M. m. latulata* [29]. Although male *M. m. latulata* could not expel adult *N. brasiliensis* from the intestine because of suppression of goblet cell hyperplasia by testosterone, the reproduction of eggs in the uterus of the surviving parasites was suppressed by host immunity. The underlying mechanisms causing in the inconsistency between numbers of worms and eggs detected from *S. venezuelensis* implanted cotton rats require further clarification.

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REFERENCES


