Characterization of experimental *Cryptosporidium parvum* infection in IFN-γ knockout mice

XIANGDONG YOU[1] *and* J. R. MEAD[1,2]*

[1] *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA*
[2] *Georgia VA Research Center on AIDS and HIV Infection, Veterans Affairs Medical Center, Decatur, GA 30033, USA*

(*Received 4 March 1998; revised 10 June 1998; accepted 11 June 1998*)

SUMMARY

Severe cryptosporidial infections were produced in gamma interferon (IFN-γ) knockout mice. Mean oocyst shedding increased from 332 to 30717 oocysts/100 μl of faecal suspension between day 4 and 9 after administration of 1×10^5 oocysts/mouse. No significant differences in oocyst shedding were observed in mice after being inoculated with 1×10^5, 1×10^4 or 1×10^3 oocysts/mouse ($P > 0{\cdot}05$). Infected mouse weights decreased an average 3–4 g before death or euthanization. Histological studies revealed heavy parasite colonization in small intestinal epithelium (approximately 250 organisms/high-power field at $\times 400$). Mesenteric lymph nodes in infected mice were markedly enlarged compared to controls ($P < 0{\cdot}05$). Both $CD4^+$ and $CD8^+$ T cell populations increased in spleens of infected mice while the B cell population increased in mesenteric lymph nodes from infected mice. No significant proliferation was observed when pooled lymphocytes from infected mice were exposed to *C. parvum* antigens *in vitro*. Addition of recombinant mouse IFN-γ did not restore antigen responsiveness. While lymphoproliferative responses to specific antigen were not significant in the short period following infection, this mouse model provides unique features to study the characteristics of acute infection and the immune response against *C. parvum*.

Key words: *Cryptosporidium parvum*, gamma interferon, knockout mice.

INTRODUCTION

Cryptosporidiosis is asymptomatic or associated with a self-limiting diarrhoeal illness in immunocompetent people but causes severe, chronic diarrhoea that can be life-threatening in immunocompromised individuals. Animal models of infection with *Cryptosporidium parvum* have shown the importance of cell-mediated immunity and production of certain cytokines. In particular, gamma interferon (IFN-γ) may have an important role since cryptosporidial infection can induce the production of IFN-γ *in vitro* and *in vivo* (Harp, Whitmire & Sacco, 1994; Tilley, McDonald & Bancroft, 1995; Harp & Sacco, 1996; Wyatt *et al*. 1997). It was found that parasite shedding was greatly enhanced in SCID mice treated with anti-IFN-γ monoclonal antibody (Tzipori, Rand & Theodos, 1995). Recombinant IFN-γ in *C. parvum*-infected rats limits cryptosporidiosis in the small intestine (Rehg, 1996). The protection of IFN-γ against *C. parvum* infection may involve more than a single mechanism.

Gene targeting and transgenic animal technology have become a widely used approach in the biomedical area especially in immunology. Mice with targeted disruptions in genes encoding proteins of immunological interest (knockout) has proved to be valuable for examining the function of these molecules *in vivo* and IFN-γ knockout mice suffer more severely than controls from experimental intracellular pathogen infections such as *Mycobacterium tuberculosis*, *Leishmania major*, and *Francisella tularensis* (Cooper *et al*. 1993; Flynn *et al*. 1993; Wang *et al*. 1994; Elkins *et al*. 1996). Recently, Theodos *et al*. (1997) reported the development of severe *C. parvum* infection in IFN-γ knockout mice. In this report, we independently confirmed and further characterized the *C. parvum* infection in C57BL/6J-Ifg$^{tm1@}$ IFN-γ knockout mice.

MATERIALS AND METHODS

Animals

Male C57BL/6J-Ifg$^{tm1@}$ gamma-interferon knockout mice (GKO), age 8–10 weeks (The Jackson Laboratory Bar Harbor, ME) were maintained at the Veterans Affairs Medical Center (Decatur, GA) animal facility in an isolation room under pathogen-free conditions. Mice were housed in micro-isolator cages (Nalgene Labware, Rochester, NY) in high-efficiency particulate air (HEPA)—filtered laminar

* Corresponding author: VA Medical Center Research 151, 1670 Clairmont Road, Decatur, GA 30033, USA. Tel: +1 404 321 6111. X2545. Fax: +1 404 728 7780. E-mail: jmead@emory.edu

https://doi.org/10.1017/S0031182098003424 Published online by Cambridge University Press

flow racks (Lab. Products Inc., Maywood, NJ). All cages, food, water and bedding were sterilized before use. Sterilized surgical clothing (i.e. mask, gown and gloves) was worn when mice were handled. All manipulations were performed in a HEPA-filtered hood.

Production of C. parvum *oocysts and inoculation*

The *C. parvum* isolate (IOWA bovine isolate) was generated in newborn Holstein bull calves. Oocysts were collected and purified through discontinuous sucrose gradients as previously described (Arrowood & Donaldson, 1996). Purified oocysts were stored at 4 °C in 2·5% potassium dichromate ($K_2Cr_2O_7$) aqueous solution. Oocyst inocula were prepared by washing purified oocysts (stored < 6 months) with PBS (pH 7·2) to remove the $K_2Cr_2O_7$. In order to determine the susceptibility of GKO mice to *C. parvum* infection, different infection doses (10^3–10^5 oocysts/mouse) were used to infect GKO mice by gastric intubation. An inoculum of 10^5 oocysts/mouse was utilized to study the immune response to *C. parvum* in GKO mice. Mouse faecal samples were collected every day after infection to determine oocyst shedding. Experiments were conducted to observe the survival time of infected GKO mice. In other groups, GKO mice were euthanized before death (determined by previous experiments) for histological and immunological studies. At least 6 GKO mice were used in each group and experiments were repeated at least twice.

Flow cytometric enumeration of faecal oocysts

Mouse faecal samples were subjected to a microscale variation of the discontinuous sucrose gradient method as described (Arrowood, Hurd & Mead, 1995). Briefly, faecal pellets were homogenized in adjusted volumes of 2·5% $K_2Cr_2O_7$. The samples were vortexed and allowed to stand for the large debris to settle. Supernatant aliquots were overlaid onto microscale sucrose gradients which were prepared by underlying a 1·103-specific gravity sucrose solution beneath a 1·064-specific gravity sucrose solution in 2·0 ml microfuge tubes. Each tube was centrifuged at 1000 ***g*** for 20 min. The interface between the 2 sucrose solutions was then collected, washed with saline, and suspended in PBS supplemented with 0·1% bovine serum albumin. The partially purified stool concentrate was incubated for 30 min at 37 °C with 5 µl of an oocyst-specific monoclonal antibody conjugated with fluorescein isothiocyanate (OW50-FITC) and then analysed by flow cytometry as previously described (Arrowood *et al*. 1995). Each experimental run included positive and negative controls that were used to calibrate the region setting necessary to discriminate the labelled oocyst population from the background debris. Absolute counts were calculated from the data files for oocysts/100 µl of sample suspension.

Histological studies

Tissue samples were removed from euthanized mice at day 9 p.i. and fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin, sectioned and stained with haematoxylin and eosin. For parasite enumeration, small intestine, colon, gallbladder, and liver tissue sections were examined at a magnification of × 400, and the number of parasites/field was determined. A total of 5 random fields were counted/sample and then averaged.

Preparation of lymphoid cell suspensions

Cell suspensions of mouse spleens and mesenteric lymph nodes (MLNs) were prepared in RPMI-1640 medium (Cellgro™, Mediatech, Herndon, VA) by gently teasing tissues over stainless steel screens. ACK lysing buffer (0·15 M NH_4Cl, 1·0 M $KHCO_3$, 0·01 M NaEDTA, pH 7·2) was used to remove the red blood cells from the cell suspensions. Cell suspensions were washed 3 times in RPMI-1640 medium and resuspended in the same medium containing 10% fetal bovine sera (FBS) to appropriate concentrations.

Lymphocyte proliferative response

Lymphocyte proliferation was determined by standard [^{3}H]thymidine incorporation. *C. parvum* antigen was prepared by washing purified oocysts 3 times with PBS and then freezing and thawing the oocysts 5 times at −70 °C. The oocyst preparation was sonicated (Sonicator W-385, Heat Systems-Ultrasonics Inc., Farmingdale, NY) at 3 bursts of 30 sec each (50% duty cycle at output 5) in an ice bath. The sonicate was centrifuged at 17100 g for 10 min and protein concentration in the supernatant was determined using the BCA protein assay kit (Pierce, Rockford, IL). After euthanizing animals at day 9 p.i., lymphocytes from spleen and mesenteric lymph nodes (2×10^5 cells/well) were cultured with or without parasite antigen (10–100 µg/ml) in 96-well tissue culture plates in 0·2 ml of RPMI-1640 medium containing 5% FBS, 50 µM 2-mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin. Control cultures consisted of lymphocytes from uninfected knockout mice. After 4 days the cultures were pulsed with 1 µCi/well [^{3}H]thymidine (Moravek Biochemicals Inc., Brea, CA) and incubated for 18 h). The cultures were harvested using Filtermate 196 harvester (Packard, Meriden, CT) and counted with Matrix™ 9600 direct beta counter (Packard, Meriden, CT). The results were expressed as the mean counts per min (cpm) ± standard error of

https://doi.org/10.1017/S0031182098003424 Published online by Cambridge University Press

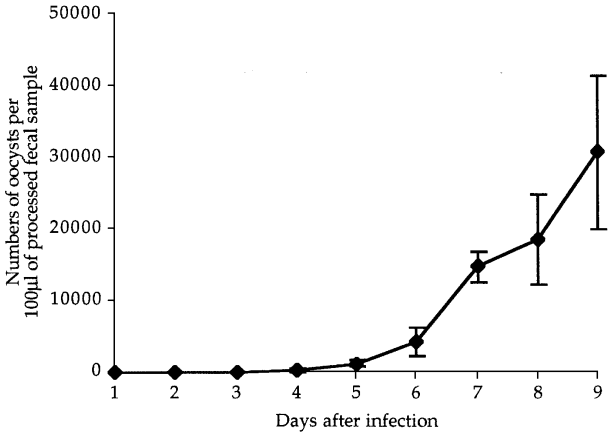

Fig. 1. Pattern of oocyst shedding in GKO mice infected with *Cryptosporidium parvum*. Each point represents the mean number of oocysts ± S.E. determined by flow-cytometric enumeration ($n = 9$).

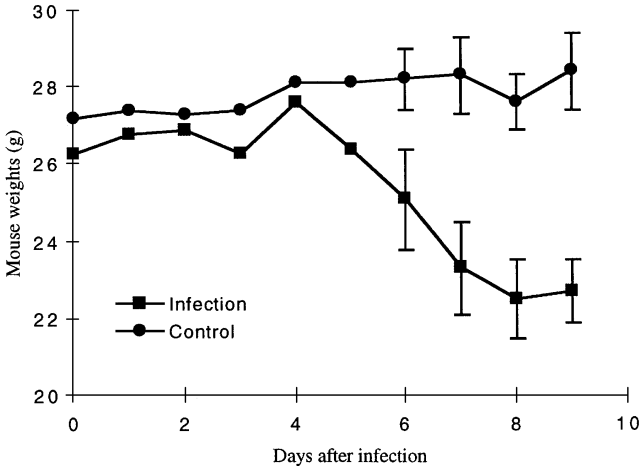

Fig. 2. Weight changes in GKO mice infected with 1×10^5 oocysts/mouse. Each group had 6 mice and the data are presented as the means ± S.D.

Table 1. Weight (mg) of spleens and mesenteric lymph nodes (MLNs) in GKO mice with/without *Cryptosporidium parvum* infection (9 days post-infection)

(Each group contains 9 animals and the data are given as mean ± S.D.)

	Spleen	MLN	MNL/spleen ratio
Infected GKO	48·1 ± 12·7	43·0 ± 16·8	0·90
Uninfected GKO	30·6 ± 6·0	8·3 ± 0·2	0·28

the mean for triplicate cultures. Mitogen phytohemagglutinin or PHA (Sigma, St Louis, MO) was used as positive control. Recombinant mouse IFN-γ (Genzyme, Cambridge, MA) was used *in vitro* to determine the role of exterior IFN-γ on the proliferation of GKO lymphocytes.

Lymphocyte population analysis

Monoclonal antibodies (mAbs) to mouse leukocyte surface markers were used with single and dual-fluorescence flow cytometry to evaluate lymphocyte populations. MAbs to mouse CD45R/B200 (RA3-6B2, IgG_{2a}), CD3 (145-2C11), CD4 (RM4-5, IgG_{2a}), CD8 (53-6·7, IgG_{2a}), α/β TCR (H57-597), γ/δ TCR (GL3) and NK cells (2B4, 129/J IgG_{2b}) were obtained from Pharmingen (San Diego, CA). Cell suspensions (10^6 cells/100 µl) were stained with 1–2 µl of mAbs at 37 °C for 30 min and then diluted 1:10 with PBS containing 0·5 % paraformaldehyde before analysis by FACScan (Becton Dickinson, Mountain View, CA).

Statistical analysis

Data were analysed by Student's *t*-test.

RESULTS

The *C. parvum* oocyst shedding pattern in IFN-γ knockout mice is shown in Fig. 1. The infection intensity in GKO mice inoculated with 1×10^5 oocysts/mouse increased markedly on day 4 after infection and continued to increase significantly over the next 6–7 days. The average parasite shedding was 332 oocysts/100 µl of faecal sample on day 4 after infection and 36081 ± 9590 (S.E.) oocysts/100 µl of faecal sample on day 9 after infection. In 1 experiment, parasite shedding in some mice reached as high as 70000 oocysts/100 µl of a faecal sample. Most of the GKO mice (75 %) died by day 10 after infection. There was little difference in survival of mice inoculated with different doses. The mean parasite shedding was 27934 ± 3881 (S.E.) and 16575 ± 6148 (S.E.) oocysts/100 µl of faecal sample on day 9 after infection, when 1×10^4 and 1×10^3 oocysts/mouse were administrated, respectively. No significant difference in oocyst shedding on day 9 after infection was observed among 3 groups ($P > 0·05$). Five out of 6 (82 %) GKO mice infected with 1×10^3 oocysts/mouse died on day 12 after infection and comparable results were observed when the experiment was repeated. Significant weight loss was evident upon examination of infected group mean weights on day 6 after infection compared with uninfected GKO mice ($P < 0·05$). Weights declined while parasite shedding increased in infected GKO mice. Mice in the infected group lost an average of 3–4 g before death or euthanization (Fig. 2). Very soft and gelatinous stools were observed beginning day 5 after infection. The average weights of solid faecal pellets decreased slightly from 3·09 to 2·76 g/mouse/day by the first 4 days after infection and markedly decreased from 0·82 to 0·46 g/mouse/day in the last 5 days of infection. At post-mortem examination, gross lesions were present in the ileum,

https://doi.org/10.1017/S0031182098003424 Published online by Cambridge University Press

Table 2. Percentage lymphocyte population of spleens and mesenteric lymph nodes (MLNs) in GKO mice with/without *Cryptosporidium parvum* infection (9 days post-infection)

(The pooled lymphocytes were from 9 GKO mice in the *C. parvum*-infected group and 8 GKO mice in the uninfected group. The numbers represent percentage of cells staining for various lymphocyte markers. CD45R/B200, B cell marker; CD3, T cell marker; CD4, T helper cell marker; CD8, T suppressor/cytotoxic cell marker.)

	Spleen cells		MNL cells	
	Infected	Uninfected	Infected	Uninfected
CD45	37·78	52·48	46·89	12·89
CD3	58·74	39·63	51·56	84·55
CD4	36·86	27·37	26·43	58·69
CD8	22·02	15·83	21·62	24·34
$\alpha\beta$-TCR	53·10	38·25	55·61	73·24
$\gamma\delta$-TCR	1·16	1·12	0·89	1·00
NK cells	8·55	2·16	16·53	3·34

Table 3. Lymphocyte proliferation of spleen cells in GKO mice with/without *Cryptosporidium parvum* infection (9 days post-infection)

(Samples were pooled spleen cells of 6 mice/group given as cpm $\pm$ S.D.)

	Group-1*		Group-2*	
	Infected	Uninfected	Infected	Uninfected
Ag 100 μg/ml†	199 $\pm$ 45	118 $\pm$ 20	94 $\pm$ 41	97 $\pm$ 63
Ag 10 μg/ml†	126 $\pm$ 19	87 $\pm$ 24	97 $\pm$ 44	96 $\pm$ 40
PHA 5 μg/ml	2576 $\pm$ 345	1253 $\pm$ 150	4584 $\pm$ 216	5907 $\pm$ 716
Medium‡	121 $\pm$ 24	126 $\pm$ 26	100 $\pm$ 26	82 $\pm$ 18

* In group-2 spleen cells were treated with 400 U/ml recombinant mouse IFN-γ, while group-1 was without treatment.
† Frozen-thawed *C. parvum* oocyst antigens were used for proliferation study.
‡ Culture medium was used as a negative control.

jejunum, caecum, and colon of infected GKO mice. The small intestines were markedly dilated and filled with gelatinous fluid contents. Enlarged gallbladders were observed in almost half of the infected GKO mice. The other organs showed no overt gross lesions. Histological studies revealed that parasites were widely distributed in the gastrointestinal tract, with heavy (intense) colonization of the mid-small intestine, terminal ileum, and caecum. Parasites were observed on the villi and in the crypts of small intestines with 247 $\pm$ 76 (S.D.) organisms/high power field ($\times$400). Villi were oedematous and infiltrated with inflammatory cells and especially plasma cells and lymphocytes. Parasite colonization was observed in the epithelium of gallbladders and hepatobiliary ducts of livers in less than one third of the infected GKO mice. The MLNs were dramatically enlarged (comparable to spleens) and the mean weights increased from 8·3 mg in uninfected GKO mice to 43·0 mg in infected GKO mice ($P < 0{\cdot}05$). The mean weights of spleens and MLNs in both infected and uninfected GKO mice are summarized in Table 1. No significant difference in spleen weights was observed between infected and uninfected GKO mice. The MLN/spleen weight ratio increased from 0·28 in the uninfected group to 0·91 in infected mice ($P < 0{\cdot}05$). The enlarged MLNs were characterized by a predominance of lymphocytes and phagocytes. The pulps were hyperaemic and the lymphoid sinus contained enormous numbers of lymphocytes and phagocytes. The normal architecture of nodes was preserved.

Immunophenotypic analyses of lymphocyte populations in spleens and MLNs are shown in Table 2. The ratio of lymphocyte populations in uninfected GKO mice was similar to those seen in other strains of mice. The T cell population ratio increased in spleens and decreased in MLNs in GKO mice infected with *C. parvum*. In contrast, the B cell population ratio decreased in spleens but increased in MLNs in *C. parvum*-infected GKO mice. In spleens, the increased T cell population comprised

https://doi.org/10.1017/S0031182098003424 Published online by Cambridge University Press

Table 4. Lymphocyte proliferation of MLN cells in GKO mice with/without *Cryptosporidium parvum* infection (9 days post-infection)

(Samples were pooled spleen cells of 6 mice/group given as cpm ± S.D.)

	Group-1*		Group-2*	
	Infected	Uninfected	Infected	Uninfected
Ag 100 μg/ml†	73 ± 34	46 ± 15	61 ± 17	45 ± 13
Ag 10 μg/ml†	59 ± 15	45 ± 4	50 ± 35	37 ± 18
PHA 5 μg/ml	14626 ± 500	6988 ± 236	18099 ± 1298	5828 ± 254
Medium‡	89 ± 17	58 ± 30	63 ± 33	50 ± 10

* In group-2 spleen cells were treated with 400 U/ml recombinant mouse IFN-γ, while group-1 was without treatment.
† Frozen-thawed *C. parvum* oocyst antigens were used for proliferation study.
‡ Culture medium was used as a negative control.

both T helper cells and T suppressor/cytotoxic cells containing α/β TCR markers. In MLNs, the ratio of $CD4^+$ to $CD8^+$ T cells decreased, while this ratio in the spleen remained constant. A 4–5 fold increase in NK cell percentages was observed in both spleens and MLNs in GKO mice infected with *C. parvum*.

No significant proliferation reaction was observed (Tables 3 and 4) when pooled lymphocytes from spleens and MLNs of infected and uninfected GKO mice were exposed to *C. parvum* antigens *in vitro* ($P > 0{\cdot}05$). Recombinant mouse IFN-γ did not influence the proliferation of lymphocytes in these groups. The mitogen PHA caused proliferation of GKO lymphocytes from both spleens and MLNs *in vitro* and this proliferation was modestly enhanced by supplementary recombinant mouse IFN-γ ($P < 0{\cdot}01$).

DISCUSSION

IFN-γ plays a very important role in the biology of monocyte/macrophage lineage of immune effector cells, which are considered the first line of host defence against infectious agents (Young & Hardy, 1995). IFN-γ induces the production of nitric oxide (NO) in macrophages and NO is thought to be a critical mechanism for macrophage cytotoxicity against a wide variety of infectious agents and tumour cells (Young & Hardy, 1995). The major sources of IFN-γ are T cells and NK cells. A predominance of Th1 cells (which produce IFN-γ and IL-2) in response to parasite antigens results in delayed hypersensitivity and activation of macrophages to kill intracellular parasites. Experimental cryptosporidiosis was accompanied by increased IFN-γ levels in spleen cells of infected BALB/c mice (Harp & Sacco, 1996) and in ileal mucosal lymphocytes of infected calves (Wyatt *et al*. 1997). $CD4^+$ cells were responsible for the *in vitro* production of IFN-γ when stimulated by *C. parvum* antigens (Harp *et al*. 1994). Similar IFN-γ production was observed in an *in vivo* model of *C. muris* infection. In the latter study, intraepithelial lymphocytes (IEL) were thought to be important in the immune response to *Cryptosporidium* through production of IFN-γ at the site of infection (Culshaw, Bancroft & McDonald, 1997). The importance of IFN-γ in resistance to *C. parvum* has also been reinforced by the observations that treatment of both immunocompetent (Ungar *et al*. 1991) and immunocompromised (Chen *et al*. 1993*a*, *b*; McDonald *et al*. 1992) mice with antibody against IFN-γ increased the severity of *C. parvum* infection. Additionally, treatment with recombinant IFN-γ reduced the severity of infection by *C. parvum* in an immunosuppressed rat model (Rehg, 1996). More recently, GKO mice were shown to be highly susceptible to *C. parvum* infection and demonstrated lymphocyte proliferation when exposed to *C. parvum* antigens (Theodos *et al*. 1997).

In general, GKO mice develop normally with no gross defects observed in the levels or phenotypes of the lymphoid populations. Although the generation of cytotoxic effector cells is not impaired, splenic NK activity is significantly suppressed in the GKO mice and splenocytes exhibit uncontrolled proliferation in response to mitogens and alloantigens. Moreover, reduced MHC II antigen expression and reduced production of macrophage antimicrobial products such as nitric oxide have been observed in GKO mice (Dalton *et al*. 1993). Consequently, GKO mice are typically quite susceptible to various infectious agents.

Our study demonstrated adult IFN-γ knockout mice are readily infected with *C. parvum*, confirming the observation by Theodos *et al*. (1997). Infections were rapidly established with heavy oocyst shedding by 4 days after inoculation when a dose of 1×10^5 oocysts/mouse was used. Infections persisted only 9–10 days before animals died from overwhelming parasitosis. Similar infections were achieved with relatively low inocula (1×10^3 oocysts/mouse), indicating IFN-γ knockout mice are very susceptible to *C. parvum* infection. Pathogenesis correlated to these overwhelming infections in the gastrointestinal tract

https://doi.org/10.1017/S0031182098003424 Published online by Cambridge University Press

as evidenced by intense parasite loads in the faeces and epithelial cell surfaces of intestinal tracts. Although parasites were found in the gallbladders in some of GKO mice infected with *C. parvum*, hepatobiliary infections were not as severe compared to infected SCID (severe combined immunodeficiency) mice probably due to insufficient time for intense colonization in these tissues. The appearance of diarrhoea-like signs (semi-solid faeces) was directly correlated with elevated faecal oocyst levels and weight loss in infected GKO mice. The exact mechanism underlying diarrhoea-like findings was unclear, but was not the result of indirect effects through secondary message systems such as cAMP, since we found no significant differences in intestinal epithelial cell cAMP levels between infected and uninfected mice (data not shown). These observations support the crucial role of IFN-γ in defence against *C. parvum* infection.

Additionally, differences in infection characteristics were noted between the present study and that of Theodos *et al*. (1997). In the previous study, after administration of the GCH1 isolate of *C. parvum* at a dose of 10^7 oocysts/mouse, parasite shedding peaked on day 8–11 post-infection and the animals generally succumbed within 2–3 weeks of infection. The extended course of infection provided sufficient time for animals to develop immune responses such as proliferation to cryptosporidial antigens. In contrast, we observed the *C. parvum* IOWA isolate, when used at smaller inoculation doses (10^5 or even 10^3 oocysts/mouse), caused overwhelming infections and resulted in a shorter infection course, suggesting there are biological differences between IOWA and GCH1 isolates.

It is believed that acquisition of resistance to *C. parvum* is generally a Th1 function of $CD4^+$ T cells which produce IFN-γ (Davami, Bancroft & McDonald 1997). Natural killer (NK) cells and other lymphocytes such as $CD8^+$ α/β T cells and double-negative γ/δ T cells play an important role in localized immunity to *C. parvum* (Aguirre, Mason & Perryman, 1994; McDonald *et al*. 1996; Waters & Harp, 1996, Abrahamsen *et al*. 1997). In our studies, *C. parvum* infections in GKO mice produced prominent inflammatory responses in the gut. In the early stages of host response to infection, proliferation and differentiation of lymphocytes in MLNs may lead to enlargement of MLNs. Since absolute numbers of cells in MLNs were not determined, it was not possible to demonstrate increases in specific cell subsets. The unusually enlarged MLNs may also be related to some unknown mechanisms that compensated for the defective capability of IFN-γ production. Both $CD4^+$ and $CD8^+$ T cells with α/β and γ/δ TCR have been found to increase substantially within the intestinal tissues of calves infected with *C. parvum* (Waters *et al*. 1996; Pasquali *et al*. 1997), while other studies showed a significant increase in $CD8^+$ T cells (Huang *et al*. 1996; Wyatt *et al*. 1997). Our results also demonstrated an increased percentage of T cells (including $CD4^+$ and $CD8^+$ T cells) in the spleen and that the cells were predominantly α/β T cells. Similar results were reported in calves (Harp *et al*. 1995). However, the T cell population in the MLNs decreased, while the percentage of B cell population increased in the MLNs. The mechanism involving increases in the ratio of B cells in the MLNs is not clear, but may be related to some localized immunity such as sIgA production. The failure to demonstrate significant lymphocyte proliferation to *C. parvum* antigens *in vitro* may be explained by short infection duration, and inhibitory activities present in the whole oocyst antigen preparation (data not shown) may also be factors.

The experimental *C. parvum*-infected GKO mouse model provides a new and useful tool to study the biology and immunology of cryptosporidiosis. This model can be used to study the characteristics of acute infection and the immune response against *C. parvum* infection *in vivo*. Interestingly, *C. parvum* infections in the GKO model may vary as different strains (isolates) of *C. parvum* or GKO mice are used. Although the cost of GKO mice is relatively high, this model may be used as an alternative *C. parvum* oocyst source when an appropriate animal facility is not available for production of *C. parvum* in calves. Other applications of the *C. parvum*-infected GKO mouse model are under investigation.

We thank K. Deanna Garmon, Kelly B. Thrasher, Amy Juodawlkis and Elizabeth Lakin for excellent technical assistance. This work was supported by grants from NIH (A1-36680) and George VA Research Center on AIDS and HIV Infections.

REFERENCES

ABRAHAMSEN, M. S., LANCTO, C. A., WALCHECK, B., LAYTON, W. & JUTILA, M. A. (1997). Localization of alpha/beta and gamma/delta T lymphocytes in *Cryptosporidium parvum*-infected tissues in naive and immune calves. *Infection and Immunity* **65**, 2428–2433.

ARROWOOD, M. J. & DONALDSON, K. (1996). Improved purification methods for calf-derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. *Journal of Eukaryotic Microbiology* **43**, 89S.

ARROWOOD, M. J., HURD, M. R. & MEAD, J. R. (1995). A new method for evaluating experimental cryptosporidial parasite loads using immunofluorescent flow cytometry. *Journal of Parasitology* **81**, 404–409.

AGUIRRE, S. A., MASON, P. H. & PERRYMAN, L. E. (1994). Susceptibility of major histocompatibility complex (MHC) class I- and MHC class II-deficient mice to *Cryptosporidium parvum* infection. *Infection and Immunity* **62**, 697–699.

CHEN, W., HARP, J. A. & HARMSEN, A. G. (1993*a*). Requirements for CD4 cells and gamma interferon in

resolution of established *Cryptosporidium parvum* infection in mice. *Infection and Immunity* **61**, 3928–3932.

CHEN, W., HARP, J. A., HARMSEN, A. G. & HAVELL, E. A. (1993*b*). Gamma interferon functions in resistance to *Cryptosporidium parvum* infection in severe combined immunodeficient mice. *Infection and Immunity* **61**, 3548–3551.

COOPER, A. M., DALTON, D. K., STEWART, T. A., GRIFFIN, J. P., RUSSELL, D. G. & ORME, I. M. (1993). Disseminated tuberculosis in interferon γ-disrupted mice. *Journal of Experimental Medicine* **178**, 2243–2247.

CULSHAW, R. J., BANCROFT, G. J. & McDONALD, V. (1997). Gut intraepithelial lymphocytes induce immunity against *Cryptosporidium* infection through a mechanism involving gamma interferon production. *Infection and Immunity* **65**, 3074–3079.

DALTON, D. K., PITTS-MEEK, S., KESHAV, S., FIGARI, I. S., BRADLEY, A. & STEWART, T. A. (1993). Multiple defects of immune cell function in mice with disrupted interferon-g genes. *Science* **259**, 1739–1742.

DAVAMI, M. H., BANCROFT, G. J. & McDONALD, V. (1997). *Cryptosporidium* infection in major histocompatibility complex congeneic strains of mice: variation in susceptibility and the role of T-cell cytokine response. *Parasitology Research* **83**, 257–263.

ELKINS, K. L., RHINEHART-JONES, T. R., CULKIN, S. J., YEE, D. & WINEGAR, R. K. (1996). Minimal requirements for murine resistance to infection with *Francisella tularensis* LVS. *Infection and Immunity* **64**, 3288–3293.

FLYNN, J. L., CHAN, J., TRIEBOLD, K. J., DALTON, D. K., STEWART, T. A. & BLOOM, B. R. (1993). An essential role for interferon-γ in resistance to *Mycobacterium tuberculosis* infection. *Journal of Experimental Medicine* **178**, 2249–2254.

HARP, J. A. & SACCO, R. E. (1996). Development of cellular immune functions in neonatal to weanling mice: relationship to *Cryptosporidium parvum* infection. *Journal of Parasitology* **82**, 245–249.

HARP, J. A., WHITMIRE, W. M. & SACCO, R. (1994). *In vitro* proliferation and production of gamma interferon by murine CD4 cells in response to *Cryptosporidium parvum* antigen. *Journal of Parasitology* **80**, 67–92.

HUANG, D. S., LOPEZ, M. C., WANG, J. Y., MARTINEZ, F. & WATSON, R. R. (1996). Alterations of the mucosal immune system due to *Cryptosporidium parvum* infection in normal mice. *Cellular Immunology* **173**, 176–182.

McDONALD, V., DEER, R., UNI, S., ISEKI, M. & BANCROFT, G. J. (1992). Immune responses to *Cryptosporidium muris* and *Cryptosporidium parvum* in adult immunocompetent or immunocompromised (nude and SCID) mice. *Infection and Immunity* **60**, 3325–3331.

McDONALD, V., ROBINSON, H. A., KELLY, J. P. & BANCROFT, G. J. (1996). Immunity to *Cryptosporidium muris* infection in mice is expressed through gut $CD4^+$ intraepithelial lymphocytes. *Infection and Immunity* **64**, 2556–2562.

PASQUALI, P., FAYER, R., ALMERIA, S., TROUT, J., POLIDORI, G. A. & GASBARRE, L. C. (1997). Lymphocyte dynamic patterns in cattle during a primary infection with *Cryptosporidium parvum*. *Journal of Parasitology* **83**, 247–250.

REHG, J. E. (1996). Effects of interferon-g in experimental *Cryptosporidium parvum* infection. *Journal of Infectious Diseases* **174**, 229–232.

THEODOS, C., SULLIVAN, K. L., GRIFFITHS, J. K. & TZIPORI, S. (1997). Profiles of healing and nonhealing *Cryptosporidium parvum* infection in C57BL/6 mice with functional B and T lymphocytes: the extent of gamma interferon modulation determines the outcome of infection. *Infection and Immunity* **65**, 4761–4769.

TILLEY, M., McDONALD, V. & BANCROFT, G. J. (1995). Resolution of cryptosporidial infection in mice correlates with parasite-specific lymphocyte proliferation associated with Th1 and Th2 cytokine secretion. *Parasite Immunology* **17**, 459–464.

TZIPORI, S., RAND, W. & THEODOS, C. (1995). Evaluation of a two-phase scid mouse model preconditioned with anti-interferon-g monoclonal antibody for drug testing against *Cryptosporidium parvum*. *Journal of Infectious Diseases* **172**, 1160–1164.

UNGAR, B. L. P., KAO, T. C., BURRIS, J. A. & FINKELMAN, F. D. (1991). *Cryptosporidium* infection in an adult mouse model. Independent roles for IFN- and CD4 T lymphocytes in protective immunity. *Journal of Immunology* **147**, 1022–1024.

WANG, Z. E., REINER, S. L., ZHENG, S., DALTON, D. K. & LOCKSLEY, R. M. (1994). CD4 effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with *Leishmania major*. *Journal of Experimental Medicine* **179**, 1367–1371.

WATERS, W. R. & HARP, J. A. (1996). *Cryptosporidium parvum* infection in T-cell receptor (TCR)-α- and TCR-δ-deficient mice. *Infection and Immunity* **64**, 1854–1857.

WYATT, C. R., BRACKETT, E. J., PERRYMAN, L. E., RICE-FICHT, A. C., BROWN, W. C. & O'ROURKE, K. I. (1997). Activation of intestinal intraepithelial T lymphocytes in calves infected with *Cryptosporidium parvum*. *Infection and Immunity* **65**, 185–190.

YOUNG, H. A. & HARDY, K. J. (1995). Role of interferon-g in immune cell regulation. *Journal of Leukocyte Biology* **58**, 373–381.