FULL PAPER *Parasitology*

Resistance of Cotton Rats, *Sigmodon hispidus*, to Primary Infection by *Nippostrongylus brasiliensis*

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(Received 15 November 2001/Accepted 6 February 2002)

ABSTRACT. The resistance of cotton rats, *Sigmodon hispidus* to *Nippostrongylus brasiliensis* infection was examined and compared the response to that of the susceptible Indian soft-furred rat, *Millardia meltada*. After a primary infection with infective third-stage *N. brasiliensis* larvae (L_3), the number of eggs in feces and adult worm recovery rates from the small intestine of cotton rats were significantly lower than in the controls. To determine whether cotton rat resistance was observed during the migratory phase or the intestinal phase, cotton rats and control animals were challenged subcutaneously with L_3 or intraduodenally with adult worms, and larval recovery from lungs and adult worm burden were evaluated. The recovery rate of larvae from the lungs of cotton rats was about five-fold lower than from controls. Adult worm recovery from the small intestine of cotton rats was also lower than that from the controls, but the difference (two-fold lower) was smaller than that observed for lung recovery. Carbon treatment at a dose of 250–500 mg/kg effectively increased larval worm recovery from the lungs of cotton rats. However, this treatment had no effect on worm recovery from the intestine after intraduodenal implantation of adult *N. brasiliensis*. These results suggest that macrophage function have important role in the expression of strong resistance during the migratory phase of *N. brasiliensis* infection in cotton rats.

KEY WORDS: carbon, cotton rat, innate protection, macrophage, *Nippostrongylus brasiliensis*.

J. Vet. Med. Sci. 64(5): 423–426, 2002

The cotton rat, *Sigmodon hispidus* is a widely used model of many viral and rickettsia infections that cause human diseases, including respiratory syncytial virus [18], measles virus [17], HIV type-1 [12] and *Bartonella* species [11]. This animal has also been used as an experimental host for several parasites, for example bovine liver fluke, *Fasciola hepatica* [14] and rodent filaria, *Litomosoides carinii* [6, 7, 24], to which the rat is moderately susceptible.

The rat intestinal nematode, *Nippostrongylus brasiliensis* is a commonly used parasite in investigations of various host immune responses to helminth infection. Several studies that used unique rodent models instead of common laboratory rodents such as mice and rats have reported significant findings on host-parasite interactions [10, 22, 23]. However, the usefulness of cotton rats as models for intestinal nematode infections is unknown. In the present study, therefore, we examined susceptibility of cotton rats to *N. brasiliensis*. Although this parasite has shown high infectivity in several rodents, cotton rats are highly resistant to *N. brasiliensis* infection. Here we characterized the susceptibility of the cotton rat to *N. brasiliensis* during the tissue migratory and intestinal phases of infection. We also evaluated the importance of macrophage function in the resistance of the cotton rat to *N. brasiliensis* infection.

MATERIALS AND METHODS

Animals: Cotton rats (*S. hispidus*) and Indian soft-furred rats (*Millardia meltada*) were raised at the Experimental Animal Center, Miyazaki Medical College, Japan. In the present study, we used sexually mature (12–18 weeks old) male animals. Male Wistar rats were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan) and housed in our laboratory under conventional conditions. Since *M. meltada* is susceptible to *N. brasiliensis* infection [22, 23], these animals were used as controls for comparison of susceptibility to the parasite.

Parasitological technique: The strain of *N. brasiliensis* used in the present study has been maintained in our laboratory by serial passage in Wistar rats over six years by subcutaneous inoculation with 3,000–4,000 infective third-stage larvae (L_3) prepared by the charcoal culture method. Adult worms were obtained from the intestine of Wistar rats at 7 days postinfection as previously described [8]. To determine susceptibility during the migratory and the intestinal phases of the parasites, cotton rats and control *M. meltada* were challenged by subcutaneous inoculation with 1,000 L_3 or by intraduodenal implantation of 300 adult worms. The degree of parasite infection was monitored by daily counting of eggs in the feces (EPG, eggs per gram of feces) and/or by counting worms recovered from lungs or small intestines at autopsy. Animals were euthanized by ether overdose on the designated days. For migrating worm recovery, both lungs were removed, cut into small pieces using a motor driven disperser, and incubated at 37°C for 3 hr in petri dishes containing saline. The number of larvae that emerged was determined under a dissecting microscope. For adult worm recovery, the small intestine was cut open longitudinally, incubated at 37°C for 2 hr in saline, and the

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worms counted [8, 9].

Carbon treatments: Carbon particles were prepared according to the method of Abe *et al.* [1]. Briefly, carbon particles in Rotring Ink (Art. 591017, Rotring, Germany) were dialyzed against distilled water for several days. Carbon concentration was adjusted to 40 mg/m*l* in distilled water, stabilized by gelatin addition (final 1%, w/v), homogenized three times, and autoclaved. The carbon suspension was sonicated just before use and injected intravenously. Cotton rats were injected once or twice with a dose of 250 mg/kg body weight on designated days (total: 250 or 500 mg/kg) and then inoculated with L_3 or adult *N. brasiliensis* worms.

Statistical analyses: Results were statistically analyzed using Student's *t*-test and a *p* value below 0.05 was considered significant. Data are presented as means ± SEM for figures and means ± SD for tables.

RESULTS

In response to subcutaneous infection with 1,000 *N. brasiliensis* L_3 obtained from fecal culture, EPG in the cotton rats was extremely low throughout the monitoring period, whereas the EPG of control animals rose drastically from Day 5 to Day 8 and then decreased. From Day 6 to Day 9, daily EPG of cotton rats was significantly ($p<0.001$) lower than that of control animals (Fig. 1a). The animals were euthanized on Day 9, and the number of adult worms in the small intestines of cotton rats and control animals were counted by the incubation method (Fig. 1b). In contrast to the high recovery rate from the intestine of control animals (38.1% of the initial dose), few adult worms (1.6%) were recovered from cotton rats.

To determine whether the low susceptibility of cotton rats to *N. brasiliensis* infection was prevalent during the migratory phase or the intestinal phase, cotton rats and control *M. meltada* were challenged subcutaneously with 1000 L_3 or intraduodenally with 300 adult worms. Larval recovery from lungs was evaluated at 24, 48, and 72 hr, and adult worm burden was evaluated 24 hr after inoculation. Larval recovery in both control and experimental animals peaked at 48 hr post-inoculation. Larval recovery rates from the lungs of cotton rats were significantly lower than those from the control *M. meltada* at all sampling points. The maximal larval count (at 48 hr) was about five-fold lower in cotton rats than in controls (Table 1). Adult worm recovery from the small intestine of cotton rats was also significantly lower (two-fold lower) than that from control animals (Table 2).

To determine whether the susceptibility during the early migratory phase of *N. brasiliensis* infection was dependent upon the mononuclear phagocyte system, macrophage function was suppressed by carbon particle injection and larval recovery from the lungs of cotton rats was examined. As shown in Table 3, carbon treatment significantly increased larval worm recovery from the lungs of cotton rats at both 250 and 500 mg/kg and at all time points examined (-2, -1, and 0 days). However, the effect was dose-dependent, and

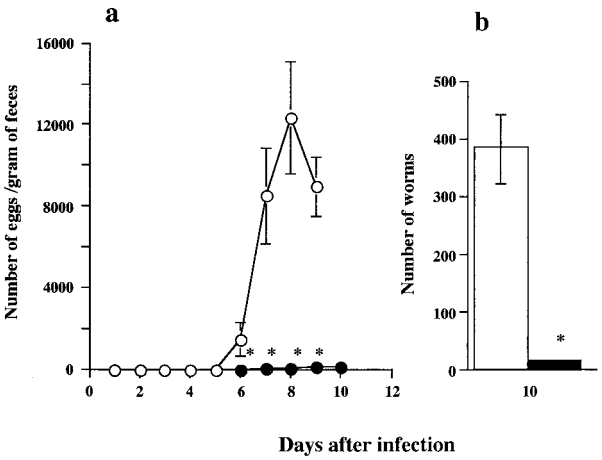

Fig. 1. EPG (eggs/gram of feces) kinetics of control *M. meltada* (○) and cotton rats (●) after subcutaneous inoculation with 1,000 *N. brasiliensis* L_3 (a). *M. meltada* (open column) and cotton rats (closed column) used for monitoring fecal egg output were autopsied on Day 10 for adult worm recovery from the intestine (b). Vertical bars represent mean ± SEM from five animals. * $p<0.001$.

Table 1. Recovery of larvae from the lungs of cotton rats and control *M. meltada* after subcutaneous inoculation with 1,000 *N. brasiliensis* L_3

Time after challenge	No. of larvae recovered from lungs	
(hr)	*M. meltada*	Cotton rat
24	92.0 ± 10.7	24.8 ± 18.2***
48	192.5 ± 65.3	43.2 ± 49.8**
72	33.0 ± 11.8	15.6 ± 4.7*

Values are the mean ± SD for five animals.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$, significantly lower than controls.

Table 2. Recovery of worms from the small intestine of cotton rats and control *M. meltada* after intraduodenal implantation of 300 adult *N. brasiliensis* worms

Time after challenge	No. of worms recovered from intestine	
(hr)	*M. meltada*	Cotton rat
24	212.6 ± 53.4	126.4 ± 26.6*

Values are the mean ± SD from five animals.
* $p<0.05$, significantly lower than controls.

Group D, which received carbon injections (250 mg/kg × 2) one day before and on the day of inoculation with L_3, showed the highest larval recovery (4.5-fold higher than untreated cotton rats, $p<0.001$).

The effect of carbon treatment on worm recovery from the small intestine of cotton rats was examined 24 hr after intraduodenal implantation of 300 *N. brasiliensis* adult worms. The number of worms recovered was 135.2 ± 19.9 and 129.6 ± 17.6 from untreated and carbon treated cotton rats, respectively. No significant effect of carbon treatment was observed ($p=0.4$) on the establishment of adult worms

Table 3. Effect of carbon treatment on larval recovery from the lungs of cotton rats after subcutaneous inoculation with 1,000 *N. brasiliensis* L_3[a)]

Group	Days of treatment[b)] -2	-1	0[c)]	Total dose (mg/kg)	No. of larvae recovered
Untreated control				0	44.2 ± 16.5
A		Yes		250	85.4 ± 14.5*
B	Yes	Yes		500	144.4 ± 20.5**
C			Yes	250	98.0 ± 15.7*
D		Yes	Yes	500	200.6 ± 30.0**

a) Larval recovery from the lungs was performed 48 hr after L_3 inoculation.
b) Carbon was intravenously injected at 250 mg/kg on designated days.
c) Time of infection.
Values are mean ± SD from five animals.
* $p<0.01$, ** $p<0.001$, significantly higher than untreated control.

in the small intestine of cotton rats.

DISCUSSION

The present study showed that cotton rats were not susceptible to infection by tissue migratory stages of *N. brasiliensis* L_3, and macrophage function was identified as an important factor in this resistance. Cotton rats were also resistant to the establishment of adult *N. brasiliensis* in the small intestine, but the intensity of resistance in this phase was smaller than that in migratory phase. These results suggest that capability of cotton rats in innate protection is different between the phases of *N. brasiliensis* infection. After *N. brasiliensis* penetrates the host skin or subcutaneously infects the host, larvae travel via the blood stream and reach the lungs of normal rats within 20 to 40 hr [3, 5]. In the present study, although larval recovery from the lungs of normal cotton rats was highest at 48 hr after L_3 infection, only a small number of larvae (4% of initial dose) were actually detected. These results suggest that most *N. brasiliensis* larvae were killed during the early migratory phase of infection, before or just after larvae entered the lungs.

The resistance of rats or mice to parasite infection during the early tissue migratory phase has been primarily attributed to activated mononuclear cell functions [1, 2] and in particular to the adherence and killing of parasites by activated macrophages [13, 19]. In a previous study, significant leukocytosis was evident in bronchoalveolar spaces following primary infection of Sprague-Dawley rats by *N. brasiliensis* larvae. In particular, the number of neutrophils significantly increased and many inflammatory cells adhered to larvae, causing disruption of the cuticle [19]. Complement-dependent cell-mediated cytotoxicity (CDCC) by alveolar macrophages plays an important role in lung resistance to resident and migrating helminths [3]. During the first 3–4 days after primary infection, *N. brasiliensis* infected-rats exhibit multinucleated giant cell responses that may be due to non-specific complement-dependent mechanisms [4]. *N. brasiliensis* larvae can directly activate complement through alternative pathways [13] and induce host immune response to recruit many inflammatory cells that can adhere to and kill the larvae [19].

In the present study, carbon treatment affected the resistance of cotton rats to *N. brasiliensis* infection during the migratory phase, but not during the intestinal phase, indicating that the protective mechanisms operating in cotton rats differed between the two phases. Intravenous injection of carbon particles could reduce either phagocytic activity or the size of the mobilizable pool of the mononuclear phagocyte system [21]. Ingestion of carbon particles by macrophages suppresses their ability to further process antigens in T cell-mediated immune mechanisms [20]. In parasitic infections, carbon treatments have suppressed the resistance of female mice to migratory stages of *Strongyloides ratti* [1] and *Brugia pahangi* [15, 16].

Resistance to early stages of parasitic infection, usually seen in female animals, is attributed to activated macrophages, which are affected by host sex hormones and thus induce gender-specific differences in host resistance [15, 16]. In contrast to previous reports, male cotton rats in the present study also showed strong resistance to infection by *N. brasiliensis* during the early migratory phase, and we observed no gender-specific differences in infection resistance (data not shown). Furthermore, this resistance was almost completely suppressed by carbon treatment. Thus, future studies must examine the roles of complement in the resistance of cotton rats to infection.

In conclusion, the cotton rat, *S. hispidus* showed strong resistance to the migratory phase of *N. brasiliensis* infection and this resistance was successfully suppressed by carbon treatment. Therefore, this animal appears to be a good model for investigating the mechanisms involved in resistance to parasitic infections during tissue migratory stages.

ACKNOWLEDGEMENTS. The authors thank the staff of the Department of Veterinary Internal Medicine for assistance. H. W. was an exchange student from Bogor Agricultural University, and his stay in Miyazaki University for this study was supported by an AIEJ Short-term Exchange Promotion Program (Inbound) Scholarship.

REFERENCES

1. Abe, T., Kiyota, M. and Nawa, Y. 1985. *Strongyloides ratti*: increase in susceptibility to infection following blockade of the mononuclear phagocyte system in female mice. *Aust. J. Exp. Biol. Med. Sci.* **63**: 651–653.
2. Dawkins, H. J., Muir, G. M. and Grove, D. I. 1981. Histopathological appearances in primary and secondary infections with *Strongyloides ratti* in mice. *Int. J. Parasitol.* **11**: 97–103.
3. Egwang, T. G., Gauldie, J. and Befus, D. 1984. Complement-dependent killing of *Nippostrongylus brasiliensis* infective larvae by rat alveolar macrophages. *Clin. Exp. Immunol.* **55**: 149–156.
4. Egwang, T. G., Richards, C. D., Stadnyk, A. W., Gauldie, J. and Befus, A. D. 1985. Multinucleate giant cells in murine and rat lungs during *Nippostrongylus brasiliensis* infections. A study of the kinetics of the response *in vivo*, cytochemistry, IgG- and C3-mediated functions. *Parasite. Immunol.* **7**: 11–18.
5. Haley, A. J. 1962. Biology of the rat nematode, *Nippostrongylus brasiliensis* (Travassos,1914). II. Preparasitic stages and development in the laboratory rat. *J. Parasitol.* **48**: 13–23.
6. Hayashi, Y., Nogami, S. and Nakamura, M. 1983. Comparison of activities of mononuclear phagocyte system between the cotton rat, *Sigmodon hispidus*, and the jird, *Meriones unguiculatus*, in relation to the treatment of filariasis. *Jpn. J. Exp. Med.* **53**: 257–262.
7. Hayashi, Y., Nogami, S. and Shibuya, T. 1984. Effect of stimulation and blockade of mononuclear phagocyte system on the treatment of microfilaremia of *Litomosoides carinii* in the cotton rat and the jird. *Jpn. J. Exp. Med.* **54**: 51–55.
8. Ishikawa, N., Horii, Y. and Nawa, Y. 1993. Immune-mediated alteration of the terminal sugars of goblet cell mucins in the small intestine of *Nippostrongylus brasiliensis*-infected rats. *Immunology* **78**: 303–307.
9. Ishikawa, N., Horii, Y. and Nawa, Y. 1994. Inhibitory effects of concurrently present 'normal' *Nippostrongylus brasiliensis* worms on expulsion of 'damaged' worms and associated goblet cell changes in rats. *Parasite Immunol.* **16**: 329–332.
10. Khan, A. I., Horii, Y. and Nawa, Y. 1993. Defective mucosal immunity and normal systemic immunity of Mongolian gerbils, *Meriones unguiculatus*, to reinfection with *Strongyloides venezuelensis*. *Parasite Immunol.* **15**: 565–571.
11. Kosoy, M. Y., Regnery, R. L., Kosaya, O. I. and Childs, J. E. 1999. Experimental infection of cotton rats with three naturally occurring *Bartonella* species. *J. Wildl. Dis.* **35**: 275–284.
12. Langley, R. J., Prince, G. A. and Ginsberg, H. S. 1998. HIV type-1 infection of the cotton rat (*Sigmodon fulviventer* and *S. hispidus*). *Proc. Natl. Acad. Sci. U.S.A.* **95**: 14355–14360.
13. Mackenzie, C. D., Jungery, M., Taylor, P. M. and Ogilvie, B. M. 1980. Activation of complement, the induction of antibodies to the surface of nematodes and the effect of these factors and cells on worm survival *in vitro*. *Eur. J. Immunol.* **10**: 594–601.
14. McKown, R. D., Ridley, R. K. and Kennedy, G. A. 2000. The hispid cotton rat (*Sigmodon hispidus*) as an experimental host for the bovine liver fluke (*Fasciola hepatica*). *Vet. Parasitol.* **87**: 125–132.
15. Nakanishi, H., Horii, Y., Terashima, K. and Fujita, K. 1989. Effect of macrophage blockade on the resistance to a primary *Brugia pahangi* infection of female BALB/c mice. *Trop. Med. Parasitol.* **40**: 75–76.
16. Nakanishi, H., Horii, Y., Terashima, K. and Fujita, K. 1989. Effect of testosterone on the susceptibility of C57BL/6 mice to infection with *Brugia pahangi* with reference to inflammatory cell response. *J. Parasitol.* **75**: 455–460.
17. Niewiesk, S. 1999. Cotton rats (*Sigmodon hispidus*): an animal model to study the pathogenesis of measles virus infection. *Immunol. Lett.* **65**: 47–50.
18. Prince, G. A., Prieels, J. P., Slaoui, M. and Porter, D. D. 1999. Pulmonary lesions in primary respiratory syncytial virus infection, reinfection, and vaccine-enhanced disease in the cotton rat (*Sigmodon hispidus*). *Lab. Invest.* **79**: 1385–1392.
19. Ramaswamy, K., De Sanctis, G. T., Green, F. and Befus, D. 1991. Pathology of pulmonary parasitic migration: morphological and bronchoalveolar cellular responses following *Nippostrongylus brasiliensis* infection in rats. *J. Parasitol.* **77**: 302–312.
20. Sabet, T., Newlin, C. and Friedman, H. 1968. The effect of RES blockade on cellular antibody formation to sheep erythrocytes. *Proc. Soc. Exp. Biol. Med.* **128**: 274–278.
21. Stuart, A. E., Habeshaw, J. A. and Davidson, A. E. 1978. Phagocytes *in vitro*. pp. 31.1–31.30. *In*: Handbook of Experimental Immunology, 3rd ed. (Weir, D.M. ed), Blackwell Scientific Pub, Oxford.
22. Tiuria, R., Horii, Y., Makimura, S., Ishikawa, N., Tsuchiya, K. and Nawa, Y. 1995. Effect of testosterone on the mucosal defence against intestinal helminths in Indian soft-furred rats, *Millardia meltada* with reference to goblet and mast cell responses. *Parasite Immunol.* **17**: 479–484.
23. Tiuria, R., Horii, Y., Tateyama, S., Tsuchiya, K. and Nawa, Y. 1994. The Indian soft-furred rat, *Millardia meltada*, a new host for *Nippostrongylus brasiliensis*, showing androgen-dependent sex difference in intestinal mucosal defence. *Int. J. Parasitol.* **24**: 1055–1057.
24. Wenk, P. and Mossinger, J. 1991. Recovery of adult stages and microfilaraemia after low dose inoculation of third stage larvae of *Litomosoides carinii* in *Sigmodon hispidus*. *J. Helminthol.* **65**: 219–225.