Susceptibility of Mast Cell-Deficient W/W¹ Mice to Cryptosporidium parvum

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Received 5 September 1990/Accepted 20 November 1990

Mast cell-deficient W/W¹ infant mice were similar to normal mice in their susceptibility to and recovery from infection with the intestinal protozoan Cryptosporidium parvum. W/W¹ adult mice were significantly more susceptible to primary infection than were normal adult mice, but both groups recovered at a similar rate.

The protozoan parasite Cryptosporidium parvum infects mucosal surfaces in many species of animals (16). Infection elicits an antibody response in humans, calves, and mice, but neither serum antibody nor maternal transfer of colostral antibody appears to protect against the disease (7, 12, 18). Chronic infections with C. parvum are seen in AIDS patients, concurrent with a decrease in T-cell function (2). Athymic mice, which lack functional T cells, are also unable to clear infections acquired in the neonatal period (9) and can be chronically infected as adults (17). Infections in adult athymic mice can be cured by adoptive transfer of T cells from normal mice (17). These observations strongly suggest that T cells are necessary for the development of specific acquired immunity to C. parvum. To further examine the mechanisms of immunity to C. parvum, we examined the susceptibility of W/W¹ mice to C. parvum infection. These mice have normal T-cell function but have a deficit in erythrocyte and granulocyte lineages, including intestinal mast cells (15).

C57BL/6J (W/+), female and WB/REJ (W/+) male breeder mice were purchased from Jackson Laboratory, Bar Harbor, Maine, and bred at the National Animal Disease Center to produce WBB6F1, infants with the genotypes W/W¹, W/+, W/W¹, and W/+, distinguishable by coat color (14). For experiments with adult mice, 4-week-old WBB6F1/J (W/W¹) and WBB6F1/J control (W/+, W/W¹, or W/+), female mice were obtained from Jackson Laboratory. Adult mice were allowed to acclimate for 3 weeks before use. C. parvum oocysts were isolated from feces collected from calves experimentally infected with C. parvum (12). Immediately before use, oocysts were washed three times in phosphate-buffered saline, and numbers of oocysts were determined by direct counts with a hemacytometer. Infant mice received 10⁵ oocysts in 0.1 ml of phosphate-buffered saline by gastric intubation, and adult mice received 10⁶ oocysts in 0.1 ml of phosphate-buffered saline per os. The level of challenge was determined by previously observed infectivity of the oocyst pool for infant mice and adjusted for adult mice on the basis of body weight relative to that of infant mice.

Infant mice were experimentally inoculated with oocysts at 1 week of age. One week later, when the mice were 2 weeks old, fecal pellets were collected from individual mice, smeared onto glass slides, stained with carbol-fuchsin, and examined for oocysts (8). Smears were examined again when the mice were 4 weeks old, and mice found negative by this technique were killed. The intestinal tract was removed from each mouse and fixed in 10% Formalin–saline. Following fixation, tissue was embedded in paraffin. Histologic sections were cut, stained with hematoxylin and eosin, and examined microscopically for C. parvum. Remaining mice were weaned. At 6 weeks of age, mice were killed, and smears were made from the contents of the distal colon of each mouse, stained, and examined. Intestinal tissue was prepared for histologic examination as described above. In experiments with adult mice, animals were experimentally inoculated with oocysts at 7 weeks of age. Groups of mice were necropsied at 8, 9, 10, 12, 14, 16, and 17 weeks of age. Some mice were challenged a second time at 16 weeks of age and necropsied 1 week later. At necropsy, smears of colon contents from individual mice were stained and examined for oocysts, and tissues were prepared for histlogic examination as described above. Tissues were also fixed in a methanol-formaldehyde-acetic acid mixture, and sections were stained with Alcian blue for detection of mast cells (10).

Infectivity scores were determined as follows: 3+, C. parvum found in stained smears of fecal pellets or colon contents; 2+, C. parvum not found in stained smears, but numerous C. parvum organisms found in intestinal tissue by histologic examination; 1+, C. parvum not found in stained smears, but a few C. parvum organisms found in intestinal tissue by histologic examination; or 0, C. parvum not found. Differences in the mean scores between groups were compared with Student’s t test.

There were no significant differences in the resistance to and rate of recovery from C. parvum infection between W/W¹ and normal mice challenged at 1 week of age (Table 1). At 2 weeks of age, virtually all mice of both phenotypes were found to be infected with C. parvum. By 4 weeks, most mice had cleared the infection; the remaining infected mice were equally distributed between W/W¹ and normal phenotypes. At 6 weeks of age, no C. parvum was found in mice of either phenotype.

Adult W/W¹ mice had significantly (P < 0.01) higher infectivity scores 1 week following challenge than did adult normal mice (Table 2). In 9 of 19 ilea from mast cell-deficient mice, parasites were numerous (Fig. 1). In the positive ilea from normal mice, only a few parasites were found, usually on the dome epithelium of a Peyer’s patch. No mice of either phenotype were found to be infected at 9, 10, 12, 14, or 16 weeks of age (three mice of each phenotype), and none of the mice (three mice of each phenotype) rechallenged at 16 weeks of age were found to be infected 1 week later. Mast

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cells were not seen in the intestinal tissues from adult mice of either phenotype following challenge with *C. parvum*.

Since infant W/W' mice were no more susceptible to *C. parvum* infection than were their phenotypically normal littermates and since both groups cleared the infection with similar kinetics, it appears that resistance to and recovery from *C. parvum* infection in infant mice is not affected by the W/W' genotype. Adult W/W' mice were much more heavily infected 1 week after challenge than were adult normal mice, but both groups recovered at a similar rate, and neither group became infected after a second challenge. These results suggest that, while the recovery of adult mice from *C. parvum* infection (and perhaps resistance to a second challenge) requires functional T cells (9, 17), other factors are involved in the initial resistance of adult mice to challenge with *C. parvum*, since W/W' mice have normal T-cell-mediated immune responses (6, 11) and adult nude (T-cell-deficient) mice do not show evidence of infection until several weeks after challenge (9, 17).

We hypothesized that mast cells might play a role in this initial resistance of adult mice to *C. parvum*, since previous studies attributed increased susceptibility to intestinal parasites to a deficiency of mast cells in W/W' mice (1, 4). In the present study, we found no evidence of recruitment of mast cells to the site of infection in either W/W' or normal mice;

### TABLE 1. Infection and recovery of W/W' and normal infant mice following challenge with *C. parvum*

<table>
<thead>
<tr>
<th>Age (wk) and group</th>
<th>No. positive/ no. of mice tested</th>
<th>Infectivity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/W'</td>
<td>14/14</td>
<td>3.00</td>
</tr>
<tr>
<td>Normal</td>
<td>24/26</td>
<td>2.77</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/W'</td>
<td>6/19</td>
<td>0.53</td>
</tr>
<tr>
<td>Normal</td>
<td>16/40</td>
<td>0.50</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/W'</td>
<td>0/3</td>
<td>0.00</td>
</tr>
<tr>
<td>Normal</td>
<td>0/5</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Mice were experimentally inoculated with oocysts at 1 week of age and examined for *C. parvum* at the ages indicated.

Therefore, it is difficult to ascribe a role for mast cells in the resistance of mice to *C. parvum*.

In addition to being mast cell deficient, W/W' mice are also infertile, lack melanocytes in the skin, and have macrocytic anemia (15). These multiple effects may be due to dysfunction of a receptor coded for by the W locus (3, 5, 13). The data from the present study suggest that the initial resistance of adult mice to *C. parvum* infection may involve a cell type or function affected by the W locus. Further studies are necessary to identify this cell type or function.

We gratefully acknowledge the assistance of Douglas Woodmansee and Bruce Pesch.

### REFERENCES


