Rat Model for Human Cryptosporidiosis

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Effective treatment for Cryptosporidium infection in immunocompromised patients has yet to be found. We report a rodent model of persistent Cryptosporidium infection. Sprague-Dawley rats were injected subcutaneously twice a week for 8 weeks with 25 mg of hydrocortisone acetate. Fed a regular low-protein diet for 9 weeks, they were challenged once with 10³ calf Cryptosporidium oocysts 5 weeks after the start of the hydrocortisone acetate regimen. Oocyst shedding was evaluated in feces daily by using a carbol fuchsin-staining method. Rats shed oocysts from days 2 to 9 after ingestion and developed a persistent infection for more than 38 days. Excretion was lower after subsequent parasite challenges, suggesting that a degree of protection developed progressively. The results suggest that this experimental model provides a procedure for screening candidate therapeutic agents.

Cryptosporidium sp. is a coccidian parasite which infects a wide variety of host species, including humans (18), invading different mucosal surfaces, especially those of the respiratory (8, 12) and digestive (11, 19) tracts. In immunocompetent humans, Cryptosporidium infection may produce a self-limited flulike gastrointestinal illness (1–3, 7) that resolves spontaneously in 1 to 4 weeks (7, 15, 16). In immunocompromised patients, particularly those with acquired immunodeficiency syndrome, Cryptosporidium sp. may produce severe and prolonged diarrhea which does not respond to treatment and which contributes to mortality. Evaluation of anti-Cryptosporidium drugs based on the therapeutic outcomes of patients with acquired immunodeficiency syndrome has methodological limitations and was not successful in identifying efficient therapy (4). The use of endodermal cells from the choioallantoic membranes of chicken embryos (6), human fetal lung cells, primary chicken kidney cells, or pig kidney cells in culture (5) has been proposed for the in vitro evaluation of antiprotozoan drugs. These methods provide oocysts for only a short period and are not easily performed.

Therefore, the development of a reliable animal model is crucial for the evaluation of various drugs for prophylaxis or therapy. In vivo, controlled animal studies were conducted with antibiotics, sulfamides, and antiparasitic agents in calves (14) and mice (21) and with ornithine decarboxylase inhibitors in pigs (13). However, immunocompetent animals often either do not become infected, exhibit a self-limiting infection (17), or die soon after becoming infected. In one successful model, nude mice challenged at 6 days of age with oocysts had persistent Cryptosporidium infections until day 56, the end of experiment (9).

The aim of our study was to establish a persistent Cryptosporidium infection in a small animal. The validity of a rodent model as a model of human cryptosporidiosis is supported (i) by the identification of the parasite in the different hosts (20) and (ii) by the similarity of infections in both immunosuppressed humans and rodents (10).

Male Sprague-Dawley rats weighing between 200 and 250 g and free of Cryptosporidium oocysts at the start of the experiments were used. Immunosuppression was induced by a regimen of 25 mg of hydrocortisone acetate subcutaneously twice weekly, 5 weeks before and 3 weeks after Cryptosporidium challenge; during the course of immunosuppression, each rat received a total dose of 400 mg of hydrocortisone acetate. During the same period, they were fed a regular low-protein (7%) diet (consisting of bread exclusively), which has been shown to enhance the development of opportunistic parasites, such as Pneumocystis carinii, in rats (22). All the rats were housed one per cage, and feces were collected daily in a plate located below the cage to avoid contact between feces and animals.

Three groups of animals were sequentially studied. Group 1 consisted of 32 rats immunosuppressed for 5 weeks (25 mg of hydrocortisone acetate twice a week subcutaneously) and fed a low-protein diet during the same period. Then the animals received one challenge of 10³ Cryptosporidium oocysts. Immunosuppression was continued for 3 weeks in all the animals. The low-protein diet was continued for 4 weeks with 29 rats and for 3 months with the 3 other rats. At the end of a 4-week low-protein-diet period, nine rats (group 2) from group 1 were left for 2 months without immunosuppression under normal feeding. Then the rats were immunosuppressed (25 mg of hydrocortisone acetate twice a week) 5 weeks before and 3 weeks after a second challenge with 10³ Cryptosporidium oocysts. They were fed a low-protein diet for 5 weeks before and 4 weeks after challenge.

Group 3 consisted of the 9 rats of group 2 which, at the end of the 4-week postchallenge low-protein-diet period, were left for 2 months without immunosuppression under normal feeding. Then they were immunosuppressed (25 mg of hydrocortisone acetate twice a week) 5 weeks before and 3 weeks after a third challenge with 10³ Cryptosporidium oocysts. They were fed a low-protein diet for 5 weeks before and 4 weeks after challenge.

In all the groups, challenge consisted of 10³ Cryptosporidium oocysts added after a 24-h water-restriction regimen to 10 ml of drinking water, which was completely ingested in less than 6 h. Preliminary experiments demonstrated that 10³ oocysts constituted the minimal dose to obtain detection of oocysts in feces (data not shown). The control group con-

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sisted of four immunosuppressed rats which were not challenged.

_Cryptosporidium_ oocysts were obtained from feces of naturally infected calves of a cattle breeding station located 20 km from Rouen, France. Feces containing oocysts were collected and stored in a 2.5% (wt/vol) solution of potassium dichromate at 4°C for up to 3 months before use. Oocysts were purified by being washed at least three times in distilled water and passed through a graded series of four sieves (pore sizes, 315, 200, 125, and 63 μm) to exclude particles larger than 63 μm. The filtrates containing oocysts were pooled, suspended in 30 ml of a 10% sodium hyochlorite solution at room temperature for 15 min, and centrifuged (400 × g for 15 min). The pellets were washed three times in distilled water, and oocysts were counted in a hemacytometer and sus-

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Day 1 of oocyst shedding after challenge</th>
<th>Day of peak shedding</th>
<th>No. of oocysts per MF at shedding peak</th>
<th>No. of oocysts per MF per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (32)</td>
<td>4 ± 2 (2-9)</td>
<td>10 (7-23)</td>
<td>25 ± 38 (0.9-120)</td>
<td>6.9 ± 8 (0.6-34)</td>
</tr>
<tr>
<td>2 (9)</td>
<td>6 ± 2 (3-11)</td>
<td>9 (4-25)</td>
<td>0.75 ± 1 (0.01-3.4)</td>
<td>0.17 ± 0.16 (0.017-0.5)</td>
</tr>
<tr>
<td>3 (9)</td>
<td>7 ± 3 (4-11)</td>
<td>7 (6-23)</td>
<td>0.23 ± 0.2 (0.01-0.41)</td>
<td>0.13 ± 0.11 (0.01-0.23)</td>
</tr>
</tbody>
</table>

TABLE 1. _Cryptosporidium_ oocyst shedding in immunosuppressed rats

*a Values are means ± standard deviations. Ranges are given in parentheses.
*b Groups are described in the text. No oocysts were found in control animals (immunosuppressed and not challenged, n = 4; not immunosuppressed and challenged, n = 4).  
* Magnification, ×400.

Although several drugs, including spiramycin, pentamidine, and tetracycline, have been clinically tested (15), effective treatment for _Cryptosporidium_ infection has yet to be found for immunocompromised hosts. Development of effective treatment has been limited by the lack of a simple method for screening the efficacies of drugs.

The lack of host specificity has been confirmed, and evidence has been provided that a single _Cryptosporidium_ species is found in mammals, including humans (20); _Cryptosporidium_ oocysts of human and calf origins produced indistinguishable infection in suckling mice, rats, and adult mice when they were challenged orally (16). In our experiments with oocysts from naturally infected calves, oocysts were detected from days 2 to 9 following inoculation, a time interval similar to that found with other animal models, such as lambs (19), nude mice (10), or calves (11). Peak oocyst counts per MF were found to be higher than in nude mice (10). The mortality rate reached 21%, and in surviving animals infection persisted as long as they received hydrocortisone acetate and a low-protein diet. In recent experiments, a low-protein diet alone was found to be more effective in maintaining _Cryptosporidium_ infection than hydrocortisone acetate alone, as previously observed in an experimental rat model for _P. carinii_ infection (22). This suggests that susceptibility to _Cryptosporidium_ infection could be enhanced by protein malnutrition.

Optimum oocyst excretion was observed in primarily infected animals, and excretion decreased after subsequent infection challenges under the same diet and immunosuppressive conditions. This suggests that a degree of protection developed progressively, and it should be further determined whether T cells are required for recovery, as previously demonstrated with mice (10).

This experimental immunosuppressed-rat model is suitable for screening candidate therapeutic agents and can be used to study either preventive or curative activities of drugs.

LITERATURE CITED


