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Comparison of Antibody and Cytokine Responses to Primary *Giardia muris* Infection in *H-2* Congenic Strains of Mice

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The course of primary infections with *Giardia muris* differs between BALB and B10 *H-2* congenic strains of mice. In the first 3 weeks of infection, there is a more rapid decline in intestinal trophozoite and fecal cyst counts in B10 strains than in BALB strains. To determine whether this difference could be explained by variation in specific antibody responses, both secretory immunoglobulin A (IgA) and serum antibody responses were compared between these strains. No significant differences in the timing, titer, or specificity of secretory or serum antibodies were found. However, on comparing specific anti-*G. muris* serum IgG subclass responses, we found that B10 strains produced IgG2a while BALB strains produced IgG1, suggesting differential involvement of T helper 1 and 2 subsets of lymphocytes. When cells harvested from mesenteric lymph nodes were stimulated with concanavalin A in vitro, both gamma interferon and interleukin-5 were secreted by cells from B10 mice, but only interleukin-5 was secreted by cells from BALB/c mice. Specific blockade of gamma interferon by monoclonal antibody administered to B10 mice resulted in an enhanced intensity of infection.

Antibody appears to play a critical role in the clearance of *Giardia* infection. In mice (1, 41), rats (35), and humans (31), infection stimulates anti-*Giardia* immunoglobulin G (IgG), IgM, and IgA serum antibodies. *Giardia*-specific secretory antibody, principally IgA, is also produced (28). In humans, infection is frequently implicated as a cause of diarrhea in subjects with hypogammaglobulinemia (20) and in AIDS patients with impaired antibody responses (21). Mice depleted of B lymphocytes and rendered deficient of IgM, IgG, and IgA experience chronic *Giardia muris* infection (39), as do B-cell-deficient mice expressing the *xid* gene (40). Passively transferred anti-*G. muris* IgM monoclonal antibody improved the clearance of trophozoites in CD-1 Swiss mice (3), but this was not confirmed in Swiss albino mice (48).

Secretory IgA (sIgA) is thought to play the major role in defense against infection. *Giardia* trophozoites reside totally within the intestinal lumen, adhering to the epithelial surface, where sIgA is the principal antibody isotype. There is a considerable reduction in the prevalence of giardiasis among infants fed breast milk containing high titers of anti-*Giardia* sIgA (29). In mice, surface binding of sIgA to trophozoites has been demonstrated (14), and there is a good temporal correlation between the appearance of specific sIgA in the intestinal lumen and the clearance of infection. How sIgA might act is unclear, but it may opsonize trophozoites for phagocytosis (22) or inhibit their attachment to the intestinal epithelium (19). Trophozoites do not normally invade the intestinal mucosa, and therefore immune clearance must operate at the epithelial surface.

Clearance of *G. muris* infections is also T-cell dependent. Prolonged primary infections occur in nude mice (42), and resistance can be conferred by adoptive transfer of lymphocytes (32, 38, 48). Heyworth et al. (15) have shown that $CD4^+$ T helper cells, but not $CD8^+$ T cytotoxic cells, are required for clearance of infection.

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We have previously reported a consistent and significant difference in the course of primary *G. muris* infection between BALB and B10 *H-2* congenic strains of mice (46). At 2 and 3 weeks postinfection, fecal cyst excretion in B10 strains is 10-fold less than in BALB strains. On a BALB genetic background, there are significant differences in fecal cyst excretion between strains of different *H-2* haplotype. Differences in fecal cyst excretion are paralleled by intestinal trophozoite counts. BALB and B10 *H-2* congenic strains therefore provide an opportunity to investigate the role of antibody and lymphocyte responses in immunity to *G. muris.*

MATERIALS AND METHODS

Animals. Six- to eight-week-old female, specific-pathogen-free mice were purchased from Harlan Olac Ltd., Bicester, United Kingdom. The strains used were BALB/c, BALB/B, BALB/K, C57BL10(B10)/ScSn (B10), B10.BR, B10.D2n, and $(BALB/c \times B10)F_1$.

G. muris. Cysts were obtained from G. Faubert, McGill University, Montreal, Quebec, Canada. They were stored at 4°C in phosphate-buffered saline (PBS; pH 7.2) and continually passaged through BALB/c mice. Mice were infected orally with 10^3 cysts in 0.2 ml of Hanks balanced salt solution.

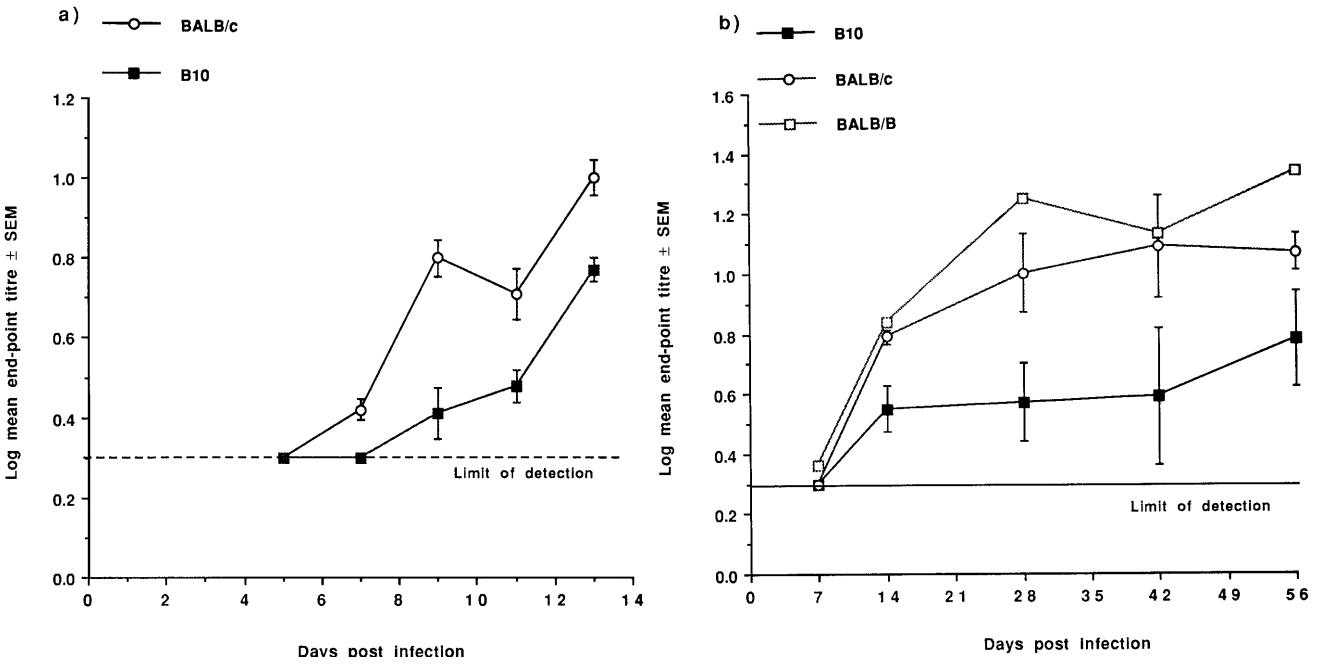
The course of infection was monitored by quantifying cysts in 2-h fecal output as described by Roberts-Thomson et al. (33). Cysts were counted in a hemocytometer, and the count was expressed as cysts per 2-h fecal collection per mouse. The limit of detection by this method is 250 cysts. Trophozoites were collected as previously described (46).

Sample collection (i) Intestinal secretory antibody. The small intestine was removed and ligated, and 3 ml of PBS containing a cocktail of proteolytic enzyme inhibitors was injected as described by Snider et al. (39). After massage for 1 min, the intestinal fluid was collected, filtered, and centrifuged, and the supernatant was stored at −30°C.

(ii) Serum antibody. Mice were bled at the time of sacrifice, and serum collected was stored at −30°C.

Trophozoite antigen preparation. Washed trophozoites were disrupted by osmotic shock in distilled water, freeze-thawed, and centrifuged, and the supernatant was stored at −80°C. Protein concentration was estimated by the Lowry method (25). The average yield of protein was 40 μg/10^6 trophozoites.

ELISA. Enzyme-linked immunosorbent assay (ELISA) was carried out by using standard techniques. Briefly, each well of 96-well plastic microtiter plates (Falcon, Oxford, United Kingdom) was coated with 50 μl of trophozoite antigen in carbonate-bicarbonate buffer, and the plates were incubated at 4°C overnight and washed with PBS-Tween. Gut washings or sera were diluted in PBS-Tween and tested in triplicate or duplicate, respectively. Each plate was controlled by using samples from uninfected mice of the same age and batch as experimental mice. After incubation (24 h, room temperature) and washing, the second antibodies, alkaline phosphatase conjugates against IgG, IgG1, IgG2a, IgA, and IgM



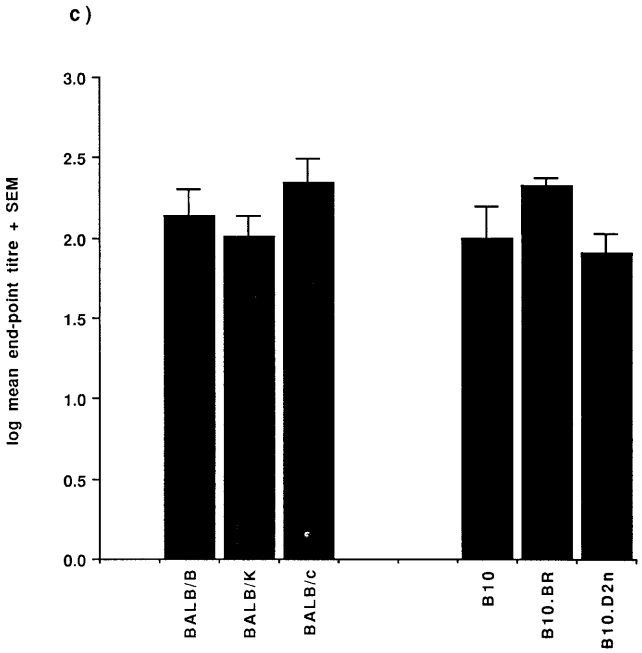


FIG. 1. sIgA response. Titers of anti-*G. muris* sIgA in gut washings, determined by ELISA, are expressed as log mean endpoint titers ± SEM. Titrations commenced at a dilution of 1:2, and therefore the lower limit of sensitivity, as depicted by the horizontal line, is equivalent to log 2 (=0.3). (a) Comparison of BALB/c and B10 strains, five mice per time point (day 7, $P = 0.004$; day 9, $P = 0.008$; day 11, $P = 0.075$; day 13, $P = 0.004$); (b) comparison of B10, BALB/c, and BALB/B strains, four mice per time point (no significant differences); (c) comparison of BALB and B10 *H-2* congenic strains, eight mice per time point (no significant differences).

(Binding Site Ltd., Birmingham, United Kingdom), were added at optimized dilutions, usually 1:1,000. After a further 24 h of incubation at room temperature, plates were washed, *p*-nitrophenol phosphate (1 mg/ml in diethanolamine buffer [pH 9.8]; Sigma, Poole, United Kingdom) was added as the substrate, and plates were then read at 490 nm.

Endpoint titers were calculated by interpolation onto an endpoint titration curve, constructed from the mean optical density + 2 standard deviations of samples from uninfected mice. Optical densities obtained with standard samples were used to calculate coefficients of variation, within plate and between plate variation ranging from 9 to 14%.

Trophozoite immobilization and agglutination assays. The assays were adapted from Belosevic and Faubert (2). Neat gut washings (50 μl) were mixed with 10^5 trophozoites (in 50 μl) in wells of round-bottom plastic microtiter plates (Dynatech, Billingshurst, United Kingdom). Following incubation at 37°C for 30 min, numbers of motile and nonmotile trophozoites were counted in a hemocytometer, and the number of nonmotile trophozoites was expressed as a percentage of the total. Agglutination was assessed at 30 and 60 min. Gut washings from uninfected mice were used as controls.

Hemagglutination inhibition assay. The assay was adapted from Farthing et al. (10). Washed and trypsinized rabbit erythrocytes were resuspended in PBS–0.5% bovine serum albumin (BSA). Trophozoites in PBS–0.5% BSA–2 mM calcium chloride (10^5 trophozoites in 25 μl) were mixed with 50 μl of a 25% solution of erythrocytes and 25 μl of PBS control or a serial dilution of gut washings. Plates were incubated at room temperature for 1 h and then examined for hemagglutination or its inhibition by gut washings.

SDS-PAGE and Western blotting (immunoblotting). The analyses were carried out by using standard techniques. Polyacrylamide gel electrophoresis (PAGE) was done with 5 to 20% gradient gels. Trophozoite proteins were solubilized by boiling in an equal volume of sodium dodecyl sulfate (SDS) sample buffer and applied in volumes of <30 μl at 10 μg/mm of gel front. Gels were stained in 0.1% Coomassie brilliant blue stain (Sigma).

For Western blotting, the method of Towbin et al. (45) was used. Proteins were electrophoretically transferred from gels to nitrocellulose (NC) in a Bio-Rad blotting chamber at 1 A for 90 min at 10°C, protein transfer being checked by staining with Coomassie blue.

NC strips were blocked with 10% skim milk overnight at room temperature and then washed with PBS. Gut washings or serum were diluted 1:20 in 10% skim milk. NC strips were incubated with 5 ml of diluted sample overnight at 4°C and then washed with PBS. Alkaline phosphatase conjugates against IgA, IgG, or IgM (Binding Site Ltd.) were used, diluted at 1:1,000 in PBS, and a 1:1 molar ratio of 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitroblue tetrazolium chloride in Tris-buffered saline (pH 9.6) was used as the substrate.

Luminal leukocyte counts. Gut washings were purified as described above and centrifuged, and the pellet was resuspended in 1 ml of PBS. One hundred microliters of this suspension was mixed with 2 μl of crystal violet, and stained leukocytes were counted in a hemocytometer.

MLNC. Mesenteric lymph node cells (MLNC) were used for proliferation assays and cytokine production. Cell suspensions were prepared in supplemented, sterile RPMI 1640 medium (Gibco, Paisley, United Kingdom) containing 10% fetal calf serum. Viable cells were counted after staining with fluorescein diacetate, viability being >90%.

MLNC proliferation. One hundred-microliter aliquots of MLNC (5×10^6/ml) were cultured in triplicate in flat-bottom 96-well plates (Dynatech). Proliferation in response to concanavalin A (ConA; 5 μg/ml; Sigma) or *G. muris* antigen (25

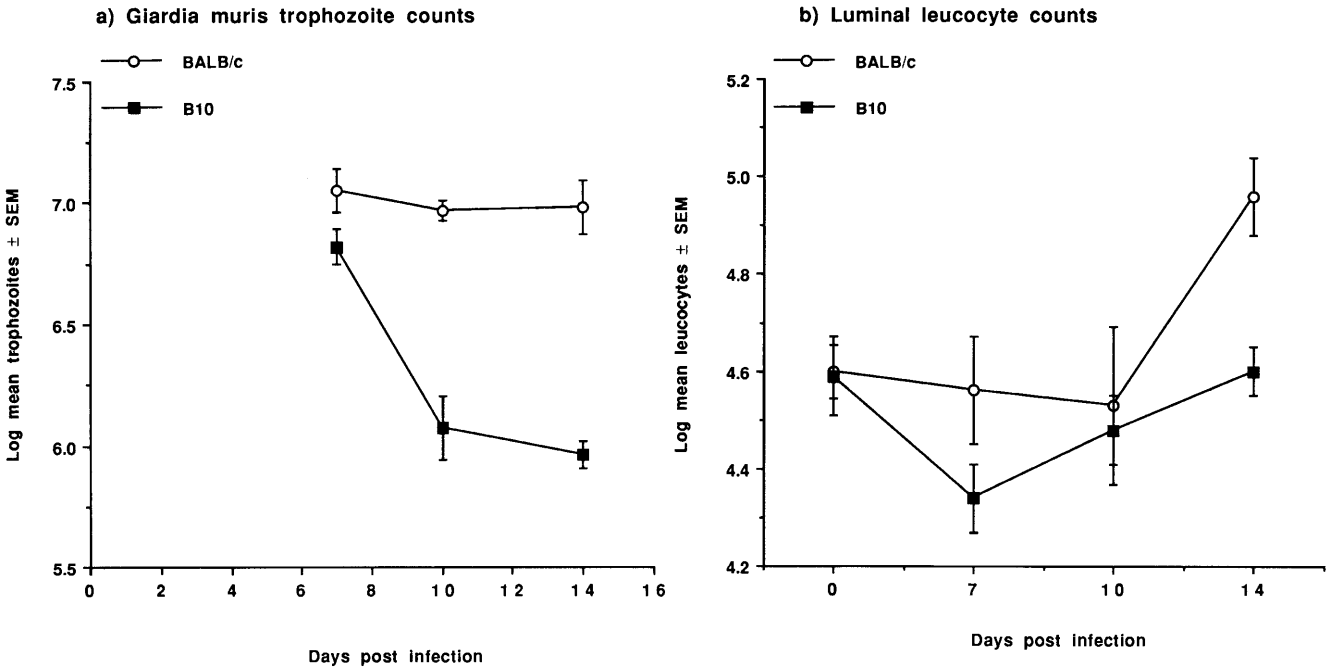


FIG. 2. Relationship between trophozoite and luminal leukocyte counts. Female BALB/c and B10 mice were infected with 10^3 *G. muris* cysts on day 0, and six mice from each strain were sacrificed at each time point. (a) Log mean trophozoite count ± SEM; (b) log mean leukocyte count ± SEM.

to 100 μg/ml) was measured by the incorporation of tritiated thymidine (Radiochemical Centre, Amersham, United Kingdom) diluted to 20 μCi/ml. After 2 h of incubation at 37°C in a 5% CO_2 atmosphere, cells were harvested onto filter paper and placed in vials containing 4 ml of scintillation fluid, and scintillations emitted per minute was measured in an automated chamber.

Cytokine assays. One-milliliter volumes of MLNC (5×10^6/ml) in supplemented RPMI 1640 were culture in 24-well plates (Falcon). Three wells were used for each cell suspension: one as a control, one with 5 μg of ConA, and one with trophozoite antigen. Plates were incubated at 37°C in 5% CO_2 for 48 h, at the end of which culture supernatants were collected.

Gamma interferon (IFN-γ) and interleukin-5 (IL-5), as representative Th1 and Th2 cytokines, were measured by capture ELISAs using the method of Else and Grencis (7). IL-5 was chosen as a marker of Th2 rather than IL-4 because consistent and higher levels are produced in tissue culture (8). The capture antibodies were R46A2 anti-IFN-γ (kindly provided by K. Robinson) and TRFK-5 anti-IL-5 (Pharmingen, San Diego, Calif.). The second antibodies were biotinylated XMG1.2 anti-IFN-γ and biotinylated TRFK-4 anti-IL-5 (Pharminagen).

In vivo blockade of cytokines. The key Th1 and Th2 regulatory cytokines IFN-γ and IL-4 were blocked in vivo by the administration of monoclonal antibodies in BALB/c and B10 mice. IFN-γ was blocked by rat anti-IFN-γ (Lee Biomolecular, San Diego, Calif.) injected intravenously in two doses of 2,500 U in 0.2 ml of saline on days 0 and 7 of infection. IL-4 was blocked by an anti-IL-4 antibody (derived from the 11B.11 hybridoma cell line and kindly donated by C. W. Reynolds, National Cancer Institute, Bethesda, Md.) injected intraperitoneally in two doses of 10 mg in 0.5 ml of saline on days 0 and 7 of infection. Such a dose can turn off a Th2-mediated IgE response although it does not completely eliminate an IgG1 response. As a control, mice were given purified rat IgG (Sigma) by the same route.

Statistical methods. The Mann-Whitney *U* test was used for statistical analysis, $P < 0.05$ being taken as the level of significance. *P* and *U* values were derived from published statistical tables (37).

RESULTS

Timing of specific sIgA production. IgA was the only isotype detected in gut washings by ELISA. sIgA appeared in BALB/c mice on day 7 postinfection but not in B10 mice until day 9 (Fig. 1a). Apart from day 11, the titer of sIgA was significantly greater in BALB/c mice than in B10 mice. In another experiment involving B10, BALB/c, and BALB/B mice sacrificed at weekly intervals, sIgA was detected in BALB/B mice on day 7 postinfection but not in the other two strains until day 14 (Fig. 1b).

Titers of sIgA as measured by ELISA. During the first 8 weeks of infection, the relatively resistant B10 strain had the lowest titers while the relatively susceptible BALB/B strain had the highest titers of sIgA (Fig. 1b). On day 14, the titer of sIgA in B10 mice was significantly lower than in BALB/c mice ($P = 0.014$, $U = 0$), but there were no other significant differences in titers. In another experiment, mice from all six *H-2* congenic strains were sacrificed 96 days after a primary infection. Across all six strains, there was no trend in sIgA titers between susceptible and resistant strains, and there were no statistically significant differences between strains (Fig. 1c).

(BALB/c × B10)F_1 mice clear infection more rapidly than either parental strain such that cysts are not detectable in feces 35 days postinfection (46). Eight F_1 hybrid mice were sacrificed on day 35. Specific anti-*G. muris* sIgA could not be detected in gut washings in five of the eight mice. Low titers (1.07, 5.25, and 5.62) were present in the remaining three.

Functional sIgA titers. To complement data on ELISA titers, functional assays were performed on gut washings obtained from all six *H-2* congenic strains 96 days following a primary infection. No differences were seen in trophozoite immobilization, and no trophozoite agglutination was observed in three experiments on separate occasions. Hemagglutination inhibition was observed with gut washings from all strains but not with naive gut washings. There was variation in the hemagglutination inhibition titer, but only one statistically significant difference emerged between strains, namely, between BALB/c and B10, the latter having a lower titer ($P = 0.041$, $U = 15$).

Antibody specificities. Western blots were performed with gut washings taken from all six *H-2* congenic strains 96 days after primary infection and subsequent to secondary and tertiary infections given 63 and 77 days, respectively, after the primary. Adjuvants were not used to enhance antibody pro-

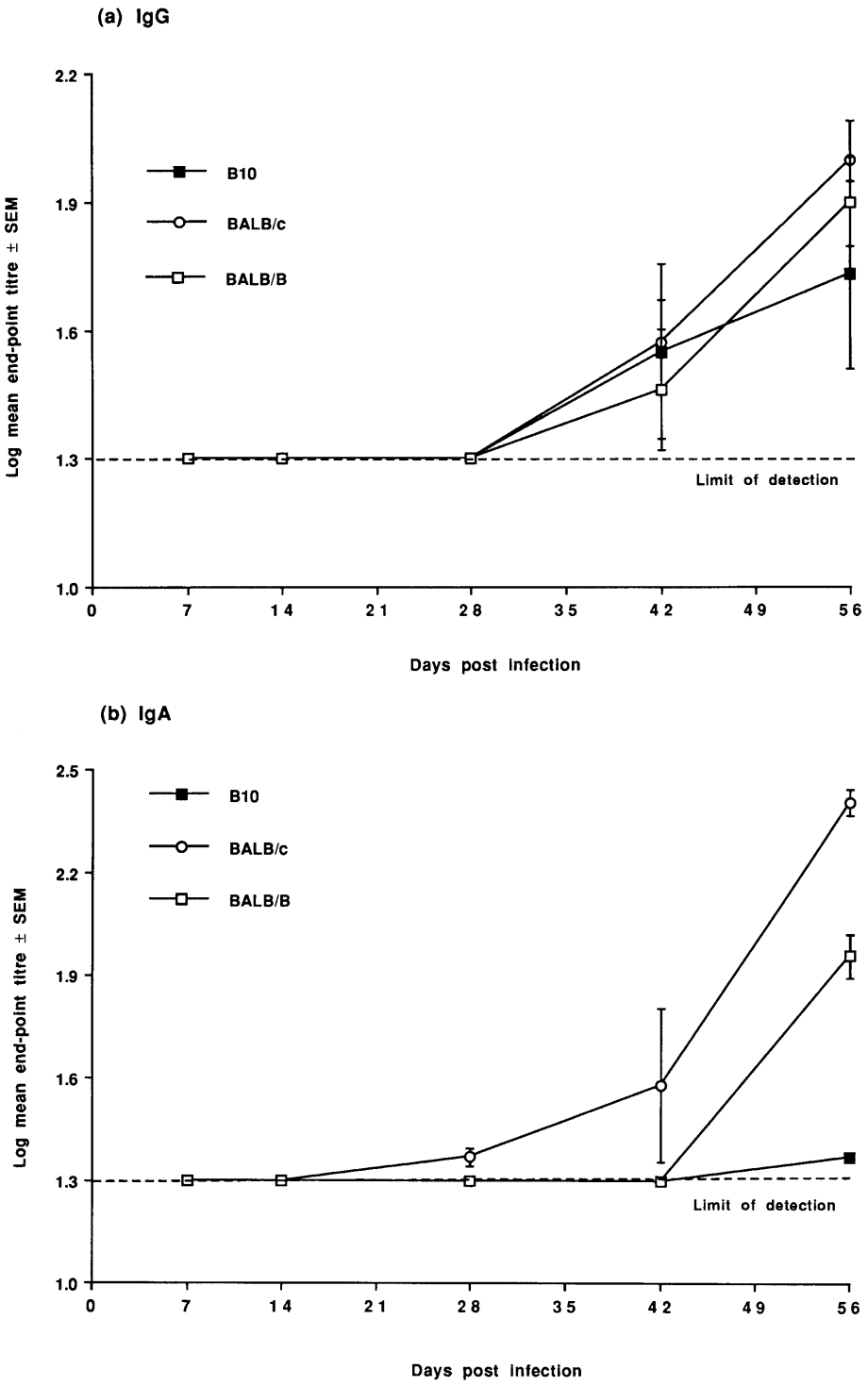


FIG. 3. Serum antibody responses. The time course of serum anti-*G. muris* IgG (a), IgA (b), and IgM (c) responses in BALB/B, BALB/c, and B10 strains. Female mice of each strain were infected with 10^3 *G. muris* cysts on day 0, and four mice were sacrificed at each time point. Titers of anti-*G. muris* antibody, determined by ELISA, are expressed as log mean endpoint titers ± SEM. Titrations commenced at a dilution of 1:20, and therefore the lower limit of sensitivity, as depicted by the horizontal line, is equivalent to log 20 (=1.3).

duction, as others have found that these alter antibody specificities, repeated infections being preferable (44). Gut washings were pooled from eight mice of each strain and used at a dilution of 1:20. Considerable difficulty was encountered in optimizing Western blots because of nonspecific background staining. Eventually results were obtained by blocking NC strips with 10% skim milk. Faint bands appeared within 30 min of incubation with substrate. All strains recognized a doublet at 30 and 33 kDa. No other bands were recognized. This doublet was not detected by naive gut washings.

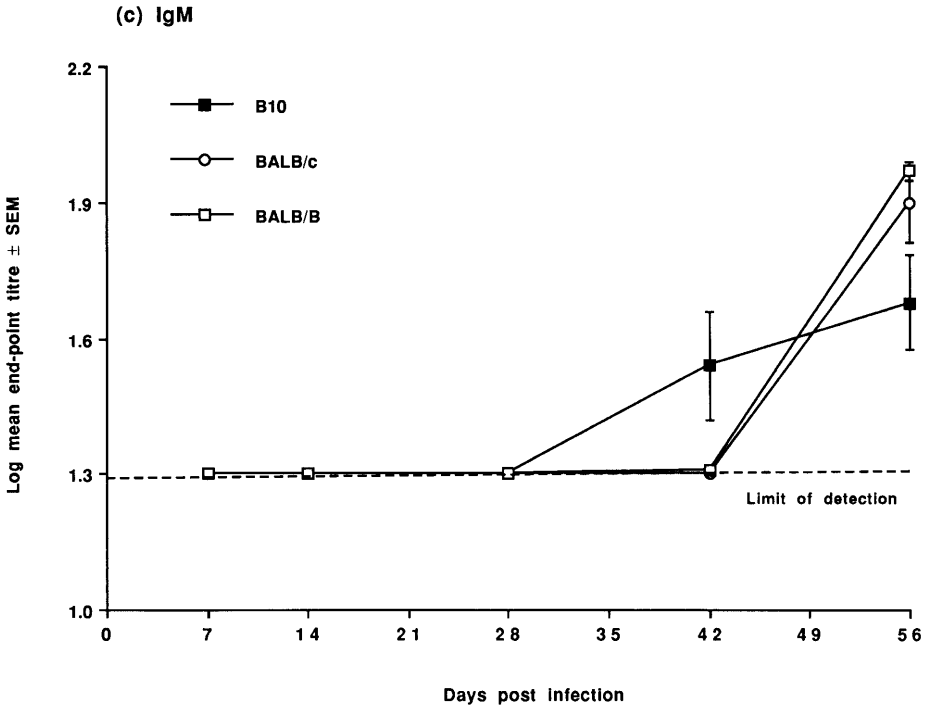


FIG. 3—*Continued.*

Luminal leukocyte counts. In the first 14 days of infection, there was a marked reduction in the number of trophozoites in B10 mice but not in BALB/c mice (Fig. 2). During the same period, there were no significant changes in luminal leukocyte numbers, which were 100- to 1,000-fold fewer than trophozoite numbers.

Serum antibody responses. *Giardia*-specific IgG, IgA, and IgM were found in sera from B10, BALB/c, and BALB/B strains (Fig. 3). Compared with sIgA in gut washings, detectable serum antibodies did not appear till after 28 days postinfection. IgA titers were significantly different between all of the strains 56 days postinfection ($P = 0.014$, $U = 0$), with the B10 strain having the lowest titer. No other significant differences in titers occurred between the strains for any isotype. Serum antibody was detectable only after the *G. muris* infection was being brought under control.

Western blotting was attempted with a variety of sera from all six *H-2* congenic strains, but despite several attempts, it was not possible to eliminate nonspecific background and define antibody specificities.

Serum IgG1 and IgG2a responses. Day 96 sera showed a clear-cut distinction between congenic strains in the IgG subclass response (Fig. 4). BALB strains mounted an IgG1, but no IgG2a, response. The converse was true for B10 strains.

MLNC proliferative responses. Peak MLNC levels of proliferation in response to ConA were similar and occurred in both BALB/c and B10 mice 5 days postinfection (proliferative indices being 9.3 ± 1.0 [standard error of the mean {SEM}] for BALB/c and 8.1 ± 0.9 for B10), with proliferative indices returning to baseline on day 10. When *G. muris* antigen was used over the concentration range of 25 to 100 μg/ml, no increase in proliferation was observed over tissue culture wells without antigen.

Cytokine assays. ConA-induced cytokine production is shown in Fig. 5 and 6. In infected BALB/c mice, IL-5 production was significantly increased on day 5 ($P = 0.01$, $U = 1$), whereas IFN-γ levels were similar in infected and uninfected mice. These findings were confirmed in two replicate experiments in which cytokine production was measured on day 5. In infected B10 mice, IL-5 levels were significantly increased on days 5 ($P = 0.005$, $U = 0$) and 13 ($P = 0.005$, $U = 0$). IFN-γ levels were significantly higher in infected mice on days 3 ($P = 0.005$, $U = 0$) and 9 ($P = 0.005$, $U = 0$). In two replicate experiments, higher levels of both IL-5 and IFN-γ were found for infected mice compared with controls on day 5. Overall B10 MLNC produced higher levels of both cytokines than BALB/c cells.

Cytokine production in response to *G. muris* antigen was also studied on day 5 postinfection in three separate experiments. Over the antigen concentration range of 25 to 100 μg/ml, MLNC from infected B10 mice but not BALB/c mice produced detectable levels of both IL-5 and IFN-γ.

In vivo blockade of cytokines. The effects of anti-IL-4 and anti-IFN-γ were studied. In BALB/c mice, neither anti-IL-4 nor anti-IFN-γ altered the course of infection in the first 2 weeks (Table 1). In B10 mice, anti-IL-4 had no effect on the course of infection but anti-IFN-γ significantly enhanced the intensity of infection on both days 7 and 14 (Table 2).

DISCUSSION

Although it is clear that sIgA is the key isotype in antibody-mediated clearance of *Giardia* infection (39), the data reported here suggest that in primary *G. muris* infection, at least, there is more to immune clearance than antibody alone.

Previous studies investigating the sIgA response to *G. muris* have used samples collected at 7-day intervals (1, 14, 16, 35, 41), which is too long to detect subtle differences in antibody production. However, in a detailed study, we have failed to explain the relative resistance of B10 mice in terms of the timing of sIgA appearance. Anders et al. (1) found higher titers of sIgA in relatively susceptible C3H/He mice than in relatively resistant BALB/c mice. However, in using both ELISA and

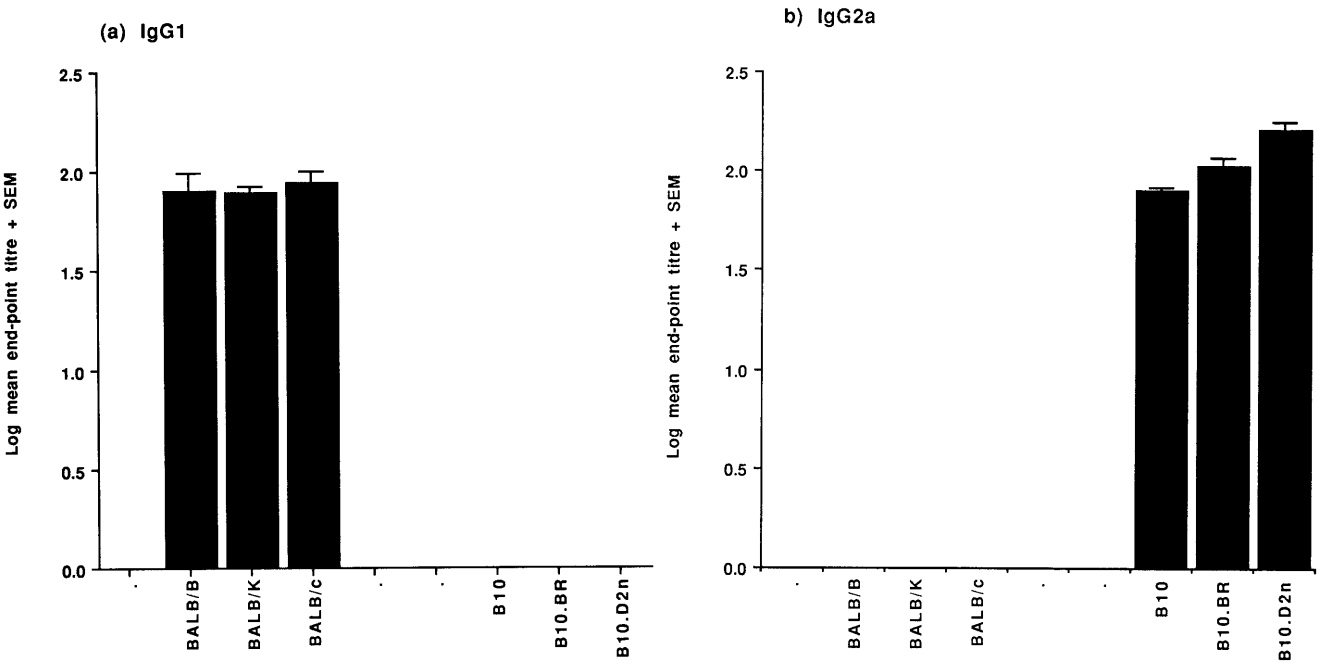


FIG. 4. Serum IgG subclass response. Titers of serum IgG1 (a) and IgG2a (b) in BALB and B10 *H-2* congenic strains. Titers of antibody are expressed as log mean endpoint titers + SEM and are derived from eight mice of each strain.

functional assays, we found no quantitative advantage in the sIgA response of the relatively resistant B10 strains. The occurrence of hemagglutination by *G. muris* trophozoites suggests the presence of a surface lectin, similar to that found in *Giardia intestinalis* (10) and which is known to cause binding of *G. intestinalis* trophozoites to isolated mouse enterocytes (24). Hemagglutination inhibition by gut washings is therefore a relevant functional assay. With this assay, as with others, there were no significant differences between strains to account for contrasting infection phenotypes. The most telling finding demonstrating that the titer of antibody is not critical to the outcome of infection was that five of eight mice (BALB/c × B10)F_1 cleared infection without detectable sIgA in the gut lumen.

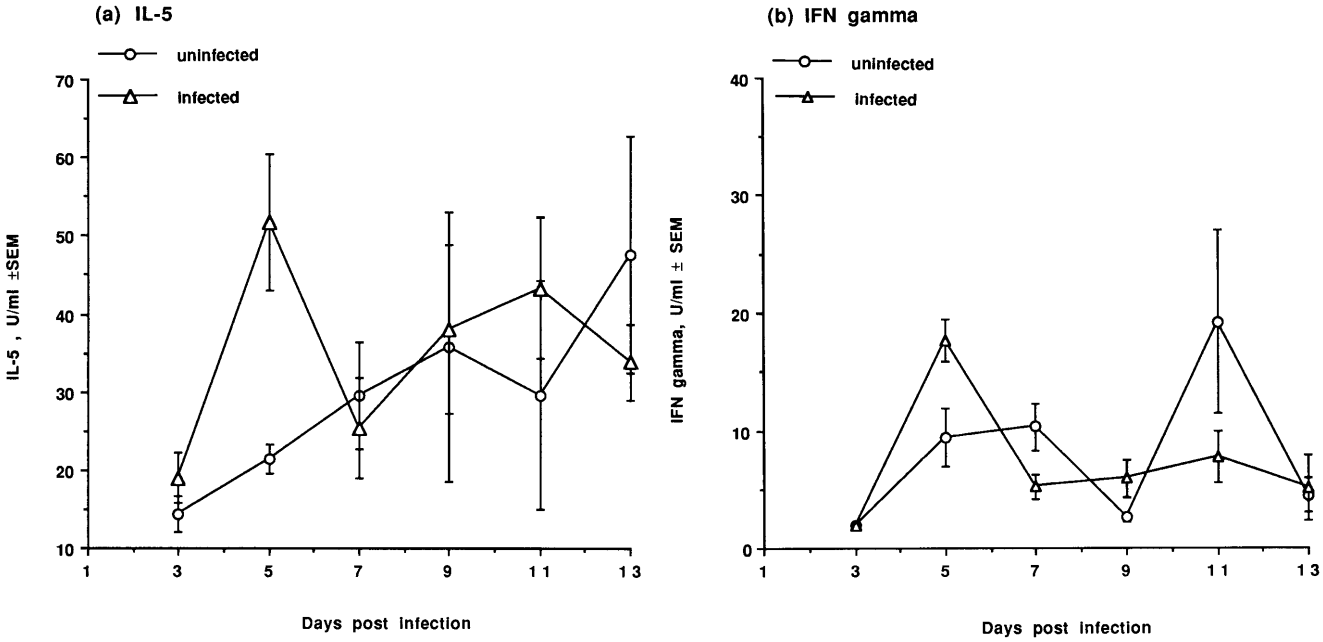


FIG. 5. Cytokine responses in BALB/c MLNC. BALB/c mice were infected on day 0 with 10^3 *G. muris* cysts. MLNC harvested on the days shown were stimulated with ConA (5 μg/ml), and tissue culture supernatants were assayed for IL-5 (a) and IFN-γ (b) after 48 h. For each time point, there were six infected and four uninfected mice. The lower limits of sensitivity in the assays were 6.3 U/ml for IL-5 and 3.6 U/ml for IFN-γ.

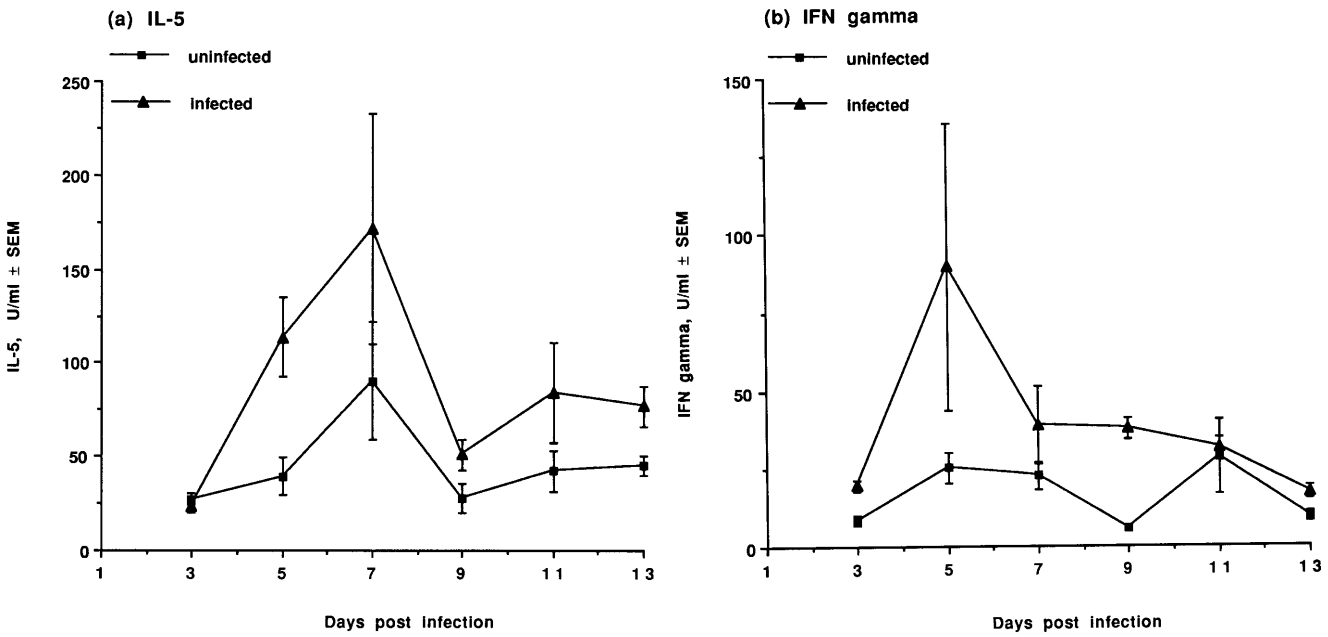


FIG. 6. Cytokine responses in B10 MLNC. B10 mice were infected on day 0 with 10^3 *G. muris* cysts. MLNC harvested on the days shown were stimulated with ConA (5 μg/ml), and tissue culture supernatants were assayed for IL-5 (a) and IFN-γ (b) after 48 h. For each time point, there were six infected and four uninfected mice. The lower limits of sensitivity in the assays were 6.3 U/ml for IL-5 and 3.6 U/ml for IFN-γ.

In qualitative studies, there was no evidence for the differential recognition of antigens to account for the relative resistance of B10 strains. It is interesting that only two of several proteins present in a trophozoite homogenate extract are recognized by immune gut washings, which confirms the data of Heyworth and Pappo (18). The situation in humans is different. Reiner and Gillin (31) have studied the secretory antibodies in human milk and showed recognition of several antigens in infected individuals.

There have been several studies of recognition of *Giardia* antigens by murine (9) and human (4–6, 21, 31, 43) sera. Erlich et al. (9) found differential recognition by BALB/c mice compared with susceptible C3H/He mice, particularly with respect to a complex of proteins of about 32 kDa. However, in (BALB/c × C3H/He)F_2 mice, susceptibility did not segregate with recognition of any particular antigen. In humans, Janoff et al. (21) found no difference in antigen recognition between immunocompetent subjects and patients with AIDS. However, Char et al. (4) have found an impaired serum IgA response to a 57-kDa heat shock protein in malnourished Gambian children with persistent giardiasis. The timing and titers of serum anti-*G. muris* IgG and IgM antibodies were broadly similar between BALB and B10 strains, but we failed to define antigen recognition.

The infection phenotypes of BALB and B10 mice therefore cannot be explained by differences in secretory and serum antibody responses. A contribution from intraluminal leukocytes also seems unlikely, as their numbers are small relative to trophozoites (17). However, the clear-cut contrasting patterns of serum IgG subclass production implies differential involvement of Th subsets. IgG1, produced by BALB strains, is regulated by IL-4 from Th2 cells (49). IgG2a, produced by B10 strains, is regulated by IFN-γ from Th1 cells (11). The lower serum IgA titer in B10 mice is compatible with a Th1 response, as IgA production is regulated in part by the Th2 cytokine IL-5 (27).

The relevance of these Th subsets and their cytokines is confirmed by the finding that blockade of IFN-γ in B10 mice enhanced the intensity of infection. Anti-IL-4 (11B.11) had no

TABLE 1. In vivo blockade of cytokines in BALB/c mice[a]

Treatment	Mean cyst count (10^{-4}) ± SD at indicated day postinfection	
	7	14
Anti-IL-4	140 ± 90	76.3 ± 29.4
Rat IgG	68 ± 43	66.3 ± 33.9
P value	NS	NS
Anti-IFN-γ	188.2 ± 90.8	21.3 ± 11.3
Rat IgG	142 ± 92.3	66.2 ± 42.3
P value	NS	NS

[a] Six female mice in each experimental group were infected with 10^3 *G. muris* cysts on day 0, and the course of infection was monitored weekly by measuring 2-h fecal excretion of cysts. NS, not significant.

TABLE 2. In vivo blockade of cytokines in B10 mice[a]

Treatment	Mean cyst count (10^{-4}) ± SD at indicated day postinfection	
	7	14
Anti-IL-4	57 ± 52.2	4.3 ± 2.9
Rat IgG	74.4 ± 44.4	3.1 ± 2.5
P value	NS	NS
Anti-IFN-γ	39 ± 16.9	7.1 ± 7.4
Rat IgG	12.4 ± 9.9	0.9 ± 0.6
P value	0.002	0.021
U value	1	5

[a] Six female mice in each experimental group were infected with 10^3 *G. muris* cysts on day 0, and the course of infection was monitored weekly by measuring 2-h fecal excretion of cysts. NS, not significant.

observable effect on infection in either strain, which may imply that IL-4 has no influence on immunity to giardiasis or that an insufficient dose of anti-IL-4 was used. The dose used is known to ablate IgE, but not IgG1, responses in parasitic infections (12, 23, 36), and it is generally felt that 11B.11 is not a potent blocker of IL-4 activity. It is unlikely that the contribution of IFN-γ to resistance is mediated by IgG2a, which is not present in the intestinal lumen where trophozoites reside. Other effector functions associated with IFN-γ have been investigated (47), but inhibition of nitric oxide and oxygen radical species with N^G-monomethyl-L-arginine and pentoxifylline does not modify the course of primary infections. The exact effector function of IFN-γ remains unclear, and it is also unclear whether it is solely derived from Th1 cells or other sources such as natural killer cells during *G. muris* infections.

While the difference in IgG subclasses between BALB and B10 strains suggests a clear-cut dichotomy of Th1 and Th2 subsets, this possibility is not supported by the cytokine data. BALB/c mice produced only the Th2 cytokine IL-5, whereas B10 mice produced both IL-5 and the Th1 cytokine IFN-γ. A possible explanation is that IgG production was detectable late in the course of infection whereas cytokines were measured very early. In the intervening period Th2 activity may have been down-regulated in B10 strains. Cross-regulation of Th1 and Th2 cells is well recognized (13, 26, 34).

There appears to have been only one other study of cytokine responses in murine giardiasis. Petro et al. studied Peyer's patch cytokine production in C57BL/6 mice with and without murine AIDS (30). *G. muris* infection was prolonged in the presence of murine AIDS. The latter was not associated with any alteration in the ability of unfractionated cells to produce IL-2, IL-4, or IL-5 in vitro but was associated with an increased ability of cells to produce IFN-γ. As murine AIDS depletes Peyer's patch cell numbers, the implications for these findings for in vivo levels of cytokines is unclear.

In conclusion, we have found that IFN-γ, in some way, contributes to immunity in B10 mice. As far as we can tell, the role of IFN-γ is independent of the recognized immune contribution of antibody.

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