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Persistent *Cryptosporidium* Infection in Congenitally Athymic (Nude) Mice

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Nude (*nu/nu*) BALB/c mice and their white (*nu/+*) littermates were experimentally infected with *Cryptosporidium* sp. at 6 days of age. In white mice, the infection was transient, but in nude mice a persistent infection developed that was characterized by diarrhea and, occasionally, death. There were villus atrophy and crypt hyperplasia in the small intestine of infected nude and white mice necropsied at 11 days of age. Persistently infected nude mice had, in addition to the above small intestinal lesions, diffuse cystic mucosal hyperplasia and crypt abscesses in the large intestine at 56 days of age. These results suggest that T cells are required for recovery from the *Cryptosporidium* infection but are not required for epithelial cell loss in cryptosporidiosis. Both nude and white mice appeared to be relatively more resistant to *Cryptosporidium* infection at 42 days of age than at 6 days of age.

Infections with coccidia of the genus *Cryptosporidium* Tyzzer 1910 (phylum *Apicomplexa*, suborder *Eimeriina*) have been reported in fishes, reptiles, birds, and mammals including humans (4, 11, 21). The protozoa attach to the mucosal epithelium of the digestive tract, the respiratory tract, and the conjunctiva, causing the loss of epithelial cells and infiltration of subjacent lamina propria with inflammatory cells (6, 14; J. Heine, H. W. Moon, D. B. Woodmansee, and J. F. L. Pohlenz, unpublished data).

Cryptosporidium infections in immunocompetent humans (1, 6) and animals (4, 9, 14, 18, 20) are transient, and attempts at experimental reinfections of animals have been unsuccessful (4, 20). Persistent cryptosporidiosis that does not respond to treatment has been reported in people with the acquired immune deficiency syndrome (3, 6). Individuals with the acquired immune deficiency syndrome apparently have aberrant T-cell and natural killer cell activities (7, 19). Athymic (nude) mice characteristically have depressed regulatory and effector T-cell activities but intact natural killer cell activities (10).

The objectives of this study were to establish persistent *Cryptosporidium* infection in an animal model and to evaluate the importance of T cells in the pathogenesis of cryptosporidiosis.

MATERIALS AND METHODS

The offspring of BALB/c nude (athymic, *nu/nu*) male and BALB/c white (*nu/+*) female mice were used in this study. The mice were kept with their dams (one litter per cage) and provided with pelleted food and water ad libitum. Dams were removed when the offspring were 3 weeks old. Cages containing principals and controls were housed in the same room. Persons showered and changed clothes before entering the room and always cared for, observed, and sampled all controls before any of the principals. Persons showered and changed clothes again after leaving the room. Clothing and equipment from the room were heated at 121°C for 30 min and washed before use in the room again.

Twenty-two nude mice and 18 of their white littermates were inoculated with *Cryptosporidium* sp. at 6 days of age. Fifteen nude mice and 17 of their white littermates served as uninfected controls. Seven nude mice and eight of their white littermates were inoculated at 42 days of age. Three nude mice and three of their white littermates served as uninfected controls.

Feces from a calf that had been experimentally infected with *Cryptosporidium* sp. of calf origin were suspended in 2 volumes of 2.5% potassium dichromate solution. This suspension was passed through a graded series of sieves to exclude particles larger than 63 μm and stored for 10 weeks at 4°C. For the preparation of inocula, *Cryptosporidium* oocysts were concentrated by flotation on a sugar solution (specific gravity, 1.18 g/cm^3). Mice were each inoculated via a stomach tube with 100 μl of concentrated material containing ca. 10^5 oocysts.

Mouse feces were examined microscopically for *Cryptosporidium* oocysts once (controls) or three times (principals) per week from day 6 until the end of the experiment at day 56. During the first 2 weeks, we examined methanol-fixed, Giemsa-stained fecal smears; later, when larger volumes of feces were available, we used the carbolfuchsin technique (8). The intensity of shedding was evaluated semiquantitatively as described in the legend to Fig. 1.

Four mice (two nude and two white) inoculated with *Cryptosporidium* sp. at 6 days of age (day 6) and four control mice (two nude and two white) were necropsied at day 11. Six inoculated mice (three nude and three white) and four control mice (two nude and two white) were necropsied at day 56, and colon contents were examined for cryptosporidia by using the carbolfuchsin technique. Tissue samples taken from the stomach, duodenum (10 mm distal to the stomach), midjejunum, ileum (5 mm proximal to the cecum), and midcolon were fixed in Formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

All 15 mice inoculated with *Cryptosporidium* sp. at day 42 and 6 control mice were necropsied at day 56. Histological sections of ilea were prepared.

RESULTS

All mice inoculated with *Cryptosporidium* sp. at day 6 began to shed oocysts on days 9 to 11. The intensity of

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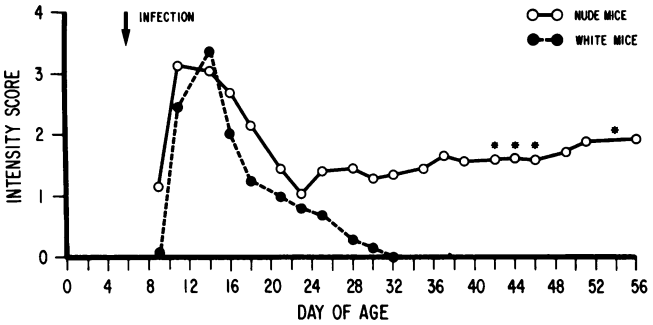

FIG. 1. Intensity of fecal shedding of *Cryptosporidium* oocysts in experimentally infected nude (athymic) mice and their white littermates. Intensity score: 1, oocysts observed but less than 1 per microscopic field (500-power magnification); 2, 1 to 5 oocysts per field; 3, 6 to 20 oocysts per field; 4, >20 oocysts per field. One mouse died at each of the indicated times (*).

shedding peaked on days 11 to 13 in both nude mice and their white littermates (Fig. 1). At necropsy on day 11, the parasites were attached to epithelial cells of the duodenum of one of the inoculated nude mice and to the jejunum and ileum of all inoculated nude and white mice. There was diffuse atrophy of villi and hyperplasia of crypts associated with the infection of the lower small intestine in all infected mice. Epithelium on atrophic villi was basophilic and cuboidal to squamous. Villus lamina propria and epithelium were infiltrated with mononuclear and occasionally polymorphonuclear leukocytes. The lengths of villi and depths of crypts for both nude and white mice are shown in Fig. 2. The extent and degree of villus atrophy and crypt hyperplasia in infected nude mice were similar to or greater than those in their infected white littermates.

In white mice the shedding of *Cryptosporidium* oocysts ceased between days 21 and 30. However, the nude mice continued to shed oocysts until the end of the experiment at day 56. At day 30, the infected nude mice began to develop diarrhea, and oocyst shedding began to increase slightly. These mice became dehydrated and lost weight, and four mice died (Fig. 1). At day 56 the average weight of infected nude mice was 16.2 g (standard deviation [SD], ±5.7); the uninfected nude mice weighed an average of 25.0 g (SD, ±2.8). This difference was significant ($P < 0.01$). White mice that recovered from infection weighed 28.5 g (SD, ±3.9) and uninfected white mice 28.7 g (SD, ±1.8). Neither parasites nor histological lesions were observed in the intestines of recovered white or uninoculated control mice necropsied at day 56. In contrast, examination of intestinal content and histological sections from the inoculated nude mice confirmed that they were still infected at day 56. Parasites were found attached to epithelial cells in the ileum, cecum, and colon of these persistently infected mice. There were also villus atrophy and crypt hyperplasia in the lower small intestine and cystic hyperplasia with abscessation of crypts in the large intestine (Fig. 3).

None of the seven nude mice or their eight white littermates inoculated with *Cryptosporidium* sp. at 42 days of age developed diarrhea. Fecal samples were taken during the first 11 days postinoculation (days 43 to 53). A few oocysts (less than one oocyst per microscopic field, 500-power magnification) could be demonstrated in samples from four mice (two nude and two white). Subsequent histological examination showed that six of seven nude mice and two of eight white mice had a few parasites attached to epithelial cells in the ileum. These infections were not associated with other morphological changes of the intestinal mucosa.

Cryptosporidium oocysts could not be demonstrated in any of the fecal samples taken from uninoculated mice. No parasites or morphological changes were seen in histological sections of stomach and intestine of control mice necropsied at 11 or 56 days of age.

DISCUSSION

Cryptosporidium infection was transient in white mice. However, in neonatally infected nude mice the infection and its associated mucosal lesions persisted until death or until the end of the experiment. Nude mice are deficient in T cells. The results of this study suggest that T cells (either regulatory or effector or both) are required for recovery from the infection in mice. Dependence on T cells for recovery from infections with other coccidia in mice and rats has also been reported (12, 13, 16).

In both nude and white mice infected with *Crytosporidium* sp., the mucosal changes in the small intestine were characterized by villus atrophy and crypt hyperplasia. These architectural changes were presumably caused by the infection. Atrophy of villi was accompanied by hyperplasia of crypts in mice necropsied at days 11 and 56. Therefore, atrophy of villi was probably due to accelerated loss or destruction, rather than to decreased production of epithelial cells. The results of this study suggest that T cells are not required for epithelial cell loss or destruction during the course of a *Cryptosporidium* infection. In contrast, with other intestinal protozoa such as *Giardia* sp. (15) and *Eimeria* sp. (17), architectural changes during infection require the presence of functional T cells.

At day 11, the *Cryptosporidium* infection was limited to the small intestine; at day 56, however, the infection extended into the large intestine, apparently causing cystic hyperplasia and abscessed crypts. To our knowledge, these lesions have not been seen previously in mammalian cryptosporidiosis, even though the infection commonly extends into the crypts of the large intestine in several species (2, 14, 23). However, they are similar to those associated with *Cryptosporidium* sp. in snakes with hypertrophic gastri-

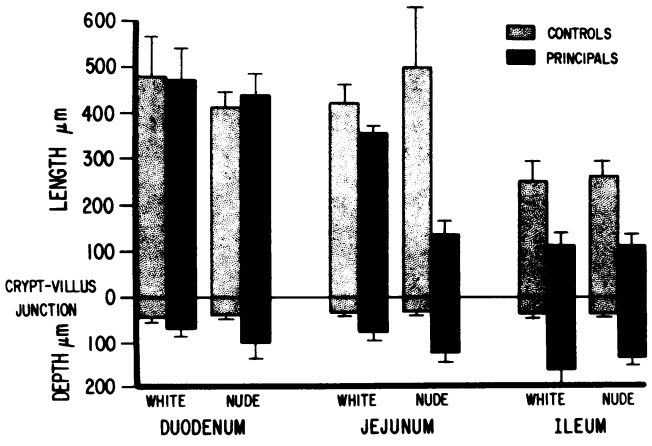

FIG. 2. Lengths of villi and depths of crypts in the small intestine of nude (athymic) mice and their white littermates 5 days after experimental *Cryptosporidium* infection. Controls were not infected. Bars represent the means ±SD of 14 to 20 well-orientated villi and crypts of two mice.

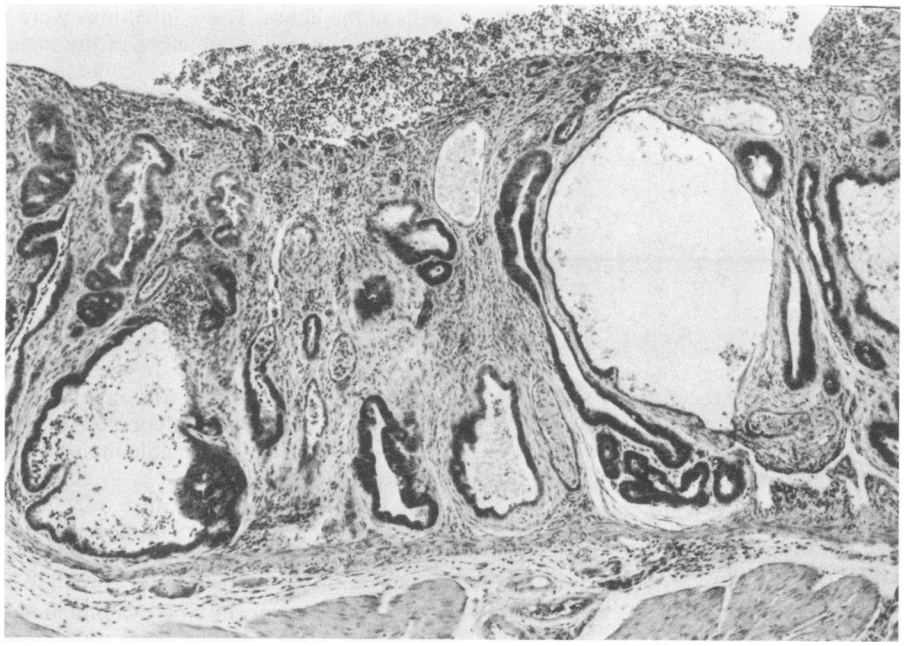

FIG. 3. Cystic hyperplasia in the colonic mucosa of a nude (athymic) mouse after 50 days of experimental *Cryptosporidium* infection.

tis (5). Hypertrophic gastritis in snakes (5) is apparently also the result of persistent cryptosporidiosis.

Cryptosporidium infection apparently caused morphological damage to the intestinal mucosa in all mice inoculated at 6 days of age. However, detectable infection could be established in only 8 of 15 mice inoculated with *Cryptosporidium* sp. at 42 days of age. These later infections were mild and without histologically detectable intestinal damage. The reason(s) for this apparent decreased susceptibility of older mice is unknown. However, it may be a general characteristic of mice, because it has also been reported by others (18).

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