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Effect of In Vivo T-Cell Depletion on the Effector T-Cell Function of Immunity to *Eimeria falciformis*

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BALB/c mice were exposed to the enteric parasite *Eimeria falciformis* to produce a natural acquired immunity. The mice were then depleted of their effector T-cell function by in vivo administration of a cytotoxic Thy-1.2 mouse monoclonal antibody. T-cell depletion was demonstrated by a reduction in concanavalin A-induced proliferation of splenic lymphocytes in treated mice compared with that in controls. Twenty-four hours following T-cell depletion, the mice were challenged with 5,000 oocysts of *E. falciformis*. Daily total oocyst counts were done for each mouse from days 6 to 21 following challenge. Our studies demonstrated that depleting mice of their effector T-cell function following establishment of immunity caused an abrogation of protective immunity to this parasite.

Enteric coccidia infect the gastrointestinal epithelial cells of their specific host (27). This infection causes damage to the gut epithelium, resulting in weight loss or mortality or both. Recovery from exposure to coccidial infection includes a natural acquired immunity, which protects the host from subsequent reinfection (20, 21, 24). The nature of this acquired immunity is of great interest to immunologists working with coccidia.

Research into the immune mechanism(s) responsible for protective immunity against coccidiosis has resulted in an understanding of the role of the T cell during the development of specific immunity. Experiments on athymic mice (14) and rats (22, 25) with T-cell immunosuppressive drugs (11) during immunization with live parasites have demonstrated that T cells are essential for the development of immunity. These studies clearly demonstrate the importance of T cells in the generation of immunity, but they do not eliminate the importance of parasite-specific antibodies, because secretory antibody in the gut also requires T-cell help (5).

Following establishment of immunity, studies have focused on the presence of T-cell reactivity and parasite-specific antibodies in animals that have already acquired natural resistance. These studies follow the presence of secretory (4, 15) and circulating (2, 16) antibodies, delayed-type hypersensitivity (7, 9), and in vitro T-cell proliferation to coccidial antigens (10, 23). These studies demonstrate measurable immune reactivity in animals exposed to the parasite. However, the effector mechanism of elimination of the parasite upon reinfection remains undefined. Further studies are needed to delineate whether secretory antibody, cellular immunity, or a combination of both these effector mechanisms is responsible for the elimination of the parasite in protected animals.

These questions can be answered either by passive transfer of immune cells into naive animals or by the in vivo depletion of immune effector cells (3). Experiments to transfer coccidial immunity to naive animals have been performed. However, these experiments transfer systemically derived antibodies (18, 19) and cells (26), instead of gut lymphocytes and secretory antibodies. It has not yet been demonstrated that transferred antibodies or cells actually migrate into the gut where the parasite replicates. In this investigation, we have depleted effector T cells from *Eimeria falciformis*-immune BALB/c mice by in vivo administration of a cytotoxic monoclonal antibody (MAb) against Thy-1.2-bearing cells. These mice were then challenged, to determine whether elimination of Thy-1.2 cells would affect the protective immune mechanism to coccidia.

MATERIALS AND METHODS

Mice. Female BALB/c mice, 19 to 21 g, were obtained from Charles River Breeding Laboratories, Inc., Portage, Mich.

Parasites. *E. falciformis* was maintained by frequent passage through coccidium-free BALB/c mice, sporulated in 2.5% potassium dichromate, and stored at 4°C for up to 8 weeks.

Immunization of mice. Mice were immunized by gavage with 10^3 sporulated oocysts of *E. falciformis* every day for 5 days. They were then rested for 2 weeks and subsequently boosted with 10^3 oocysts. Two weeks following the boost, the mice were used for the in vivo T-cell depletion experiments.

In vivo depletion of T cells. Immune and nonimmune mice were administered 0.1 ml of mouse ascitic fluid containing the MAb anti-mouse Thy-1.2 of the mouse immunoglobulin G2a isotype (Sigma Chemical Co., St. Louis, Mo.). This MAb was mixed with 0.1 ml of Low-Tox-M rabbit complement (Cederlane Labs, Hornby, Ontario, Canada) before being administered to the mice. Mice were injected in the tail vein with 0.2 ml of the complement-MAb mixture 24 h before challenge and on day 4 postchallenge. Control mice were given complement alone in saline.

T-cell proliferation assay. Spleens were removed from mice aseptically 24 h after in vivo administration of MAb. The spleens were passed through a no. 60 wire mesh. Cells were rinsed from the wire mesh and centrifuged at 200 × *g* for 15 min. The cells were cultured in round-bottomed microdilution plates (Nunclon; Nunc, Roskilde, Denmark). The culture medium was RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with penicillin G (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic

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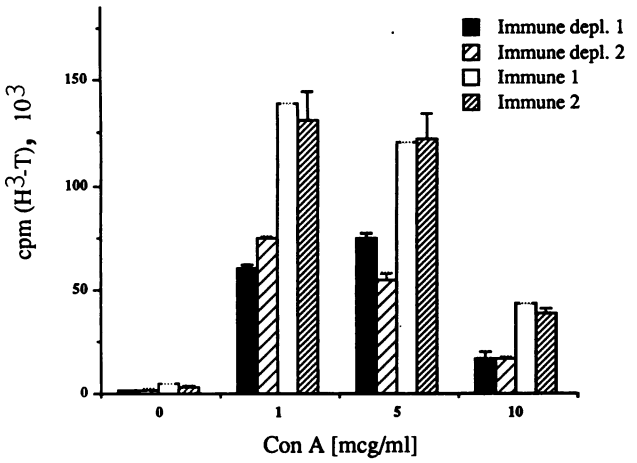

FIG. 1. Effect of in vivo administration of mouse anti-Thy-1.2 MAb on ConA responsiveness of splenocytes. BALB/c mice (two mice per group) were injected intravenously with MAb and complement (Immune depl. 1 and 2) or complement alone (Immune 1 and 2) following immunization with *E. falciformis*. After 24 h, splenocytes were removed and stimulated with ConA. [^{3}H]TdR was added after 72 h in culture. Cells were harvested 18 h later, and [^{3}H]TdR counts were recorded.

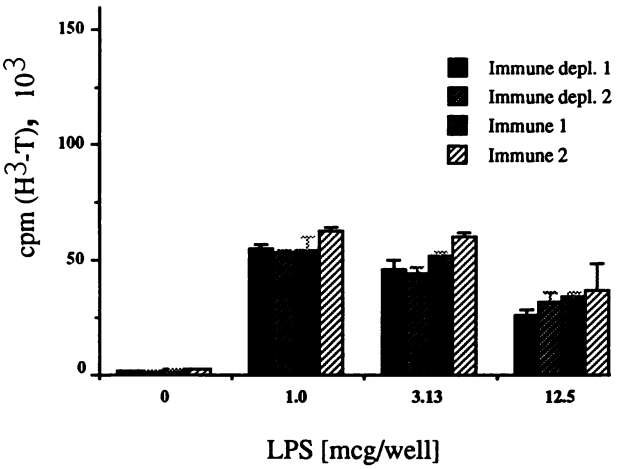

FIG. 2. Effect of in vivo administration of mouse anti-Thy-1.2 MAb on lipopolysaccharide (LPS) responsiveness of splenocytes. BALB/c mice (two mice per group) were injected intravenously with MAb and complement (Immune depl. 1 and 2) or complement alone (Immune 1 and 2) after immunization with *E. falciformis*. After 24 h, splenocytes were removed and stimulated with lipopolysaccharide. [^{3}H]TdR was added after 72 h in culture. Cells were harvested 18 h later, and [^{3}H]TdR counts were recorded.

acid) buffer (10 mM), 2-mercaptoethanol (2-ME; 5×10^{-2} M), and 10% fetal calf serum (GIBCO). In a 200-μl volume, there were 10^5 cells with concentrations of concanavalin A (ConA) (Pharmacia Diagnostics, Piscataway, N.J.) at 1, 5, and 10 μg/ml or concentrations of lipopolysaccharide (Sigma) at 2, 6.25, and 25 μg/ml. All cultures were done in triplicate. Plates were incubated at 37°C in a humidified 5% CO_2 incubator for 3 days. During the final 18 h of incubation, 10 μl of medium containing 0.5 μCi of [^{3}H]thymidine ([^{3}H]TdR; 6.7 Ci/mol; New England Nuclear Corp., Boston, Mass.) was added. Cells were collected on glass filter paper with a semiautomatic processor (Skatron, Sterling, Va.) and assayed for radioactivity by liquid scintillation (Ready Gel; Beckman Instruments, Inc., Fullerton, Calif.). Counts per minute were obtained by a B scintillation counter (Beckman Instruments, Inc., Columbia, Md.). Each sample was counted for 5 min, and net counts per minute of triplicate wells were recorded.

Oocyst counting. Mice were challenged by gavage with 5,000 oocysts of *E. falciformis*. Feces were collected daily starting at 7 days postchallenge and continuing until day 25 and were quantitatively examined for oocysts by established methods with a McMaster chamber (13). In all experiments, oocyst production was heteroscedastic, and some oocyst counts were zero; therefore, the natural logarithm transformation (oocyst count + 1) was made on the data for graphic representation (1).

RESULTS

In vivo depletion of mouse Thy-1.2 cells. Twenty-four hours after mice were injected intravenously with the anti-Thy-1.2 MAb, ConA lymphoproliferation assays were performed using splenocytes. These assays demonstrated that the cytotoxic MAb partially depleted the ConA reactivity of immune mouse splenocytes (Fig. 1). Lipopolysaccharide was also used in these functional assays to demonstrate that B-cell function remained intact following MAb treatment (Fig. 2).

Challenge studies in immune and immune Thy-1.2-depleted mice. Immune and immune T-cell-depleted mice were challenged with 5,000 oocysts of *E. falciformis* 24 h following administration of the complement or the complement-MAb mixture. Oocyst output in the immune-depleted mice began 7 days postchallenge and continued until day 22 (Fig. 3). Immune mice which were challenged produced no oocysts (data not shown).

Challenge studies in normal versus normal Thy-1.2-depleted mice. Infection control studies were done with normal nonimmune mice. These mice started oocyst production 7 days postchallenge and stopped producing oocysts by day 15 (Fig. 3). Normal mice which were depleted of their Thy-1.2 cells produced more oocysts and had an extended patency, to day 24 postchallenge (Fig. 3).

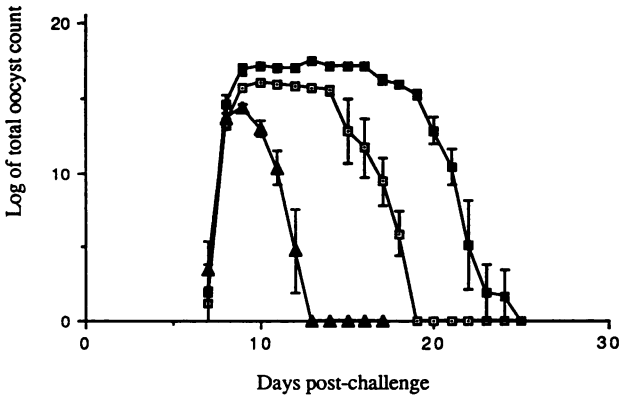

FIG. 3. Average total daily oocyst output (natural logarithm) per day per group of mice challenged with 5,000 oocysts with or without administration of in vivo cytotoxic MAb anti-Thy-1.2. Standard error bars included. Four normal BALB/c mice were not previously immunized (▲). Four normal nonimmunized mice were depleted 24 h prior to challenge by intravenous administration of anti-Thy-1.2 and complement (■). Six immune-depleted mice were immunized with *E. falciformis*, depleted of Thy-1.2 cells by administration of anti-Thy-1.2 and complement, and challenged 24 h later (⊡).

DISCUSSION

The occurrence of a natural acquired immunity to coccidia has encouraged the pursuit of a recombinant vaccine for poultry. Coccidia have a complicated life cycle, consisting of both intracellular and extracellular stages (27). Deciphering the effector mechanism of host immunity to coccidia may elucidate which life cycle stage(s) is the target for immune elimination of the parasite. This information could then concentrate vaccine discovery efforts on that life cycle stage. For instance, if secretory antibody plays a major role in immunity, then one might suppose that the extracellular stage(s) was the target of elimination. Alternatively, if a direct cytotoxic event by immune cells on infected host cells occurred, then the intracellular stage of the parasite life cycle would be suspect. The mechanism of immunity may also be a combination of events, including both antibodies and effector T cells (e.g., antibody-dependent cellular cytotoxicity).

Experiments to describe the lymphoid cells necessary to establish protective immunity have included studies of coccidiosis in T-cell-deficient (14, 17, 22, 25) and B-cell-deficient (6, 17) animals. Studies of T-cell-deficient animals indicate the importance of the T cell in generation of immunity. However, secretory antibody involvement in the effector phase of immunity cannot be ruled out in these experiments, since T cells are also essential for secretory antibody production (5). To more specifically address the involvement of antibody in protective immunity, classical studies using hormonal bursectomy with B-cell-deficient chickens have been done (6, 17). These studies demonstrate development of immunity despite measurably lower levels of circulating antibody. However, the ability to generate secretory antibody to the coccidia on these bursaless chickens has not been determined.

To study the effector mechanism of immunity to coccidia, adoptive transfer experiments have been performed using spleen and mesenteric lymph node cells obtained from *Eimeria vermiformis*-immune mice (26). Measurable protection was obtained by transfer of mesenteric lymph node cells, which probably contain both B and T cells (15). The passive transfer experiments with immune secretory or circulating antibody from coccidium-immune chickens have not demonstrated consistent and repeatable protection (4, 18, 19).

Another approach to answering questions about the effector mechanism of immunity to coccidia is to specifically deplete immune animals of their effector cells with in vivo cytotoxic antibodies (3). These studies circumvent the isolation and purification of the necessary cell types from the gut mucosa of coccidium-immune animals to adoptively transfer immunity. Studies which test whether these cells home in on the specific areas in the gut where the coccidia replicate would also have to be incorporated. With the use of in vivo cytotoxic antibodies, the depletion of effector cells can be done in situ once mice have acquired immunity.

In our studies, *E. falciformis*-immune mice were depleted of their Thy-1.2 cells with an in vivo cytotoxic MAb. This in vivo Thy-1.2 cell depletion resulted in approximately a 50% reduction of a ConA-induced spleen cell lymphoproliferation. Since this lymphoproliferation was only 50%, we assume that Thy-1.2 depletion was not complete. However, this T-cell depletion was enough to completely abrogate protective immunity. It is also important to note that the naive Thy-1.2-depleted mice that were exposed to the coccidia extended the period of parasite replication, which suggests a lack of developing immunity. Similar findings have also been described for animals with deficient T-cell development, athymic animals (14), and animals experimentally immunosuppressed by drug treatment (12).

Depleting immune mice of their Thy-1.2 effector cells, which resulted in abrogation of protective immunity, demonstrates that the level of secretory and circulating antibodies present in immune animals is not enough by itself to afford protection. Instead, antigen-specific T-cell activation is necessary for the effector phase of immunity to *E. falciformis*. Secretory and circulating antibodies may still be important participants in the effector mechanism of immunity by promoting phagocytosis or mediating cytotoxic events. These studies do not rule this out. Also, the possibility of NK cells coated with antibody killing the parasite cannot be ruled out, since NK cells also carry the Thy-1.2 marker and are depleted by the MAb (8).

Further investigation into identification of the T-cell subpopulation(s) responsible for protective immunity to coccidia may contribute further information on the life cycle stage(s) of the parasite targeted during immunity. This information will be invaluable for research concerned with the production of a coccidiosis vaccine.

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