INFECTION AND IMMUNITY, Aug. 1997, p. 3165–3170 Vol. 65, No. 8
0019-9567/97/$04.00+0

Nonspecific Immune Responses and Mechanisms of Resistance to *Eimeria papillata* Infections in Mice

M. L. SCHITO AND J. R. BARTA*

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W2

Received 29 January 1997/Returned for modification 26 March 1997/Accepted 28 May 1997

Severe combined immunodeficient (SCID)-beige mice inoculated with the intracellular parasite *Eimeria papillata* produced significantly more oocysts during primary infections than inoculated immunodeficient SCID mice. Therefore, the addition of the beige mutation, which detrimentally affects neutrophil and natural killer (NK) cell functions, enhanced the parasites' ability to reproduce within the small intestine. To identify which of these two cell types is responsible for a protective immune response during primary infection, the following groups of mice were inoculated: (i) SCID mice depleted of neutrophils with antigranulocyte monoclonal antibody (RB6-8C5), (ii) C57BL/6 mice depleted of NK cells with the anti-NK-1.1 monoclonal antibody (PK136), and (iii) transgenic Tgε26^{++} mice (T and NK cell deficient). To identify the mechanisms of immunity during primary and secondary infections, gamma interferon (IFN-γ) knockout and perforin knockout mice were inoculated. Oocyst output was found to be significantly higher during primary infection for mice depleted of NK cells by administration of anti-NK-1.1 antibodies, for Tgε26^{++} mice, and for IFN-γ knockout mice. During secondary infections, only perforin knockout mice produced significantly more oocysts compared to control mice. Our observations suggest that NK cells inhibit *E. papillata* oocyst output during primary infection by the production of IFN-γ and that this inhibition is independent of perforin. Immunity to reinfection does not require IFN-γ but appears to be mediated, at least in part, by a perforin-dependent mechanism.

Coccidiosis, caused by eimerian parasites, produces an enteritis of varying severity depending on the species of parasite and the infectious dose. The disease detrimentally affects meat and milk production by disrupting nutrient absorption from the intestinal tract of domestic agricultural animals. These obligate intracellular protozoan parasites exhibit host, tissue, and cell specificity within each host species. Each host can harbor multiple eimerian species. Although immunity is afforded by previous exposure, there is a lack of cross-protective immunity between parasite species even within a single host (6, 29). Chronic infections, in the absence of reinfection, do not occur because eimerian parasites have defined single-host life cycles which end by the formation of oocysts within infected host cells. Nonetheless, normal T-cell-mediated immune responses can reduce parasite replication, as measured by oocyst output, for most species during primary infection and for all eimerian species infecting mice examined thus far during subsequent infections (36).

Immune responses of laboratory rodents to eimerian parasites have been studied because of the availability of immunologic reagents and genetically immune-deficient hosts. *Eimeria papillata* and *Eimeria vermiformis* are two eimerian parasites that replicate within cells of the murine small intestine. Previous studies of *E. vermiformis* with recombinant cytokine and cytokine-depleting antibodies indicated that resistance against primary infection involves gamma interferon (IFN-γ) (31–33). Immunity appears to be mediated by CD4$^+$ T cells but not CD8$^+$ T cells (30, 34) or by natural killer (NK) cells (39). These results have been in dispute because eimerian parasites infect mucosal tissues within the gastrointestinal tract, which raises the possibility that the method of depletion may not significantly affect targets within this site. However, resistance to challenge infection was found to be mediated in an IFN-γ-independent manner (32). How murine hosts inhibit parasite replication during reinfection remains unknown.

Recently, we studied *E. papillata* infections in severe combined immunodeficient (SCID), SCID-beige (SCID-bg), and immunocompetent BALB/c mice (36). We found that immunity to primary infection was not mediated by T or B cells because oocyst output from infected SCID mice and that from BALB/c mice were not significantly different. These results were unique because SCID mice inoculated with other eimerian coccidia (*E. vermiformis*, *Eimeria falciformis*, and *Eimeria ferrisi*) produced 10-fold more oocysts compared to BALB/c controls. Compared to SCID mice, the additional beige mutation in SCID-bg mice enhanced oocyst output three- to fourfold, which suggests that neutrophil cytotoxicity and/or NK cell activity may inhibit *E. papillata* replication during primary infections. Immunity to reinfection with *E. papillata* was shown to be mediated by lymphocytes in immunocompetent mice (36). Therefore, we attempted to determine the importance of NK cells and neutrophils during primary infection and the mechanisms involved in resistance during primary and secondary infections.

MATERIALS AND METHODS

Animals. Six- to eight-week-old male mice were used. BALB/c, C57BL/6, and C.B-17 *scid/scid* (SCID) mice were purchased from Charles River Laboratories (Sainte Constant, Quebec, Canada, and Wilmington, Mass.). The (C57BL/6 × CBA)F_1 and the IFN-γ knockout (BALB/c-*Ifg^{tm1}*) mice were purchased from Jackson Laboratories (Bar Harbor, Maine). Perforin knockout [(C57BL/6 × 129SvEv)TacfBR-(KO)*pfp*] mice on a C57BL/6 background were purchased from Taconic (Germantown, N.Y.). Transgenic Tgε26^{++} (C57BL/6 × CBA)F_2 mice were a kind gift from B. A. Croy (Department of Biomedical Sciences, University of Guelph). These latter mice have 30 to 35 copies of the human CD3-ε gene, resulting in severe T- and NK-cell deficiencies (42). The *scid/scid.beige/beige* (SCID-bg) mice were obtained from the breeding colony at the University of Guelph (Ontario Veterinary College Isolation Facility). Immunodeficient mice were fed sterile food and water and kept in HEPA filtered barrier-isolated facilities. Manipulations for immunodeficient mice were performed in HEPA filtered biological containment hoods. Immunocompetent mice

* Corresponding author. Mailing address: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Phone: (519) 824-4120, ext. 4017. Fax: (519) 824-5930. E-mail: jbarta@uoguelph.ca.

were housed in conventional isolation rooms. All mice were kept in microisolator cages and acclimatized for 1 week prior to oocyst inoculation.

Parasite inoculation and enumeration. Mice were inoculated per os with 10^3 sporulated oocysts of *E. papillata* suspended in 10 μl of sterile saline. Oocysts were surface sterilized with sodium hypochlorite and washed at least four times in saline prior to oral inoculation in mice. Once every 24 h, fresh fecal pellets (two to three pellets) were collected and weighed for each mouse and the bedding was changed to eliminate reinfection. Oocyst output was measured as previously described (36). Briefly, fecal pellets were suspended in 10 volumes (1:10) of potassium dichromate (2.5% [wt/vol]). Samples were vortexed to homogenize them and diluted in saturated sodium chloride (specific gravity, 1.20) for oocyst flotation. Oocysts were enumerated in a McMaster chamber and expressed as number of oocysts per gram of wet feces. For reinfection studies, mice were inoculated 30 days after primary inoculation with the same dose of oocysts.

In vivo antibody depletion of NK cells and neutrophils. C57BL/6 mice were injected intraperitoneally (i.p.) with 0.5 mg of anti-NK-1.1 (PK136; mouse immunoglobulin G2a [IgG2a]) (from the American Type Culture Collection, Rockville, Md., as HB191) for NK-cell depletion. Control mice received 0.5 mg of murine IgG2a (UPC-10 myeloma) (Sigma ImmunoChemicals, Mississauga, Ontario, Canada). For NK-1.1 experiments, mice were injected at the fourth day and first day prior to oocyst inoculation. To deplete neutrophils, SCID mice were injected i.p. with 0.15 mg of rat antigranulocyte (RB6-8C5; rat IgG2b) monoclonal antibody (9) (RB6-8C5 was a kind gift from J. Yager, Department of Pathobiology, Ontario Veterinary College), with control SCID mice receiving 0.15 mg of purified rat IgG (Sigma ImmunoChemicals). The antibody reacts specifically with murine granulocytes but not with monocytes or lymphocytes. For neutrophil depletion studies, mice were injected 2 h prior to oocyst inoculation (Fig. 1C) or on −4, −1, and +2 days with respect to oocyst inoculation (Fig. 1D).

Lymphocyte isolation from spleen, mesenteric lymph node, and intestinal intraepithelium. Splenic lymphocytes were isolated by standard methods. Briefly, individual spleens were disrupted in Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) with 10% fetal bovine serum (FBS) (referred to as complete DMEM) by pressing the tissue through a metal sieve. Cells were centrifuged to a pellet at 400 × *g*. Erythrocytes were lysed by resuspending the cell pellet briefly in sterile distilled water and then adding an equal volume of 2× phosphate-buffered saline. Adherent cells were removed by incubating splenocytes at 37°C for 30 min in 60- by 15-mm tissue culture dishes (Becton Dickinson, Lincoln Park, N.J.) containing complete DMEM. Nonadherent lymphocytes were gently removed, washed, and enumerated with a hemocytometer. Mesenteric lymph node lymphocytes were isolated by a similar procedure, but the erythrocyte lysis step was omitted.

Intestinal intraepithelial lymphocytes (i-IEL) were isolated from the small intestines of C57BL/6 mice by procedures described previously (4, 25). Briefly, small intestines were flushed with ice-cold Ca^{2+}-Mg^{2+}-free Hanks' balanced salt solution (CMF-HBSS) containing 2% FBS to remove intestinal debris. Peyer's patches were excised, and the intestine was cut longitudinally. Intestines from three mice were cut into 1-cm pieces and washed by being shaken in CMF-HBSS. Tissues were transferred to siliconized 250-ml flasks containing 25 ml of CMF-HBSS with 10% FBS and 0.1 mM EDTA warmed to 37°C. Flasks were shaken for 20 min in a 37°C water bath. The supernatant was removed and allowed to stand for 10 min to remove heavy debris. The shaking procedure was repeated to release the remaining epithelial cells from the intestinal tissues. The supernatant containing dead epithelial cells and i-IEL was centrifuged at 400 × *g*. Cells were resuspended in 5 ml of cold complete DMEM and passed through a nylon wool (Cellular Products Inc., Buffalo, N.Y.) column. The column was immediately washed with 10 ml of complete DMEM, and the collected cell suspension was centrifuged at 400 × *g*. Cells were resuspended in 40% Percoll (Pharmacia, Uppsala, Sweden), layered over 70% Percoll in a 15-ml conical centrifuge tube, and centrifuged for 20 min at 1,500 × *g*. A band of cells collected between the 40 and 70% Percoll interface was washed three times in complete DMEM and enumerated with a hemocytometer. Cell viability for splenocytes, lymph node lymphocytes, and i-IEL was greater than 95% by trypan blue dye exclusion.

Flow cytometric assessment of NK cells. For determining NK-cell depletions, splenic lymphocytes from depleted and control C57BL/6 mice ($n = 3$) were examined by flow cytometry on the day of oocyst inoculation (day 0). The percentage of lymphocytes that stained positive for NK-1.1, αβ T-cell receptor (TcR), or both was determined for three tissue sources (spleen, mesenteric lymph node, and small intestine) from normal C57BL/6 mice ($n = 3$). A total of 10^6 lymphocytes were incubated at 4°C in the absence of light. Available Fc receptors on lymphocytes were blocked for 30 min with 10% normal horse serum. Cells were stained with fluorescein isothiocyanate-conjugated hamster anti-αβ TcR (Sigma ImmunoChemicals) and R-phycoerythrin-conjugated mouse anti-NK-1.1 (PharMingen, San Diego, Calif.) for 45 min. A nonstained sample was also included as a control for background fluorescence. All samples were processed immediately by flow cytometry (FACScan; Becton Dickinson, San Jose, Calif.) and analyzed with Lysis II software (Becton Dickinson).

Assessment of neutrophil depletion. Four groups of SCID mice, injected at time zero with 0.15 mg of rat antigranulocyte monoclonal antibodies, were compared with a SCID control group (time zero) ($n = 3$ mice per group) at various time points after injection (2, 36, 72, and 120 h). All mice were anesthetized with Metofane (Janssen Pharmaceutica, North York, Ontario, Canada) prior to the withdrawal of 250 to 500 μl of blood by cardiac puncture. Blood samples were analyzed on a Technicon H1 hematology system (Technicon Instruments Corp., Tarrytown, N.Y.) to assess leukocyte differential counts.

Statistical analysis. Data were analyzed by the Student *t* test ($P < 0.05$) and expressed as means ± standard errors of the mean.

RESULTS

Effect of neutrophils and NK cells during primary infection. Oocyst output in SCID-bg mice was significantly higher during peak output (day 4 postinoculation [p.i.]) compared to that of SCID mice (Fig. 1A). To determine which cell type played a role, C57BL/6 mice were treated with an anti-NK cell monoclonal antibody (PK136) (Fig. 1B) and SCID mice were treated with an antigranulocyte monoclonal antibody (RB6-8C5) (Fig. 1C and D). When antibody-treated mice were inoculated with *E. papillata*, oocyst output increased significantly for NK-1.1-depleted mice on day 4 p.i. (Fig. 1B) and for both neutrophil depletions on day 5 p.i. (Fig. 1C and D).

By use of the PK136 monoclonal antibody, the percentage of splenic $\alpha\beta^-$ NK-1.1^+ cells was significantly reduced (3.94 ± 0.5) compared to that for control mice (6.64 ± 1.8). NK-1.1 depletion did not affect other splenic lymphocytes because the percentage of $\alpha\beta^+$ NK-1.1^- T cells (29.0 ± 2.8) was not significantly different from control values (33.3 ± 3.0). Determinations were not made for i-IEL because less than 1% of the i-IEL population expresses NK-1.1 in normal mice (13). Similarly, less than 2% of the mesenteric lymph node lymphocytes express NK-1.1 (data not shown). By the RB6-8C5 monoclonal antibody, neutrophil depletion ranged from 73 to 96% [100 − (number of neutrophils from treated mice/number of neutrophils from control mice) × 100] compared to control-treated mice with a duration of at least 5 days without significantly affecting lymphocyte or monocyte numbers (Fig. 2). Mice treated with either anti-NK-1.1 or antigranulocyte monoclonal antibodies produced higher numbers of oocysts compared to controls.

In order to determine the extent of NK cell involvement, T- and NK cell-deficient (Tgε26^{++}) mice were inoculated with *E. papillata* (Fig. 3). Although Tgε26^{++} mice are devoid of T cells, parasite output during primary infections has been previously shown not to be mediated by T and B lymphocytes (36). Thus, these transgenic mice permit assessment of NK cell involvement during infection because the NK cell deficiency approaches 100%. Oocyst output was significantly higher for Tgε26^{++} mice compared to control mice at all time points with the exception of day 4 p.i. These data suggest that NK cells control primary *E. papillata* infections.

Immune mechanisms active during primary infections. To determine if IFN-γ can affect oocyst output, IFN-γ knockout mice were inoculated and compared to inoculated BALB/c control mice (Fig. 4A). During primary infection, IFN-γ knockout mice produced significantly more oocysts at all time points compared to controls.

To examine if NK cells mediate immunity by perforin-dependent cytolysis, perforin knockout mice and C57BL/6 control mice were inoculated with *E. papillata*. Oocyst output did not differ significantly between the two types of hosts throughout primary infection (Fig. 4C).

Immune mechanisms active during secondary infections. To determine the mechanisms responsible for T-cell-mediated resistance, perforin and IFN-γ knockout mice were reinfected with 10^3 oocysts of *E. papillata* 30 days after the initial inoculation. Oocyst output was compared to that of reinfected control mice. For IFN-γ knockout mice, oocyst output was not significantly different from that of control mice with the exception of day 4 p.i. due to the extension of the patent period by

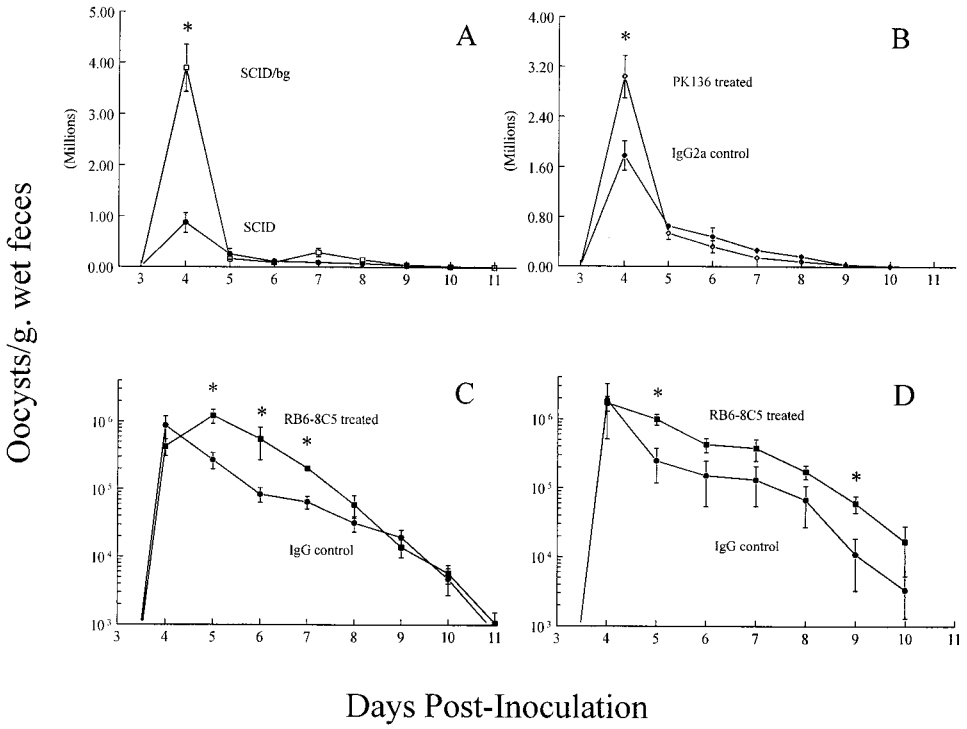

FIG. 1. Comparison of oocyst output from NK- and/or neutrophil-deficient mice infected at day 0 with 10^3 sporulated *E. papillata* oocysts. (A) Immunodeficient SCID mice (●) ($n = 4$) versus SCID-bg mice (□) ($n = 4$). (B) C57BL/6 mice injected with mouse anti-NK-1.1 (◇) ($n = 4$) versus control C57BL/6 mice injected with mouse IgG2a (◆) ($n = 4$). (C) SCID mice injected with antigranulocyte monoclonal antibody (RB6-8C5) 2 h prior to oocyst inoculation (■) ($n = 6$) versus control SCID mice injected with rat IgG (●) ($n = 6$). (D) SCID mice injected with antigranulocyte monoclonal antibody (RB6-8C5) (■) ($n = 4$) versus control SCID mice injected with rat IgG (●) on days −4, −1, and +2 in relation to oocyst inoculation ($n = 4$). Each datum point represents mean number of oocysts per gram of wet feces ± standard error of the mean. The asterisks indicate that the values are significantly different ($P < 0.05$) from those for control. Duplicate experiments gave similar results.

1 day (Fig. 4B). Therefore, IFN-γ knockout mice were as resistant as the BALB/c control mice. For perforin knockout mice, oocyst output was significantly higher for all time points except for days 5 and 9 p.i. (Fig. 4D). Thus, perforin knockout mice appeared to be more susceptible to reinfection compared to control mice.

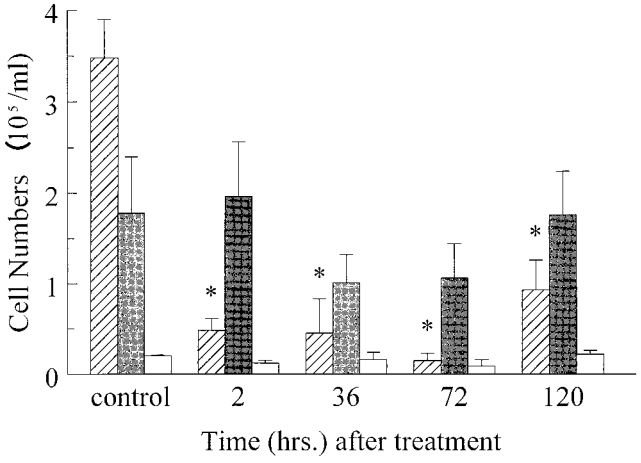

FIG. 2. Numbers of peripheral blood neutrophils (hatched bars), lymphocytes (gray bars), and monocytes (empty bars) from day 0 control SCID mice and antibody-treated SCID mice at various times posttreatment. Treated mice received 0.15 mg of the monoclonal antibody RB6-8C5 at time zero. The results are expressed as mean cell numbers (10^5) ± standard deviations ($n = 3$ mice per group). The asterisks indicate that the values are significantly different ($P < 0.05$) from those for day 0 control SCID mice.

DISCUSSION

Our previous studies suggested that immunity to *E. papillata* during primary infection was not mediated by T or B lymphocytes but was affected by mutations in NK cell cytotoxicity or neutrophil function (36). To identify which of these two cell types limits coccidial replication, we depleted C57BL/6 mice of NK cells and SCID mice of neutrophils by using monoclonal antibodies prior to oocyst inoculation.

Neutrophils have been shown to play an important role in host immunity to a variety of pathogens including *Mycobacterium avium* (2), *Chlamydia trachomatis* (3), *Candida albicans* (16), *Listeria monocytogenes* (9, 28), and *Francisella tularensis* (38) in vivo and *E. falciformis* (5) in vitro. Treating SCID mice with the antigranulocyte antibody significantly reduced the number of circulating neutrophils without significantly affecting the number of lymphocytes or monocytes. Neutrophil-depleted mice produced significantly more oocysts compared to control mice on days 5, 6, and 7 for the first experiment and days 5 and 9 for the second experiment, which suggests that recruited peripheral blood neutrophils partially inhibited parasite replication within the intestinal tract. Neutrophils can significantly affect parasite replication without the participation of parasite-specific antibodies since SCID mice were used for the neutrophil depletion studies.

Neutrophil depletion may have detrimentally affected host physiology to an extent such that oocyst output was delayed.

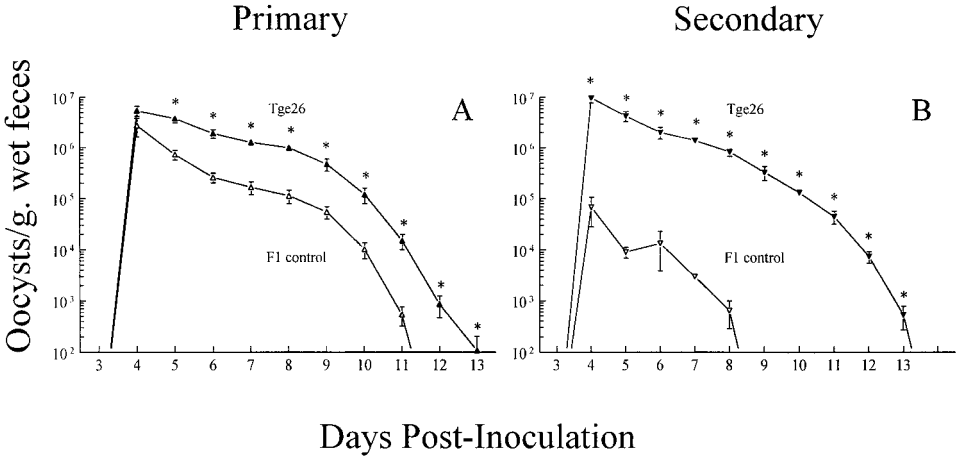

FIG. 3. Oocyst output in T- and NK cell-deficient Tgε26^{++} mice infected at day 0 with 10^3 sporulated *E. papillata* oocysts. (A) Primary infection in Tgε26^{++} mice (▲) versus (C57BL/6 × CBA)F_1 control mice (△). (B) Secondary infection in Tgε26^{++} mice (▼) versus (C57BL/6 × CBA)F_1 control mice (▽). Each datum point represents mean number of oocysts per gram of wet feces ± standard error of the mean ($n = 6$). The asterisks indicate differences in oocyst output that are statistically significant from those for control mice ($P < 0.05$).

Evidence for this comes from studies in which irritants, such as proteose peptone injected i.p. at the time of infection, delayed oocyst output in chickens infected with *Eimeria necatrix* (1). Indeed, when mice were injected 2 h prior to oocyst inoculation, peak oocyst output was delayed by 1 day. However, if depletion commences 4 days prior to oocyst inoculation, peak oocyst output in depleted SCID mice is not delayed but does remain higher throughout patency. This suggests that there is a small but significant neutrophil-specific effect and not a nonspecific response to i.p. irritation.

Although anti-NK-1.1 treatment depleted only 41% of splenic NK cells, there was a significant increase in oocyst output on day 4 (twofold) in infected treated mice compared to infected control mice. It is noteworthy that the beige mutation

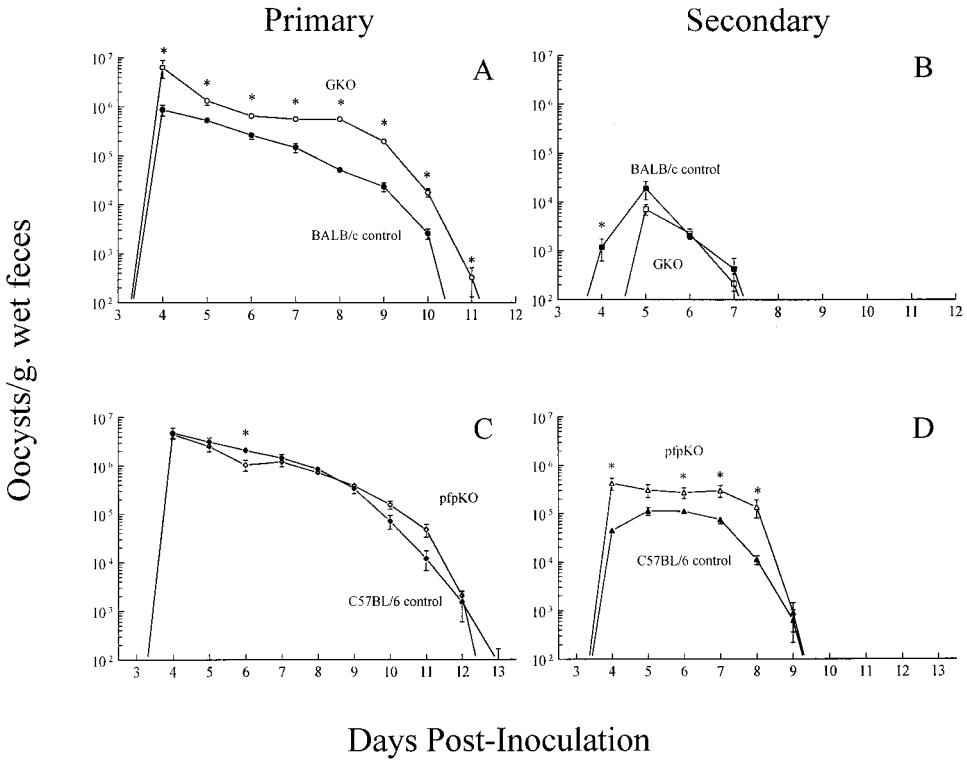

FIG. 4. Oocyst output in IFN-γ knockout (GKO) and perforin knockout (pfpKO) mice infected at day 0 with 10^3 sporulated *E. papillata* oocysts. (A) Primary infection in GKO (○) versus BALB/c (●) mice. (B) Secondary infection 30 days later in GKO (□) versus BALB/c (■) mice. Each GKO datum point represents mean number of oocysts per gram of wet feces ± standard error of the mean ($n = 5$). (C) Primary infection in pfpKO (◇) versus C57BL/6 (◆) mice. (D) Secondary infection 30 days later in pfpKO (△) versus C57BL/6 (▲) mice. Each pfpKO datum point represents mean number of oocysts per gram of wet feces ± standard error of the mean ($n = 6$). The asterisks indicate differences in oocyst output that are statistically significant from those for control mice ($P < 0.05$).

in SCID-bg mice results in only a 50% reduction in NK cell activity from levels in SCID mice (24). Peak oocyst output in the anti-NK-1.1-treated C57BL/6 mice increased, just as it did for SCID-bg mice infected with this parasite relative to intact congenic controls.

To confirm the role of NK cells in immunity to primary infections, Tgε26^{++} mice were also inoculated to determine if resident tissue NK cells which (i) were not totally removed by antibody depletions, (ii) were not affected by the beige mutation, or (iii) lack NK-1.1 receptors could affect oocyst output. Although these mice are deficient for both T and NK cells, the inability of T cells to control primary *E. papillata* infections (36) allows evaluation of the NK cell contribution to immunity during primary infections. With the exception of day 4 p.i., Tgε26^{++} mice produced significantly more oocysts throughout patency compared to control animals. Thus, oocyst output was higher for all three experimental systems (NK-1.1 depleted, SCID-bg, and Tgε26^{++} mice) that have NK cell deficiencies. This suggests that NK cells play an important role in mediating resistance to *E. papillata* during primary infections.

IFN-γ has received considerable attention because of its role in immunity to intracellular pathogens (20). The production of IFN-γ by stimulated NK cells has been observed to control pathogens such as *Toxoplasma gondii* (11, 15, 17, 37), *Cryptosporidium parvum* (8), *Leishmania major* (21), *Trypanosoma cruzi* (7), *Listeria monocytogenes* (43), *Cryptococcus neoformans* (35), and murine cytomegalovirus (26). Resistance to primary infections with *E. papillata* also appears to be mediated by NK cell-derived IFN-γ. In contrast, T cells (34), but not NK cells (39), mediate immunity to primary infections with *E. vermiformis*. IFN-γ was observed to directly affect intracellular survival of *E. vermiformis* in vitro without the aid of lymphoid or myeloid cells (31). This suggests that the IFN-γ-dependent antiparasitic effect is mediated directly by host cells infected with *Eimeria* species.

Immunity to reinfection was found to be IFN-γ independent for *E. papillata*, *E. vermiformis* (32), and *L. monocytogenes* (14). IFN-γ was not necessary during primary infection in order to prime the effector cells responsible for mediating immunity to reinfection with these pathogens.

Mice lacking IFN-γ or its receptor have a number of defects including reduced NK cell activity and increased cytotoxic T-lymphocyte activity (10). Since IFN-γ knockout mice were slightly more resistant than control mice when reinfected (patency delayed by 1 day), perforin knockout mice were used to determine the importance of perforin-mediated T-cell cytotoxicity. Cytotoxic T lymphocytes and NK cells contain granzymes and perforin granules that can be released upon activation to lyse target cells in a major histocompatibility complex-dependent or major histocompatibility complex-independent mechanism, respectively (22, 27, 40). Although NK cells play a role in controlling *E. papillata* replication during primary infection by an IFN-γ-dependent mechanism, perforin does not appear to be involved.

When reinfected, perforin knockout mice shed significantly more oocysts than control mice. This suggests that immunity to secondary infection is mediated, in part, by a perforin-dependent mechanism. Partial resistance to reinfection in perforin knockout mice, compared to control mice, suggests that Fas-mediated cytolysis or other unrelated cytotoxic mechanisms may also be involved. The induction of perforin mRNA by CD8$^+$ αβ$^+$ lymphocytes is dependent on interleukin 2 (IL-2) (23). A perforin-dependent, CD8$^+$ T-cell cytotoxic mechanism has been observed to control pathogens such as *L. monocytogenes* (18) and lymphocytic choriomeningitis virus (19, 41). Alternatively, perforin-dependent cytotoxicity may be only one component of a multifactorial immune response.

Cytotoxic CD8$^+$ T cells are the most likely candidate to mediate immunity to *E. papillata* by a perforin-dependent mechanism for the following reasons. First, T cells have been shown to mediate immunity to secondary infections (36). Second, higher numbers of αβ$^+$ TcR and CD8$^+$ T cells were found within the intestinal mucosa in immune mice compared to uninfected control mice (unpublished observation). Last, levels of IL-2 produced by mesenteric lymph node lymphocytes were highest during the first few days of reinfection (unpublished observation). Thus, the data support an IL-2-dependent perforin-mediated mechanism of immunity to reinfection by cytotoxic T cells, probably αβ$^+$ CD8$^+$ T cells (12, 13).

In summary, our observations suggest that NK cells mediate immunity possibly by producing IFN-γ during primary infections with *E. papillata*. Neutrophils may also participate, but to a lesser extent, as nonspecific effector cells acting by an antibody-independent mechanism to control infection. During secondary infections, immunity is not mediated by IFN-γ and is partially dependent on perforin, possibly produced by intestinal αβ$^+$ CD8$^+$ T cells.

ACKNOWLEDGMENTS

The work was supported in part by a grant from the Natural Sciences and Engineering Research Council of Canada (OGP0106453) and by funding from the Ontario Ministry of Agriculture, Food, and Rural Affairs Research Program to J.R.B.

We thank Bill Chobotar for additional support towards the project; Brenda Ludlow and Susan Atkinson for processing blood samples on the Technicon H1; and M. A. Fernando, D. Martin, M. Tahir, R. Carreno, S. Kopko, K. Strickler, and B. Coles for constructive criticisms.

REFERENCES

1. **Al-Attar, M. A., and M. A. Fernando.** 1987. Transport of *Eimeria necatrix* sporozoites in the chicken: effects of irritants injected intraperiotoneally. J. Parasitol. **73:**494–502.
2. **Appelberg, R., A. G. Castro, S. Gomes, J. Pedrosa, and M. T. Silva.** 1995. Susceptibility of beige mice to *Mycobacterium avium*: role of neutrophils. Infect. Immun. **63:**3381–3387.
3. **Barteneva, N., I. Theodor, E. M. Peterson, and L. M. de la Maza.** 1996. Role of neutrophils in controlling early stages of a *Chlamydia trachomatis* infection. Infect. Immun. **64:**4830–4833.
4. **Beagley, K. W., K. Fujihashi, A. S. Lagoo, S. Lagoo-Deenadaylan, C. A. Black, A. M. Murray, A. T. Sharmanov, M. Yamamoto, J. R. McGhee, C. O. Elson, and H. Kiyoni.** 1995. Differences in intraepithelial lymphocyte T cell subsets isolated from murine small versus large intestine. J. Immunol. **154:** 5611–5619.
5. **Bekhti, K., M. Kazanji, and P. Péry.** 1992. *In vitro* interactions between murine neutrophils and *Eimeria falciformis* sporozoites. Res. Immunol. **143:** 909–917.
6. **Blagburn, B. L., and K. S. Todd.** 1984. Pathological changes and immunity associated with experimental *Eimeria vermiformis* infections in *Mus musculis*. J. Protozool. **31:**556–561.
7. **Cardillo, F., J. C. Voltarelli, S. G. Reed, and J. S. Silva.** 1996. Regulation of *Trypanosoma cruzi* infection in mice by gamma interferon and interleukin 10: role of NK cells. Infect. Immun. **64:**128–134.
8. **Chen, W., J. A. Harp, A. G. Harmsen, and E. A. Havell.** 1993. Gamma interferon functions in resistance to *Cryptosporidium parvum* infection in severe combined immunodeficient mice. Infect. Immun. **61:**3548–3551.
9. **Czuprynski, C. J., J. F. Brown, N. Maroushek, K. D. Wagner, and H. Steinberg.** 1994. Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to *Listeria monocytogenes* infection. J. Immunol. **152:** 1836–1846.
10. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science **259:**1739–1742.
11. **Gazzinelli, R. T., M. Wysocka, S. Hayashi, E. Y. Denkers, S. Hieny, P. Casper, G. Trinchieri, and A. Sher.** 1994. Parasite-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii*. J. Immunol. **153:**2533–2543.
12. **Gelfanov, V., V. Gelfanova, Y.-G. Lai, and N.-S. Liao.** 1996. Activated αβ-CD8$^+$, but not αα-CD8$^+$, TcR-αβ$^+$ murine intestinal intraepithelial lym-

phocytes can mediate perforin-based cytotoxicity, whereas both subsets are active in fas-based cytotoxicity. J. Immunol. **156:**35–41.
13. **Guy-Grand, D., B. Cuénod-Jabri, M. Malassis-Seris, F. Selz, and P. Vassalli.** 1996. Complexity of the mouse gut T cell immune system: identification of two distinct natural killer T cell intraepithelial lineages. Eur. J. Immunol. **26:**2248–2256.
14. **Harty, J. T., and M. J. Bevan.** 1995. Specific immunity to *Listeria monocytogenes* in the absence of IFN-γ. Immunity **3:**109–117.
15. **Hunter, C. A., C. S. Subauste, V. H. Cleave, and J. S. Remington.** 1994. Production of gamma interferon by natural killer cells from *Toxoplasma gondii*-infected SCID mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha. Infect. Immun. **62:**2818–2824.
16. **Jensen, J., T. Warner, and E. Balish.** 1993. Resistance of SCID mice to *Candida albicans* administered intravenously or colonizing the gut: role of polymorphonuclear leukocytes and macrophages. J. Infect. Dis. **167:**912–919.
17. **Johnson, L. L., F. P. VanderVegt, and E. A. Havell.** 1993. Gamma interferon-dependent temporary resistance to acute *Toxoplasma gondii* infection independent of $CD4^+$ or $CD8^+$ lymphocytes. Infect. Immun. **61:**5174–5180.
18. **Kägi, D., B. Ledermann, K. Bürki, H. Hengartner, and R. M. Zinkernagel.** 1994. CD8+ T cell-mediated protection against an intracellular bacterium by perforin-dependent cytotoxicity. Eur. J. Immunol. **24:**3068–3072.
19. **Kägi, D., B. Ledermann, K. Bürki, P. Seiler, B. Odermatt, K. J. Olsen, E. R. Podack, R. M. Zinkernagel, and H. Hengartner.** 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature **369:**31–37.
20. **Kaufmann, S. H. E.** 1994. Bacterial and protozoal infections in genetically disrupted mice. Curr. Opin. Immunol. **6:**518–525.
21. **Laskay, T., M. Rollinghoff, and W. Solbach.** 1993. Natural killer cells participate in the early defense against *Leishmania major* infection in mice. Eur. J. Immunol. **23:**2237–2241.
22. **Liu, C.-C., C. M. Walsh, and J. D.-E. Young.** 1995. Perforin: structure and function. Immunol. Today **16:**194–201.
23. **Liu, C.-C., S. Rafii, A. Granelli-Piperno, J. A. Trapani, and J. D.-E. Young.** 1989. Perforin and serine esterase gene expression in stimulated human T cells: kinetics, mitogen requirements, and effects of cyclosporin A. J. Exp. Med. **170:**2105–2118.
24. **MacDougall, J. R., B. A. Croy, C. Chapeau, and D. A. Clark.** 1990. Demonstration of a splenic cytotoxic effector cell in mice of genotype *scid/scid.bg/bg*. Cell. Immunol. **130:**106–117.
25. **McDonald, V., H. A. Robinson, J. P. Kelly, and G. J. Bancroft.** 1996. Immunity to *Cryptosporidium muris* infection in mice is expressed through gut $CD4^+$ intraepithelial lymphocytes. Infect. Immun. **64:**2556–2562.
26. **Orange, J. S., B. Wang, C. Terhorst, and C. A. Biron.** 1995. Requirement for natural killer cell-produced interferon gamma in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. J. Exp. Med. **182:**1045–1056.
27. **Podack, E. R., H. Hengartner, and M. G. Lichtenheld.** 1991. A central role of perforin in cytolysis? Annu. Rev. Immunol. **9:**129–157.
28. **Rogers, H. W., and E. R. Unanue.** 1993. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. Infect. Immun. **61:**5090–5096.
29. **Rose, M. E.** 1973. Immunity, p. 295–341. *In* D. M. Hammond and P. L. Long (ed.), The coccidia: *Eimeria*, *Isospora*, *Toxoplasma*, and related genera. University Park Press, Baltimore, Md.
30. **Rose, M. E., P. Hesketh, and D. Wakelin.** 1992. Immune control of murine coccidiosis: $CD4^+$ and $CD8^+$ T lymphocytes contribute differentially in resistance to primary and secondary infection. Parasitology **105:**349–354.
31. **Rose, M. E., A. L. Smith, and D. Wakelin.** 1991. Gamma interferon-mediated inhibition of *Eimeria vermiformis* growth in cultured fibroblasts and epithelial cells. Infect. Immun. **59:**580–586.
32. **Rose, M. E., D. Wakelin, and P. Hesketh.** 1991. Interferon-gamma-mediated effects upon immunity to coccidial infections in the mouse. Parasite Immunol. **13:**63–74.
33. **Rose, M. E., D. Wakelin, and P. Hesketh.** 1989. Gamma interferon controls *Eimeria vermiformis* primary infection in BALB/c mice. Infect. Immun. **57:**1599–1603.
34. **Rose, M. E., H. S. Joysey, P. Hesketh, R. K. Grencis, and D. Wakelin.** 1988. Mediation of immunity to *Eimeria vermiformis* in mice by $L3T4^+$ T cells. Infect. Immun. **56:**1760–1765.
35. **Salkowski, C. A., and E. Balish.** 1991. A monoclonal antibody to gamma interferon blocks augmentation of natural killer cell activity induced during systemic cryptococcosis. Infect. Immun. **59:**486–493.
36. **Schito, M. L., J. R. Barta, and B. Chobotar.** 1996. Comparison of four murine *Eimeria* species in immunocompetent and immunodeficient mice. J. Parasitol. **82:**255–262.
37. **Sher, A., I. P. Oswald, S. Hieny, and R. T. Gazzinelli.** 1993. *Toxoplasma gondii* induces a T-independent IFN-γ response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-α. J. Immunol. **150:**3982–3989.
38. **Sjöstedt, A., J. W. Conlan, and R. J. North.** 1994. Neutrophils are critical for host defense against primary infection with the facultative intracellular bacterium *Francisella tularensis* in mice and participate in defense against reinfection. Infect. Immun. **62:**2779–2783.
39. **Smith, A. L., M. E. Rose, and D. Wakelin.** 1994. The role of natural killer cells in resistance to coccidiosis: investigations in a murine model. Clin. Exp. Immunol. **97:**273–279.
40. **Smyth, M. J., and J. A. Trapani.** 1995. Granzymes: exogenous proteinases that induce target cell apoptosis. Immunol. Today **16:**202–206.
41. **Walsh, C. M., M. Matloubian, C. C. Liu, R. Ueda, C. G. Kurahara, J. L. Christensen, M. T. Huang, J. D. Young, R. Ahmed, and W. R. Clark.** 1994. Immune function in mice lacking the perforin gene. Proc. Natl. Acad. Sci. USA **91:**10854–10858.
42. **Wang, B., C. Biron, J. She, K. Higgins, M. Sunshine, E. Lacy, N. Lonberg, and C. Terhorst.** 1994. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3E gene. Proc. Natl. Acad. Sci. USA **91:**9402–9406.
43. **Wherry, J. C., R. D. Schreiber, and E. R. Unanue.** 1991. Regulation of gamma interferon production by natural killer cells in *scid* mice: roles of tumor necrosis factor and bacterial stimuli. Infect. Immun. **59:**1709–1715.

Editor: J. M. Mansfield