Dietary lipids containing gangliosides reduce *Giardia muris* infection *in vivo* and survival of *Giardia lamblia* trophozoites *in vitro*

M. SUH[1], M. BELOSEVIC[2,3] *and* M. T. CLANDININ[1,4]*

[1] *Nutrition and Metabolism Research Group, Departments of Agricultural, Food and Nutritional Science,* [2] *Departments of Biological Sciences,* [3] *Medical Microbiology and Immunology and* [4] *Medicine, University of Alberta, Edmonton, Alberta T6G 2E9, Canada*

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SUMMARY

We examined whether a ganglioside supplemented diet affected the course of *Giardia muris* infection in mice and survival of *Giardia lamblia* trophozoites *in vitro*. Female CD-1 mice were fed 1 of 5 experimental diets: standard lab chow as a control diet; semi-synthetic diets containing 20% (w/w) triglyceride based on the fat composition of a conventional infant formula; triglyceride diet; triglyceride diet containing a low level of ganglioside (0·1% w/w); and triglyceride diet containing a high level of ganglioside (1·0% w/w of diet). After 2 weeks of feeding, mice were inoculated with *G. muris* by gastric intubation and fed the experimental diets during the course of the infection. Cysts released in the faeces and trophozoites present in the small intestine were enumerated at various times post-infection. The average cyst output and the number of trophozoites during the course of the infection in mice fed ganglioside-containing diet were found to be significantly lower (3-$\log_{10}$ reduction) compared to animals fed control diets. The results of *in vitro* growth studies indicated that gangliosides may be directly toxic to the parasites. Thus, gangliosides have a protective effect against *G. muris* infection *in vivo* and affect the survival of *G. lamblia* trophozoites *in vitro*.

Key words: diet, ganglioside, *Giardia*, protozoa, intestine, infection.

INTRODUCTION

Giardia is a protozoan parasite that inhabits the upper small intestine of a wide range of vertebrates including humans. It is spread via contaminated food and water and by direct host-to-host contact. After entering the host, the parasites emerge from the cysts, and adhere to the epithelial brush border of the small intestine as flagellated trophozoites. The trophozoites multiply in the small intestine, eventually encyst and are passed in the faeces of infected individuals. The number of cysts released in faeces was reported to be related to the trophozoite burden in the small intestine and degree of pathology in infections of mice with *Giardia muris* (Gillon, Thamery & Ferguson, 1982; Belosevic & Faubert, 1983; Daniels & Belosevic, 1995). In humans, clinical manifestations of giardiasis range from asymptomatic to symptomatic. Symptoms include diarrhoea, weight loss, abdominal distension, vomiting and abdominal pain (Wolfe, 1992; Farthing, 1996). The severity of symptoms may vary and was found to be related to the initial number of cysts ingested, the age of the host, and the state of the host immune system (Wolfe, 1992; Farthing, 1996). Disaccharidase deficiency causing malabsorption has been observed in both humans (Jennings *et al*. 1976) and animals (Gillon *et al*. 1982; Belosevic, Faubert & MacLean, 1989; Buret, Gall & Olson, 1991; Daniels & Belosevic, 1995), and was related to the parasite burden in the small intestine (Belosevic *et al*. 1989; Daniels & Belosevic, 1995).

Gangliosides, sialic acid-containing glycosphingolipids, are located at the surface of the cell membrane with the hydrophilic oligosaccharide chain extending into the extracellular space. Glycosphingolipid constitutes approximately 20% of the brush border membrane lipids (Forstner & Wherrett, 1973). The dominant ganglioside is GM3 (Iwamori *et al*. 1984), which is 7 times more concentrated in the neonatal compared to adult intestine of rats (Bouhours & Bouhours, 1983). The specific physiological roles of gangliosides are poorly understood. Studies have shown, however, that gangliosides provide binding sites for a wide range of pathogens including viruses, bacteria and fungi (Holmgren *et al*. 1985; Laegreid & Otnaess, 1987; Kyogashima, Ginsburg & Krivan, 1989; Rolsma *et al*. 1998). For example, ganglioside GM3 acts as a natural receptor in pig small intestine for rotavirus (Rolsma *et al*. 1998) and the enterotoxigenic bacteria *Escherichia coli* K99 (Kyogashima *et al*. 1989). Ganglioside GM1 in human intestine (Holmgren *et al*. 1985) and in human milk (Laegreid

* Corresponding author: Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada. Tel: +780 492 1266. Fax: +780 492 2216. E-mail: mike.belosevic@ualberta.ca

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Table 1. Experimental diets

	Chow*	TG†	TG + PUFA‡	Gang-Low¶	Gang-High
(g/100 g)					
Basal diet§	Chow	80·0	80·0	80·0	80·0
Fat	5·0	20·0	20·0	20·0	20·0
Triglyceride	5·0	20·0	20·0	19·6	16·5
Gangliosides	—	—	—	0·1	1·0
Phospholipids	—	—	—	0·25	2·5

* Standard mouse chow serving as a control.
† Fatty acid composition reflecting the fat blend of an existing infant formula.
‡ TG fat blend with addition of arachidonic acid (C20 : 4*n*-6) and docsahexaenoic acid (C22 : 6*n*-3) mixture.
¶ TG fat blend with ganglioside-containing nutrients.
§ Clandinin & Yamashiro (1982). TG, triglyceride; TG + PUFA, triglyceride containing long-chain polyunsaturated fatty acids; Gang-Low, low concentration of ganglioside; Gang-High, high concentration of ganglioside.

& Otnaess, 1987) is a receptor for enterotoxin of *Vibrio cholerae* and the heat-labile *E. coli*, thereby acting as a physiological guard for protection against these enteric infections. Pre-term newborn infants fed ganglioside supplemented formula at a concentration of 1·43 mg/100 Kcal, were shown to have significantly lower numbers of *E. coli* and higher bifidobacteria in their faeces (Rueda *et al*. 1998). Recent studies showed that gangliosides exist in clusters in the plasma membrane forming glycosphingolipid enriched domains (Brown & Rose, 1992), and that these domains are the preferential interaction sites between target cells and pathogens (Karlsson, 1995).

Decreased prevalence of giardiasis among infants fed breast milk containing high titres of anti-*Giardia* secretory IgA (sIgA) has been reported (Walterspiel *et al*. 1994). Studies showed that non-immune components of human milk such as unsaturated fatty acids (Rohrer *et al*. 1986) and free fatty acids (Reiner, Wang & Gillin, 1986) produced by the action of bile salt-stimulated lipase (Gillin, 1987), may be involved in the elimination of the parasites.

Since breast milk also contains a significant amount of gangliosides (Rueda *et al*. 1996), we hypothesized that gangliosides may also play a protective role in giardiasis. To test this hypothesis, we examined whether dietary ganglioside affected the course of *G. muris* infection in mice by enumerating cyst output in the faeces and trophozoite burden in the small intestine of mice fed ganglioside supplemented diet. We also determined the effect of different ganglioside concentrations (ganglioside-enriched preparation and isolated ganglioside fractions) on *in vitro* survival of *G. lamblia* (= *G. duodenalis*) trophozoites.

MATERIALS AND METHODS

The effect of ganglioside supplemented diet on the course of Giardia muris *infection*

Animals and diets. This study was approved by the University of Alberta Animal Welfare Committee and the procedures used adhered to the Canadian Council for Animal Care Guidelines. Five- to six-week-old female pathogen-free CD-1 mice weighing 20·4 ± 1·1 g, were obtained from Charles River Laboratories (St Constant, Quebec) and were randomly divided into 5 groups of 5 mice each. The control group was fed a standard lab chow diet (Chow) (Table 1). The other 4 groups were fed semi-synthetic experimental diets (Clandinin & Yamashiro, 1982) containing 20% (w/w) fat as triglyceride. The fat composition of the semi-synthetic diet reflects the fat composition of a conventional infant formula providing a ratio of 18 : 2*n*-2 to 18 : 3*n*-3 of 7 : 1 (TG). Three additional experimental diets were prepared by addition of long-chain polyunsaturated fatty acids, C20 : 4*n*-6 (1%, w/w) and C22 : 6*n*-3 (0·5%, w/w of total fatty acid) (TG + PUFA), or low (0 ·1% ganglioside, w/w of total diet, Gang-Low) or high (1% ganglioside, w/w of total diet, Gang-High) ganglioside preparation (New Zealand Dairy, New Zealand) to TG diet. The lipid composition of the ganglioside preparation consists of about 45–50% (w/w) phospholipids and 15–20% (w/w) gangliosides. The ganglioside fraction contained GD3, GD1b, GM3 and other gangliosides (80%, 9%, 5% and 6% w/w, respectively). The ganglioside preparation also contained lactose and minerals (60–70%, 10–12% w/w of ganglioside preparation, respectively) and the level of these amounts was adjusted in the basal diet (Table 1). After feeding for 2 weeks, animals were inoculated orally with 10^4 *G. muris* cysts/mouse suspended in 0·2 ml of de-ionized water and were maintained on different diets during the course of the infection (25 days).

Enumeration of G. muris *cysts in faeces*. Fresh faeces from each mouse were collected for 2 h between 7:00 am and 9:00 am every 5 days until day 25 post-infection for determination of cysts released in the faeces. The mean wet weight of faeces at different days post-infection were as follows: Day 5: 0·98 ± 0·08, 0·24 ± 0·13, 1·22 ± 0·10, 1·31 ± 0·10 and 1·05 ± 0·05 for Chow, TG, TG + PUFA, Gang-Low and Gang-High, respectively; Day 10: 1·23 ± 0·06, 0·30 ± 0·06,

$0{\cdot}32 \pm 0{\cdot}05$, $0{\cdot}30 \pm 0{\cdot}06$, $0{\cdot}29 \pm 0{\cdot}08$ for Chow, TG, TG + PUFA, Gang-Low and Gang-High, respectively; Day 15: $1{\cdot}22 \pm 0{\cdot}03$, $0{\cdot}22 \pm 0{\cdot}05$, $0{\cdot}23 \pm 0{\cdot}03$, $0{\cdot}24 \pm 0{\cdot}05$, $0{\cdot}19 \pm 0{\cdot}05$ for Chow, TG, TG + PUFA, Gang-Low and Gang-High, respectively; Day 20: $1{\cdot}31 \pm 0{\cdot}05$, $0{\cdot}39 \pm 0{\cdot}08$, $0{\cdot}48 \pm 0{\cdot}08$, $0{\cdot}34 \pm 0{\cdot}05$, $0{\cdot}31 \pm 0{\cdot}03$ for Chow, TG, TG + PUFA, Gang-Low and Gang-High, respectively; Day 25: $1{\cdot}05 \pm 0{\cdot}06$, 0.4 ± 0.06, $0{\cdot}42 \pm 0{\cdot}10$, $0{\cdot}54 \pm 0{\cdot}05$, $0{\cdot}39 \pm 0{\cdot}08$ for Chow, TG, TG + PUFA, Gang-Low and Gang-High, respectively. Cysts were isolated using the sucrose gradient centrifugation described by Roberts-Thompson *et al*. (1976), and enumerated using procedures described previously (Daniels & Belosevic, 1995). Briefly, faeces were weighed, emulsified in de-ionized water and gently layered on 1 M sucrose solution in a glass test-tube. Samples were centrifuged for 15 min at 400 ***g***. The cysts present at the water–sucrose interface were carefully removed with a pipette and washed in de-ionized water by centrifugation for 10 min at 600 ***g***. The supernatant fraction was discarded and the pellet containing the cysts re-suspended in 1 ml of de-ionized water. Cysts were enumerated using a haemocytometer and expressed as the number of cysts per gram of faeces.

Enumeration of G. muris *trophozoites in the small intestine*. The enumeration of trophozoites present in the small intestine of 10–12 mice for each experimental group was done on day 10 post-infection using the procedures we described previously (Daniels & Belosveic, 1995). Briefly, the small intestine was removed and divided into 4 equal sections. Intestinal segments were placed in ice-cold phosphate-buffered saline (PBS, pH 7·2) and incubated on ice for 30 min. The intestinal segments were then slit longitudinally and mucosa scraped using glass microscope slides. The mucosal scrapings, including the remainder of the intestinal segment, were placed in 6 ml of ice-cold PBS, mixed vigorously and filtered through a double layer of moist cheese cloth. The volume of the filtered solution was adjusted to 6 ml and the total number of trophozoites in each segment determined using a haemocytometer.

The effect of ganglioside-enriched preparation and ganglioside fractions on growth of Giardia lamblia *trophozoites* in vitro

Preparation of culture medium containing ganglioside. The preparation containing gangliosides was vortexed and sonicated (Sonic 300 Dismembrator, Artek System Corp.) in 10 ml of TYI-S-33 culture medium (Diamond, Harlow & Cunnick, 1978), and further diluted by adding 990 ml of culture medium. This stock culture medium containing a known concentration of gangliosides was filtered in succession through Whatman No. 1 filter paper, $0{\cdot}8\ \mu$m, $0{\cdot}45\ \mu$m (Milli-Fil-P.F. Millipore Corp.), and $0{\cdot}22\ \mu$m (Sterivex-GS filters with filling bell, Millipore Corp.) filters connected to a peristaltic pump. The stock solution was kept at $-30\ ^{\circ}$C and was diluted to appropriate test concentrations of ganglioside before use in the *in vitro* assays.

G. lamblia (WB strain) trophozoites were cultured in Diamond's TYI-S-33 (Diamond *et al*. 1978). *Giardia lamblia* trophozoites (5×10^5) were inoculated in $12{\cdot}5\ \text{cm}^2$ tissue culture flasks in the total volume of 40 ml. Nutrient stock solution containing gangliosides was diluted to provide a concentration of ganglioside (as N-acetyl neuraminic acid amounts, NANA) at 0 (control), 0·001, 0·01, 1, 2, and $4\ \mu$g/ml. The cultures were incubated for 24 and 48 h at $37\ ^{\circ}$C in 5% CO_2, and the number of live and dead (no flagellar movement) trophozoites determined using a haemocytometer.

Preparation of culture medium containing ganglioside fraction. Total lipids were extracted from the ganglioside-enriched preparation using the Folch method (Folch & Sloane-Stanley, 1957). The ganglioside-containing upper phase was transferred, and the lower phase was washed once with Folch upper phase solution (chloroform/methanol/water, 3/48/47 by volume). The combined ganglioside-containing fractions were passed through Sep-Pak C18 reverse-phase cartridges (Waters Corporation, Milford, MA, USA), eluted with methanol and chloroform and methanol 2 : 1 (v/v), and dried completely under vacuum at $23\ ^{\circ}$C using a rotary evaporator (Williams & McCluer, 1980). Ganglioside (NANA) content was measured as described by Suzuki (1964). Gangliosides were then diluted using the culture medium and filtered as described above.

G. lamblia trophozoites (5×10^5) were incubated for 24 and 48 h with ganglioside fraction at the concentration of 0 (control), 4, 8, 10, 12, 14, 16, and $20\ \mu$g/ml in $12{\cdot}5\ \text{cm}^2$ tissue-culture flasks as described above.

Preparation of culture medium containing ganglioside GD3. Individual ganglioside from the ganglioside fraction was separated by thin layer chromatography on silica-gel G-plates (20×20 cm) using a developing system, chloroform/methanol/28% (w/v) NH_4OH/H_2O (60 : 35 : 7 : 3, by volume). The corresponding GD3 band was eluted with chloroform/methanol (2 : 1, v/v) and dried under nitrogen. GD3 was further purified using silica-gel high performance thin-layer chromatography (HPTLC; Whatman Inc, Clifton, NJ, USA) in a solvent system of chloroform/methanol/0·2% (w/v) $CaCl_2 . 2H_2O$ (55/45/10, by volume). GD3 was eluted with the Folch upper phase by chloroform/methanol/H_2O (3 : 48 : 47) and dried under nitrogen. GD3 was then diluted with H_2O and filtered through $0{\cdot}22\ \mu$m filters (Millex-GP filters, Millipore Corp.) fitted to a 3 ml syringe. Silica-gel

containing no ganglioside was extracted from the beginning using the same procedure. This was the control for potential carry-over of solvent used in the extraction.

G. lamblia trophozoites (5×10^5) were incubated for 24 and 48 h with GD3 at the concentration of 0 (control), 10 and 20 μg/ml in 12·5 cm^2 tissue-culture flasks as described above.

Statistical analysis

The effects of diets on *G. muris* infection by enumerating cyst output were examined in two identical independent experiments. Since no significant differences were found between the two experiments the data were combined and analysed using one-way analysis of variance, using Abacus software for the Power Macintosh. The effect of the ganglioside-enriched preparation and ganglioside fractions on the replication of *G. lamblia in vitro* were carried out in duplicate and repeated 5 and 3 times, respectively. These data were analysed using one-way analysis of variance and Duncan's multiple range test (Steel & Torrie, 1990). All results were expressed as mean $\pm$ standard error of the mean (S.E.M.). Probability level of $P<0·05$ was considered significant.

RESULTS

Mice in each group were fed 1 of the 5 experimental diets for 14 days before exposure to *G. muris* and during the course of the infection (25 days). At the beginning of the experiment the mean body weights (in g) of mice in different experimental groups were $21·0 \pm 0·3$, $20·1 \pm 0·3$, $20·0 \pm 0·3$, $20·9 \pm 0·03$, $20·1 \pm 0·4$ for Chow, TG, TG+PUFA, Gang-Low and Gang-High, respectively. The body weight of mice fed TG, TG+PUFA or Gang-Low diet increased slightly during the post-infection experimental period, whereas those given standard lab chow (Chow) and Gang-High diet maintained their weight during the course of the infection. The mean body weights of mice on Day 25 post-infection for different experimental groups were $24·8 \pm 0·3$, $26·2 \pm 1·2$, $31·1 \pm 2·2$, $26·8 \pm 1·0$, $25·0 \pm 1·0$ for Chow, TG, TG+PUFA, Gang-Low and Gang-High, respectively. Statistically significant increases in body weight were observed in mice fed TG+PUFA compared to those fed Chow or Gang-High diet ($P<0·02$).

The effect of ganglioside-supplemented diet on the course of G. muris *infection*

Two independent experiments were conducted to assess the effect of dietary ganglioside on the *G. muris* infection by measuring cyst output in CD-1 mice. Feeding mice diets containing different levels of dietary gangliosides significantly affected *G. muris* infection. The average combined cyst output ($\log_{10}$) during the 25 days in which mice were fed either Gang-Low or Gang-High diet was $1·3 \pm 0·3$ and $1·8 \pm 0·3$ cysts/g faeces, respectively, and that of mice fed Chow was $4·8 \pm 0·4$ cysts/g faeces. Animals fed TG or TG+PUFA diet released similar numbers of cysts in the faeces compared to the control mice fed Chow diet. The onset of cyst release in mice fed Gang-High and Gang-Low diets was delayed as indicated by lack of cyst release in these mice on day 5 post-infection compared to other treatment groups (Fig. 1). Mice fed either Gang-High or Gang-Low diet exhibited significantly reduced cyst output ($P<0·0001$) during the course of the infection. In all experimental groups, the highest cyst output was observed on day 10 post-infection. No differences in the average cyst output were observed between mice fed Chow and mice fed TG or TG+PUFA diets, suggesting that triglyceride with and without long chain fatty acids did not influence the course of *G. muris* infection in mice. The duration of cyst release was also affected by Gang-High and Gang-Low diets. It was found that 88% of mice fed Gang-High and 80% of mice fed Gang-Low diet did not release cysts in the faeces on days 20 and 25 post-infection, respectively, whereas the faeces of most mice fed Chow, TG or TG+PUFA contained cysts, 100%, 70% and 60%, respectively, on day 20 post-infection.

The effects of dietary gangliosides on the *G. muris* infection was also assessed by enumerating trophozoite load in the small intestine on day 10 post-infection (Fig. 2). Since no differences in cyst output were observed in animals fed TG or TG+PUFA diet compared to mice fed the Chow diet, only the ganglioside-containing diets were tested in this experiment. Diet containing gangliosides significantly reduced the trophozoite load in the small intestine (Fig. 2).

The total numbers of trophozoites in all sections of small intestine were drastically reduced in mice fed either Gang-High or Gang-Low diet compared to mice fed the Chow diet (Fig. 2). A further decrease in the number of trophozoites was observed in sections 3 and 4 of the small intestine in mice fed Gang-High diet compared to mice fed Gang-Low diet.

The effect of ganglioside-enriched preparation and isolated ganglioside fractions on the growth of G. lamblia *trophozoites* in vitro

To determine whether ganglioside-containing nutrients inhibited parasite growth, we used an *in vitro* cultured WB strain of *G. lamblia* (WB strain, ATCC). Gangliosides (as NANA) were provided at the level of 0 (control), 0·001, 0·01, 1, 2, and 4 μg/ml to each flask containing 5×10^5 trophozoites and incubated for 24 and 48 h. The growth of trophozoites *in vitro* was significantly reduced in the presence of gangliosides. After a 24 h incubation, there was a 20% reduction of live trophozoites in cultures containing

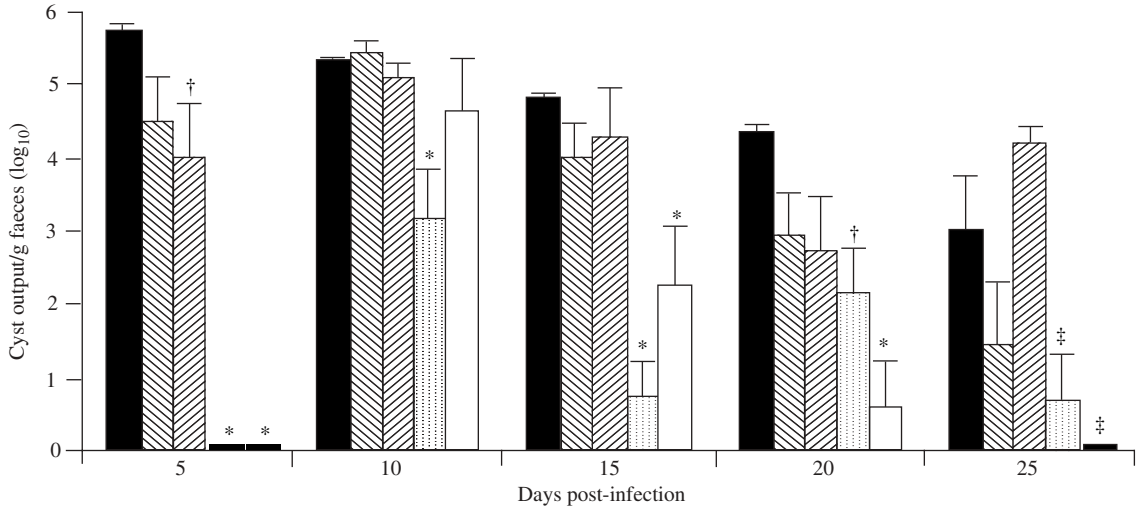

Fig. 1. Effect of dietary gangliosides on the course of *Giardia muris* infection in mice measured by enumerating cyst output in faeces. Values (means ± S.E.M., $n = 8$–10 except day 25, $n = 5$) were from 2 independent experiments. Significant effects of diets were identified by one-way analysis of variance procedures on each post-infection day: day 5, $P < 0{\cdot}0001$; day 10, $P < 0{\cdot}004$; day 15, $P < 0{\cdot}0001$; day 20, $P < 0{\cdot}003$; day 25, $P < 0{\cdot}004$. Values with * were significantly different from Chow, TG and TG + PUFA diets. Values with † were significantly different from chow diet. Values with ‡ were significantly different from chow and TG + PUFA diets. Values given as (▬) on day 5 and 25 post-infection represents zero output. ■, Chow; ▧, TG; ▨, TG + PUFA; ⬚, Gang-Low; □, Gang-High.

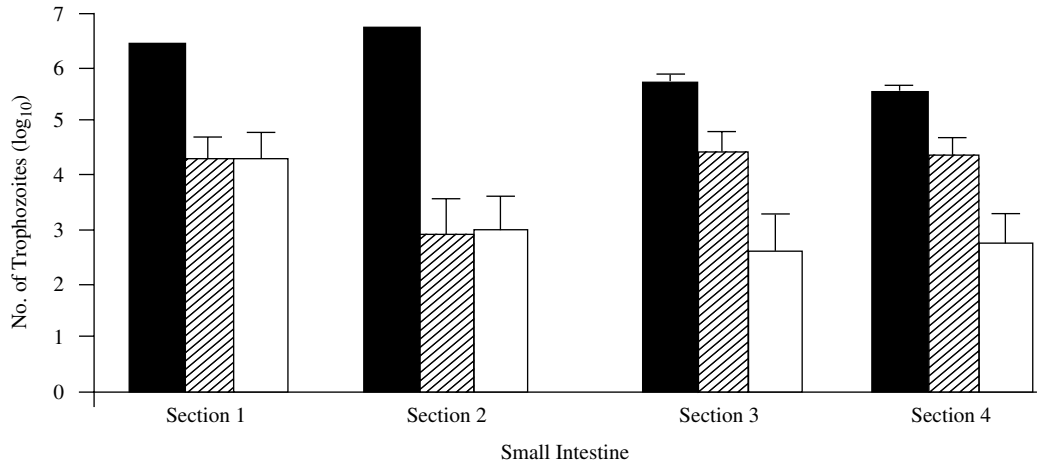

Fig. 2. Effect of dietary gangliosides on *Giardia muris* replication in the small intestine of mice on Day 10 post-infection. Values represent means ± S.E.M. ($n = 10$–12). The enumeration of trophozoites present in the small intestine was done on day 10 post-infection. The small intestine was removed and divided into 4 equal sections. Significant effects of diet were identified in each section of small intestine by one-way analysis of variance procedures; Section 1, $P < 0{\cdot}001$; Section 2, $P < 0{\cdot}0001$; Section 3, $P < 0{\cdot}0002$; Section 4, $P < 0{\cdot}0002$. Values with * at each section were significantly different from chow. Values with † at each section were significantly different Gang-Low diet. Values where S.E.M. is not shown indicates very small S.E.M. ■, Chow; ▨, Gang-Low; □, Gang-High.

4 μg/ml of ganglioside, compared to control cultures ($3{\cdot}5 \times 10^6$ and $2{\cdot}8 \times 10^6$, control and 4 μg/ml of ganglioside, respectively). After 48 h incubation, in cultures containing 2 and 4 μg/ml ganglioside, the number of live trophozoites decreased by 40% and 91%, respectively, compared to controls; $2{\cdot}5 \times 10^7$, $1{\cdot}4 \times 10^7$, and $2{\cdot}2 \times 10^6$, control, 2 and 4 μg/ml of ganglioside, respectively.

Different ganglioside species extracted from the whole ganglioside-enriched preparation were tested to determine whether the effects are due only to ganglioside constituents of the diet. After 24 h incubation, significant reduction of trophozoite growth was observed in cultures containing more than 12 μg/ml of ganglioside (data not shown). After 48 h incubation, in cultures containing 8, 10, 12, 14, and 16 μg/ml ganglioside, the number of live trophozoites decreased by 36%, 45%, 77%, 98%, and 99%, respectively, compared to control cultures (Table 2; Fig. 3). No live trophozoites were found at a ganglioside concentration of 20 μg/ml (Fig. 3). The addition of only one ganglioside, GD3, to the culture medium

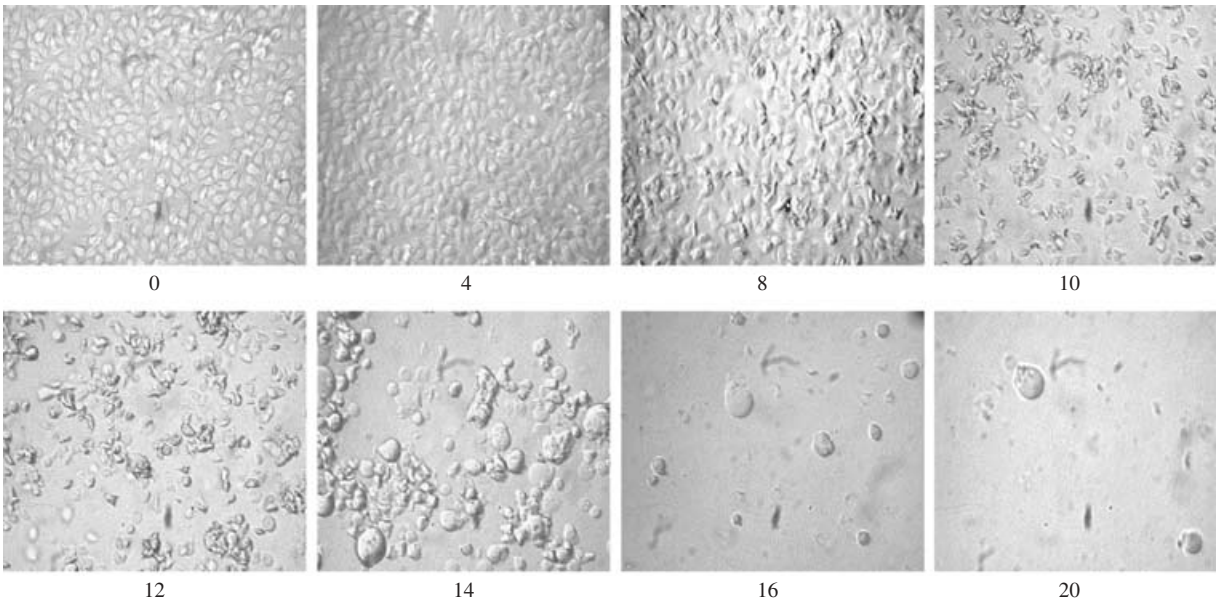

Fig. 3. Effect of gangliosides on the growth of *Giardia lamblia* trophozoites during 48 h incubation *in vitro*. Trophozoites were incubated for 48 h with gangliosides extracted from the crude ganglioside preparation at a concentration of 0 (control), 4, 8, 10, 12, 14, 16, and 20 μg/ml (N-acetyl neuraminic acid). (Magnification × 200.)

Table 2. Effect of gangliosides on the growth of *Giardia lamblia* trophozoites *in vitro*

(Values represent means ± S.E.M. ($n=3$). *G. lamblia* trophozoites were incubated with gangliosides extracted from the crude ganglioside preparation at a concentration of 0 (control), 4, 8, 10, 12, 14, 16, and 20 μg/ml (N-acetyl neuraminic acid) for 48 h.)

Ganglioside conc. (μg/ml)	Live trophozoites* ($P<0{\cdot}0003$)	Dead trophozoites† ($P<0{\cdot}002$)
0	100	$0{\cdot}8 \pm 0{\cdot}5^{b}$
4	$90{\cdot}5 \pm 3{\cdot}4^{a}$§	$1{\cdot}2 \pm 0{\cdot}6^{b}$
8	$63{\cdot}8 \pm 3{\cdot}2^{b}$	$1{\cdot}2 \pm 0{\cdot}7^{b}$
10	$55{\cdot}0 \pm 5{\cdot}7^{b}$	$3{\cdot}9 \pm 1{\cdot}7^{b}$
12	$22{\cdot}6 \pm 8{\cdot}0^{c}$	$45{\cdot}6 \pm 9{\cdot}6^{b}$
14	$1{\cdot}3 \pm 0{\cdot}8^{d}$	$884{\cdot}1 \pm 351{\cdot}8^{b}$
16	$0{\cdot}2 \pm 0{\cdot}1^{d}$	$6232{\cdot}6 \pm 1362{\cdot}4^{a}$
20‡	$0{\cdot}0 \pm 0{\cdot}0^{d}$	–

* Expressed as a percentage of controls containing zero ganglioside ($P<0{\cdot}0003$, one-way analysis of variance).
† Expressed as a percentage of live trophozoites at each concentration ($P<0{\cdot}002$, one-way analysis of variance).
‡ No live trophozoites were found at the concentration of 20 μg/ml, where no values (–) were given (Fig. 4).
§ Values without a common letter in each column are significantly different between ganglioside concentrations.

resulted in a significant reduction of live trophozoites after 48 h of cultivation. The reduction in the number of live parasites was 25% and 55% for GD3 concentrations of 10 and 20 μg/ml, respectively, when compared to control cultures not containing ganglioside. A higher concentration of GD3 alone was required to cause significant death of the parasites *in vitro*, suggesting that different ganglioside species present in complete diet may have an additive anti-parasite lytic effect.

DISCUSSION

This is the first study to examine the effects of dietary gangliosides on the course of not only *Giardia muris* but also any other gastrointestinal protozoan infection. Dietary gangliosides significantly altered the course of *G. muris* infection in mice as indicated by (i) delay in the onset of cyst release; (ii) reduced cyst output during the course of the infection; (iii) decrease in trophozoite load in the small intestine during the acute phase of infection and; (iv) accelerated elimination of the parasites from the host. Our results indicate that ganglioside content in the diet of ~0·1% (w/w, 0·02% as NANA) was sufficient to significantly alter the course of giardiasis in mice. The mechanisms of this anti-parasite effect of gangliosides remain to be elucidated. It is possible that gangliosides (i) may inhibit the adherence of the trophozoites to the intestinal epithelium by changing the membrane lipid environment of the mucosa; (ii) affect the metabolic machinery of the parasites, influencing multiplication and/or encystment; (iii) are directly toxic to the trophozoites by altering their membranes and (iv) modulate host immune function in the small intestine.

The brush border membrane contains approximately 20% glycosphingolipid (Forstner & Wherrett, 1973) and a dominant intestinal ganglioside is GM3 (Iwamori *et al*. 1984). The pattern and concentration of ganglioside appears to be both species and tissue-specific and can be influenced by the age of the host

(Iwamori *et al*. 1984). Dietary manipulation also affects the ganglioside profiles of the intestinal mucosa. We have recently shown that (Park *et al*. manuscript submitted for publication) dietary gangliosides can change the normal distribution of gangliosides in the intestinal mucosa of rats. In the present study, mice were fed a ganglioside diet for 2 weeks prior to exposure to *G. muris*, providing ample time for a change in ganglioside content of the mouse small intestine to occur. It is possible that introduction of a more acidic sugar, GD3, and proportional reduction of GM3 in the mucosa may alter the ability of trophozoites to attach to the mucosal surface, thereby affecting the normal reproduction behaviour of the parasites. Our results suggest that not only dietary mixtures of gangliosides but also isolated GD3, affect parasite survival *in vivo* and *in vitro*. That gangliosides can act as parasite receptors has been reported for *Theileria sergenti* (Watarai *et al*. 1995). The identification of specific ganglioside species that affect other gastrointestinal protozoan infections such as *Cryptosporidium parvum*, is currently under investigation in our laboratory.

Although not tested in our study, dietary gangliosides may also change anti-parasite host immune responses. Vazquez, Gil & Rueda (2001) have shown that immune cell function can be affected by dietary gangliosides. They reported that gangliosides in the diet not only increased the number of Th1 and Th2 cytokine-secreting lymphocytes but also stimulated earlier development and maturation of the number of cytokine-secreting cells. It is also possible that incorporation of dietary gangliosides into the enterocyte plasma membrane may interfere with the transport and expression of sIgA, which has been shown to be important in the protection against *G. muris* infection (Underdown *et al*. 1981). The effects of dietary gangliosides on the anti-*Giardia* immune response is currently under investigation in our laboratory.

The hypothesis that gangliosides directly affect parasite growth was tested in this study. Cultivation of *G. lamblia* trophozoites for 24 and 48 h, in the presence of different concentrations of gangliosides significantly reduced the number of live trophozoites in the cultures. GD3 alone also inhibited the growth of *G. lamblia* trophozoites, but a higher concentration (approximately 1·7 to 2-fold) was required to produce a similar inhibitory effect to that of the ganglioside mixture. These results suggest that there may be either an additive or a synergistic interaction between gangliosides present in the experimental diet for maximal anti-parasite toxic effect. Unlike wheat germ agglutinin arresting the trophozoite cell cycle (Ortega-Barria *et al*. 1994), the effect of gangliosides on *G. lamblia* trophozoites *in vitro* was irreversible, because the results indicate that the majority of trophozoites in cultures were lysed. Lysis of *G. lamblia* trophozoites by lipolytic product of non-immune component of human milk has been reported (Gillin, Reiner & Gault, 1985; Reiner *et al*. 1986). These studies did not examine the potential anti-parasite lytic effect of gangliosides present in human milk. Our results suggest that gangliosides present in milk may also participate in lysis of *G. lamblia* trophozoites.

G. lamblia trophozoites are unable to synthesize their own phospholipids and sterols *de novo* (Jarrol *et al*. 1981), but are able to take up exogenous lipids into the membrane by trans- and inter-esterification (Stevens *et al*. 1997; Gibson *et al*. 1999). This suggests that host lipids play a critical role in metabolism and long-term survival of the parasite. Gangliosides, GM2 and GD1a, have been identified as membrane components of another gastrointestinal parasite, *Entamoeba histolytica* (Sorice *et al*. 1996). It is possible that the exogenous gangliosides are taken up by trophozoites which could affect the ganglioside composition in the trophozoite cell membrane and disturb the structural components of the trophozoites, leading to lysis of the parasite.

In conclusion, the results of this study demonstrate that dietary gangliosides administered before and during giardiasis, significantly alter the course of *G. muris* infection in mice and influence the survival of *G. lamblia* trophozoites *in vitro*, by causing lysis of the parasite. These results suggest that increasing ganglioside content in the diet may have beneficial effects for control of giardiasis.

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