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# Susceptibility of Major Histocompatibility Complex (MHC) Class I- and MHC Class II-Deficient Mice to *Cryptosporidium parvum* Infection

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**Major histocompatibility complex (MHC) class I-deficient and MHC class II-deficient mice lack functional CD8 T cells and CD4 T cells, respectively. These mice were evaluated for infection following oral administration of $10^7$ *Cryptosporidium parvum* oocysts. MHC class II-deficient (but not MHC class I-deficient) mice dosed with *C. parvum* oocysts at 3 to 5 days of age remained infected 8 weeks postexposure. MHC class II-deficient mice exposed to *C. parvum* oocysts at 5 to 6 weeks of age were significantly more susceptible to infection than control mice ($P < 0.0001$).**

*Cryptosporidium parvum* is a protozoal agent that infects intestinal epithelial cells and causes diarrhea in several mammalian hosts (8). Infection follows ingestion of *C. parvum* oocysts, and the resulting disease is dependent on the immunocompetence of the host (6, 21). Immunodeficient individuals, particularly those with AIDS, develop persistent and sometimes life-threatening diarrheal disease (5–9, 15, 16, 21). Control of persistent infection in immunodeficient hosts is complicated by the lack of treatment options. Definition of protective immune responses may improve the ability to intervene immunotherapeutically.

Investigations in athymic nude mice provide evidence for the importance of T lymphocytes in recovery from cryptosporidial infection (3, 11, 22, 23). Additional studies have addressed the role of T-lymphocyte subsets in immunity to cryptosporidiosis. BALB/c mice depleted of CD4 T cells through in vivo treatment with monoclonal antibody remained persistently infected with *C. parvum*. Depletion of CD8 cells did not result in persistent infection (22, 23). These results indicate that CD4 T cells are necessary for protection against cryptosporidiosis. However, in vivo treatment with monoclonal antibody causes substantial but incomplete reduction of CD4 (1, 24) and CD8 (19) T cells. Therefore, we utilized another approach to test the hypothesis that CD4 T cells are required to prevent persistent cryptosporidial infection. Major histocompatibility complex (MHC) class I-deficient and MHC class II-deficient mice, lacking functional CD8 and CD4 T cells, respectively, were orally challenged with *C. parvum* oocysts. Because of the age-related susceptibility of immunocompetent mice to *C. parvum* infection (18, 20), both 3- to 5-day-old and 5- to 6-week-old mice were studied.

MHC class I- and MHC class II-deficient C57BL/6J mice were purchased from GenPharm International (Mountain View, Calif.). Mice deficient in MHC class I antigens, as a result of targeted gene disruption at the $\beta_2$-microglobulin allele, also lack CD8 T cells (25). MHC class II-deficient mice, produced by disruption of the $A\beta^b$ allele, lack mature CD4 T lymphocytes (4, 10). Wild-type (control) C57BL/6J mice were also obtained from GenPharm International. To obtain 3- to 5-day-old mice, breeding pairs of MHC class I-deficient, MHC class II-deficient, and wild-type mice were purchased and then bred at the Washington State University Laboratory Animal Resource Center. Severe combined immunodeficient (SCID) mice (C.B-17/lc Tac-scidfDF) were obtained from Taconic (Germantown, N.Y.), and BALB/c mice were obtained from a breeding colony at Washington State University. All mice were maintained under barrier conditions in Microisolator cages (Laboratory Products, Inc., Maywood, N.J.).

The *C. parvum* isolate utilized in these experiments was originally obtained from H. Moon and D. Woodmansee (Ames, Iowa). *C. parvum* oocysts were maintained by passage in neonatal calves and were prepared for administration to mice as described previously (3). Intestinal infection scores were assigned, and differences between scores for immunodeficient and immunocompetent mice of the same strain were analyzed by the Mann-Whitney rank sum test (2, 18).

To determine the 50% mouse infective dose (17) for this

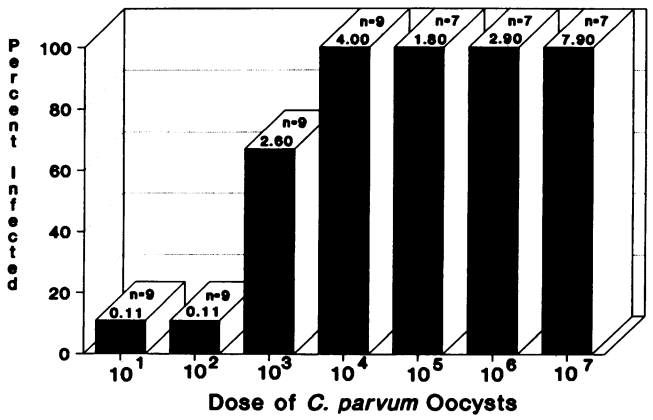


FIG. 1. Dose response of C57BL/6J neonatal mice to *C. parvum* oocyst administration. Bar heights indicate percentage of mice infected. The numbers at the top of each bar indicate the number of mice and the average infection score for the mice within the group. Infection scores were assigned as follows: 1, <33% of mucosal epithelium parasitized; 2, 33 to 66% of epithelium parasitized; 3, >66% of epithelium parasitized.

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TABLE 1. *C. parvum* infection scores in MHC class I-deficient C57BL/6J mice

| Expt | Age at infection | Wk post-infection | Infection score, mean ± SD (no. of mice) | | *P* value[b] |
|---|---|---|---|---|---|
| | | | MHC class I deficient | Controls[a] | |
| 1 | 3–5 days | 4 | 0.50 ± 0.75 (8) | 0.25 ± 0.50 (4) | 0.362 |
| | 5–6 wk | 4 | 1.08 ± 0.99 (12) | 0.50 ± 0.52 (12) | 0.175 |
| 2 | 3–5 days | 4 | 0.83 ± 0.98 (6) | 0.67 ± 1.03 (6) | 0.498 |
| | 3–5 days | 8 | 0.00 ± 0.00 (6) | 0.00 ± 0.00 (5) | 0.629 |

[a] C57BL/6J wild-type mice.
[b] Determined by Mann-Whitney rank sum test.

strain, 3- to 5-day-old C57BL/6J mice were orally dosed with serial log dilutions of *C. parvum* oocysts. One control and seven experimental groups were inoculated by gastric gavage, using a sterile 22-gauge straight feeding needle (Thomas Scientific, Swedesboro, N.J.). Mice were euthanized 96 h postinoculation. Tissues were collected and fixed in 10% buffered formalin. Slides of gastrointestinal tissues were prepared, coded, and then scored for the presence and number of *C. parvum* organisms without knowledge of treatment group. The results are shown in Fig. 1. The calculated 50% mouse infective dose for C57BL/6J mice was approximately 600 *C. parvum* oocysts, which is similar to that for BALB/c mice infected with this isolate (18). Organisms were numerous in the ileum, cecum, and colon. Intestinal infection scores were variable when the inoculum contained fewer than $10^6$ oocysts. Therefore, a dose of $10^7$ *C. parvum* oocysts was selected for further experiments to ensure consistent intestinal infection.

Groups of 3- to 5-day-old MHC class I-deficient, MHC class II-deficient, and wild-type mice were orally dosed with $10^7$ *C. parvum* oocysts in 10 μl of sterile phosphate-buffered saline (PBS). Mice were terminated 4 and 8 weeks postinfection. Gastrointestinal tissues were collected and prepared as described above. Spleens were removed, splenocytes were dissociated by mincing with forceps in Hanks balanced salt solution, and erythrocytes were removed by hypotonic lysis with Tris-buffered $NH_4Cl$ (pH 7.2). Remaining cells were washed and reacted with a panel of monoclonal antibodies. Lymphocyte surface markers (CD4, CD8, and CD45) were labeled with monoclonal antibodies GK1.5, 2.43 (courtesy of Linda Perry, Pullman, Wash.), and B220 (Pharmingen, San Diego, Calif.), respectively. After incubation with mouse anti-rat fluorescein isothiocyanate-labeled antibody (Jackson Labs, Westgrove, Pa.), spleen cells were analyzed by flow cytometry (FACScan; Becton-Dickinson, Mountain View, Calif.).

TABLE 2. *C. parvum* infection scores in MHC class II-deficient C57BL/6J mice

| Expt | Age at infection | Wk post-infection | Infection score, mean ± SD (no. of mice) | | *P* value[b] |
|---|---|---|---|---|---|
| | | | MHC class II deficient | Controls[a] | |
| 1 | 3–5 days | 4 | 9.14 ± 1.79 (14) | 0.25 ± 0.50 (4) | 0.0003 |
| | 3–5 days | 8 | 7.73 ± 1.53 (15) | 0.00 ± 0.00 (11) | <0.0001 |
| | 5–6 wk | 4 | 6.50 ± 1.00 (12) | 0.50 ± 0.52 (12) | <0.0001 |
| 2 | 3–5 days | 4 | 10.18 ± 0.75 (11) | 0.67 ± 1.03 (6) | 0.0001 |
| | 3–5 days | 8 | 7.54 ± 1.04 (11) | 0.00 ± 0.00 (5) | 0.0002 |
| | 5–6 wk | 4 | 5.73 ± 0.90 (11) | 0.50 ± 0.70 (10) | <0.0001 |

[a] C57BL/6J wild-type mice.
[b] Determined by Mann-Whitney rank sum test.

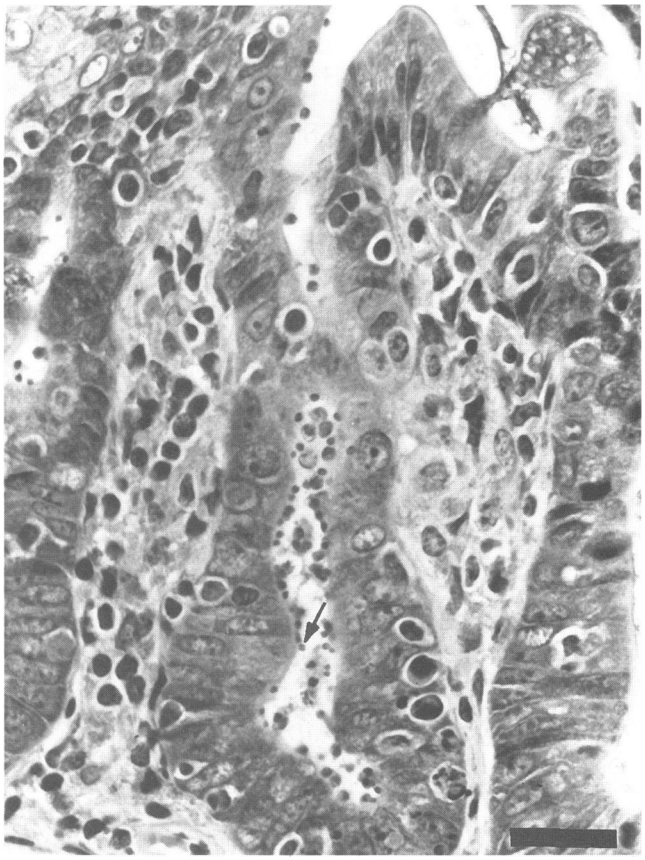

FIG. 2. Photomicrograph of the ileum from an MHC class II-deficient C57BL/6J mouse infected with $10^7$ *C. parvum* oocysts at 5 to 6 weeks of age and examined 4 weeks later. Arrow indicates location of *C. parvum* organisms. Giemsa stain was used. Bar, 20 μm.

MHC class I-deficient, MHC class II-deficient, C57BL/6J wild-type, SCID, and BALB/c mice challenged at 5 to 6 weeks of age were anesthetized with 0.01 mg of ketamine-xylazine per kg administered by intraperitoneal injection. Mice were then orally dosed with $10^7$ *C. parvum* oocysts in 50 μl of sterile PBS. Spleens and gastrointestinal tissues were analyzed 4 weeks postinfection, as described above.

Flow cytometric analysis of spleen cells confirmed CD8 T-cell deficiency in MHC class I-deficient mice and CD4 T-cell deficiency in MHC class II-deficient mice. SCID mice were deficient in T and B cells. T and B lymphocytes were present in C57BL/6J wild-type and BALB/c mice, as expected (data not shown).

The results of two independent experiments with MHC class I-deficient mice are shown in Table 1. MHC class I-deficient mice infected at 3 to 5 days of age cleared *C. parvum* infection similarly to age-matched controls at both 4 and 8 weeks. MHC class I-deficient mice infected at 5 to 6 weeks of age were no more susceptible to *C. parvum* infection than age-matched controls. Hence, CD8 T cells are not required to prevent persistent *C. parvum* infection in neonatal or adult C57BL/6J mice.

In replicate experiments with MHC class II-deficient mice, intestinal infections were substantial following exposure to $10^7$ *C. parvum* oocysts (Table 2). Mice infected at 3 to 5 days of age

TABLE 3. *C. parvum* infection scores in SCID and BALB/c control mice

| Expt | Age at infection (wk) | Wk post-infection | Infection score, mean ± SD (no. of mice) | | *P* value[a] |
|---|---|---|---|---|---|
| | | | SCID mice | BALB/c mice | |
| 1 | 5–6 | 4 | 6.17 ± 1.27 (12) | 0.89 ± 0.93 (9) | <0.0001 |
| 2 | 5–6 | 4 | 5.12 ± 0.83 (8) | 0.40 ± 0.55 (5) | 0.0008 |

[a] Determined by Mann-Whitney rank sum test.

had abundant *C. parvum* organisms within the villous and crypt mucosa of the distal ileum, cecum, and proximal colon. Mononuclear cell infiltration of the lamina propria, blunting of the villi, crypt dilatation, and epithelial necrosis were evident. Mice infected at 5 to 6 weeks of age had ample *C. parvum* organisms in the villous and crypt mucosa of the distal ileum, apex of the cecum, and proximal colon (Fig. 2). Infiltration of the lamina propria with mononuclear cells, crypt dilatation, and epithetial cell necrosis were multifocal and less severe compared with mice infected at 3 to 5 days of age.

SCID mice infected at 5 to 6 weeks of age remained infected with *C. parvum* 4 weeks postexposure, as reported previously (12, 14), and served as the positive infection control for this experiment (Table 3). SCID mice had significantly more *C. parvum* organisms than BALB/c mice.

The results of this study indicate that MHC class II-deficient, but not MHC class I-deficient, mice are susceptible to chronic cryptosporidiosis. In these mice, interruption of normal MHC class II surface expression results in lack of functionally mature CD4 T cells in the thymus and a paucity of CD4 T cells in the spleen and lymph nodes (4, 10). These mice possess normal B-cell numbers, demonstrable B-cell function, and increased CD8 T-cell proportions (4, 10, 13). Therefore, persistent cryptosporidial infection in MHC class II-deficient mice is attributed to a deficiency of CD4 T cells. The mechanisms by which CD4 T cells clear *C. parvum* infection are unresolved. The contribution of CD4 T cells to production of effector cytokines and protective mucosal antibodies is yet to be defined.

The distribution of *C. parvum* infection in MHC class II-deficient mice and that in SCID mice were dissimilar. Both MHC class II-deficient mice and SCID mice were consistently infected at the distal ileum, apex of the cecum, and proximal colon. However, all SCID mice had abundant *C. parvum* organisms at the pylorus, while in MHC class II-deficient mice this finding was variable.

MHC class I-deficient and class II-deficient mice provide unequivocal evidence that functional CD4 T cells are required to prevent persistent cryptosporidial infection. MHC class II-deficient mice should prove useful for evaluating therapeutic approaches to the management of persistent infection.

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