Rat Model for Human Cryptosporidiosis

PHILIPPE BRASSEUR,1* DENIS LEMETEIL,1 AND JEAN JACQUES BALLET2

Department of Parasitology, Hôtel Dieu, Centre Hospitalier Universitaire, 51 rue de Lecat, 76031 Rouen,1 and Laboratory of Immunochemistry and Immunopathology, Institut National de la Santé et de la Recherche Médicale U. 108, Hôpital Saint Louis, 75010 Paris,2 France

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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 chorioallantoic 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, sulfonamides, and antiparasitic agents in calves (14) and mice (21) and with orotic acid 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 10⁵ 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 10⁵ 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 10⁵ 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 10⁵ 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 10⁴ oocysts constituted the minimal dose to obtain detection of oocysts in feces (data not shown). The control group con-

* Corresponding author.
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 (porosizes, 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 hypochlorite 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 suspended at a concentration of 10^7/10 ml of water.

Rat feces were collected daily and stored suspended in a 10% (wt/vol) Formalin solution at 4°C. Oocysts were detected by phase-contrast microscopy in smears from 10 μl of homogenized fecal suspension specimen mixed with 10 μl of a carbol-fuchsin solution (9). Cryptosporidium shedding was measured by counting oocysts per microscopic field (MF) under ×400 magnification. A total of 10 to 100 MF were observed, depending on the density of parasites in each specimen. Shedding was evaluated for each rat as the mean numbers of oocysts per MF and per day.

With five infected rats, gastrointestinal tract, jejunal, and ileal tissue sections, performed 2 weeks after challenge, were examined after normal Formalin fixation, paraffin embedding, and hematoxylin-eosin staining. In the jejunum and ileal sections of the infected rats, diffuse atrophy of villi and hyperplasia of crypts were observed, associated with the presence of oocysts fixed to epithelial cells. These features were absent in three noninfected control rats.

Statistical comparisons between groups were performed by using the Fisher and Yates chi-square tests, and variance analysis was done with the Mann-Whitney U test.

In group 1, all animals began to shed oocysts on days 2 to 9 after challenge. The shedding peaked on days 7 to 23. Of 32 rats, 25 survived; the others died between days 17 and 24. No significant difference in the counts of oocysts shed was noted between the survivors and dead animals. Of 25 surviving rats, 22 ceased to shed oocysts 10 to 13 days following restoration of a normal diet (38 to 41 days after challenge). Of the three others with a continued low-protein diet, two died and one was still shedding oocysts after day 100. As shown in Table 1, excretion of oocysts occurred earlier in group 1 than in groups 2 and 3. The peak and mean values of shedding were significantly higher in group 1 than in group 2 (P = 0.05 and P < 0.01, respectively). For group 3, the difference with group 2 was not significant for the peak value of excretion or for the mean value of shedding (P = 0.3 and P = 0.2, respectively). With the control group which did not receive hydrocortisone acetate and which had a normal diet, no oocysts were found in the feces during the time of the experiment (days 0 to 30), and the unchallenged immunosuppressed control rats did not excrete oocysts in any fecal specimen.

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