Immune effector responses to an excretory-secretory product of *Giardia lamblia*

H. Kaur [a], H. Samra [a], S. Ghosh [a], Virender K. Vinayak [b], Nirmal K. Ganguly [a],*

[a] *Division of Experimental Parasitology and Parasitic Immunology, Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India*
[b] *Department of Biotechnology, Government of India, New Delhi, India*

Abstract

The prior immunisation of mice with purified excretory-secretory product (ESP) led to a complete failure of *Giardia lamblia* colonisation following challenge inoculation of these animals with trophozoites. The prior immunisation of mice with ESP resulted in a significant stimulation of local immunity as evidenced by a significant enhancement of T helper/inducer activity along with a significant increase in immunoglobulin A-bearing cells. Further, the presence of anti-ESP antibodies in the serum of immunised as well as immunised-challenged animals indicated the stimulation of the systemic lymphoid system. This suggests that the ESP is highly immunogenic and it could be one of the major antigens of *G. lamblia* responsible for protection against the infection. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: *Giardia lamblia*; Excretory-secretory product; Mouse; Lymphocyte subsetting; Flow cytometry

1. Introduction

Giardia lamblia has been implicated as one of the causative agents of diarrhoea and malabsorption especially in children [1]. Identification of the parasite antigen responsible for initiating protective immunity is essential to complete our understanding of the natural history of *G. lamblia* infection and the development of an enteric vaccine. Several groups have investigated the antigenic determinants of *Giardia* [2–11]. These studies provide evidence that surface molecules serve as anti-parasite targets for the host immune system. However, the importance of these antigens in promoting clearance of *G. lamblia* from the intestine and the development of protective immunity remains to be established. The immune system appears to have a pivotal role in not only affecting parasite clearance and protective immunity but possibly also in the pathogenesis of the T cell-mediated mucosal damage.

Since *G. lamblia* is primarily a gut parasite, alterations of gut-associated immune effector mechanisms would be important in permitting *G. lamblia* trophozoites to colonise while a stimulation of such responses would eliminate the parasite from the gut.

---

* Corresponding author. Present address: Indian Council of Medical Research, Ansari Nagar, New Delhi 110029, India. Tel.: +91 (11) 6517204, 6963980 ext. 264; Fax: (Off.) +91 (11) 6868662.

The factors that mediate the clearance of *G. lamblia* infection in some individuals while allowing persistence of infection in others remain elusive. Current evidence suggests that both humoral and cellular immune responses are important in clearing the parasite and providing immunity to reinfection [12–15]. Though the trophozoites of *G. lamblia* have a complex mosaic of antigens, the surface-associated antigens would be important for initiation of host immune responses and activation of immunological effector mechanisms. Such responses may destroy the parasites or inhibit their multiplication [16,17]. Therefore knowledge of the antigenic composition of the parasite and the role that these antigens play in the immune response during infection is important for understanding the pathogenesis of the disease. We have earlier identified and characterised an immunodominant excretory-secretory (ES) antigen (ESP, 58 kDa) of *G. lamblia* (paper communicated: Article No. IAI 1179-98) and the main aim of the present study was to investigate the biological significance of this antigen in gut-associated effector immune responses leading to the clearance of the parasite. This paper thus focuses its attention to the immune responses developed against the purified ES product of *G. lamblia* in an experimental mouse model to understand the clearance of infection.

2. Materials and methods

2.1. Purification of ESP

Cell-free supernatants of *G. lamblia* collected after 12 h of culture in MEM were successively precipitated with different percentage saturations of $(NH_4)_2SO_4$ and assessed for immunoreactivity by micro-ELISA assay. The 25% and 40% saturated $(NH_4)_2SO_4$-precipitated ES fractions having maximum immunoreactivity with anti-CGE antibodies were pooled, labelled as crude ES antigen (CESA), which was successfully eluted out first from the anti-CGE IgG-Sepharose CL-4B column and further fractionated to homogeneity by gel filtration using Protein Pak SW 300 column in the FPLC system. Both SDS-PAGE and alkaline PAGE of the purified protein (labelled as ESP) revealed a single band of M_r 58 kDa (Fig. 1).

2.2. Groups of animals

Inbred BALB/c mice aged 3–4 weeks (15–18 g) of either sex maintained in the animal facility room of our department were employed in the study. These animals were checked for *G. muris* infection [18]. They were grouped (10 mice per group) as follows.
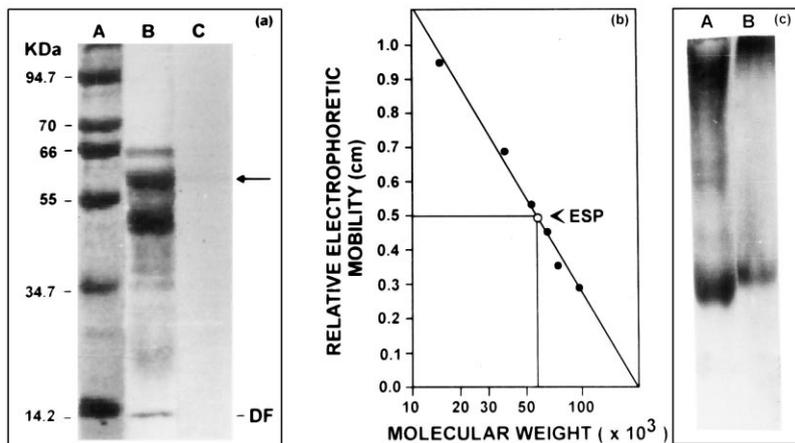
Fig. 1. Characterisation of purified ESP. a: Molecular weight markers, i.e., phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), glutamate dehydrogenase (55 kDa), pepsin (34.7 kDa) and lysozyme (14.2 kDa), CESA (10 μg) and the purified ESP (5 μg) were applied in lanes A, B and C respectively in SDS-PAGE (10%). b: The molecular mass of the purified ESP was determined from the plot of relative electrophoretic mobility of the marker proteins against their corresponding molecular weights. c: The CESA (10 μg) and the purified ESP (7.5 μg) were applied on alkaline PAGE (7.5%) in lanes A and B respectively.

Group 1: immunised group. These mice received four oral doses (50 μg total) of purified ESP at intervals of 7 days each, after neutralising the stomach acidity with 0.25 ml NaHCO$_3$ (100 mg ml^{-1}, pH 8.5). The antiserum from these animals was tested by immunodiffusion [19] using 1.5% agarose in 0.05 M sodium barbital buffer (pH 8.3), to assess to the status of immunisation.

Group 2: immunised-challenged group. These animals were immunised as described for group 1. However, this group of animals was challenged with *G. lamblia* trophozoites 7 days after the last immunising dose.

Group 3: infected group. The animals of this group were infected with *G. lamblia* trophozoites after receiving NaHCO$_3$ (as above).

Group 4: control group. These animals received the same treatment of NaHCO$_3$ (as above) prior to receiving normal saline and served as control animals. This is to control for the effect of bicarbonate treatment on trophozoite infectivity.

2.3. Infection/challenge of the experimental animals

Axenic *G. lamblia* trophozoites (Portland-1 strain) were harvested from 48–72-h culture and washed with 0.15 M phosphate-buffered saline, pH 7.2 (PBS). The count was adjusted to 2×10^7 viable trophozoites per millilitre of PBS and the animals were challenged intraoesophageally with 0.5 ml of inoculum with a catheter [20].

2.4. Follow-up of animals

The animals from each group were killed at various post immunisation/post challenge (PI/PC) or post infection (p.i.) days, i.e., 3–5 days (establishment phase), 9–11 days (acute phase) and 17–21 days (decline phase).

2.5. Trophozoite load in the intestine

The trophozoites were counted in the intestinal perfusate of infected as well as immunised-challenged animals by the method of Vasudeva et al. [21]. After laparotomy, the small intestine was excised and flushed with a fixed volume of ice-cold normal saline. The perfusate was centrifuged at $50 \times g$ for 2–3 min

to remove the tissue fragments and food particles. The supernatant was centrifuged at $600 \times g$ for 10 min. The pellet was washed with normal saline and the total number of trophozoites was counted in a haemocytometer.

2.6. Isolation of gut lymphocytes

Intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) from control and experimental mice were isolated by the modified method of Davies and Parrot [22] and Tagliabue et al. [23].

Briefly, a 12–15 cm long segment of the small intestine, cut longitudinally after surgical removal of Peyer's patches, was fragmented into 1–2 cm long pieces. These pieces after washing in Hanks' balanced salt solution (HBSS) were incubated in HBSS with 5 mM dithiothreitol (DTT) (Sisco, India) for 5–10 min at room temperature to remove mucus. The gut pieces incubated at 37°C in Ca^{2+},Mg^{2+}-free HBSS with 10^{-4} M EDTA for 20 min under constant stirring were washed 3–4 times in Ca^{2+},Mg^{2+}-free HBSS containing 5% foetal calf serum (FCS), at room temperature. The cells in the supernatant were washed twice, suspended in RPMI 1640 medium with 5% FCS and passed through a loosely packed glass wool column (GWC). They were then subjected to Ficoll Isopaque density gradient (Sigma Chemical Co., USA) centrifugation. The cells at the interface were harvested, washed and resuspended in RPMI 1640 medium with 10% FCS. These cells were labelled 'IEL'.

The gut pieces after the complete recovery of IEL were suspended in RPMI 1640 medium containing 10% FCS and 20 U ml^{-1} of collagenase (type IV, Sigma Chemical Co., USA). The cell suspension after incubation at 37°C for 10 min was passed through GWC before being subjected to Ficoll Isopaque density gradient centrifugation. The cells recovered at the interface were labelled 'LPL'.

2.7. Preparation of lymphocytes from Peyer's patches (PP) and spleen

The lymphocytes from spleen and PP were isolated by the method of Lycke et al. [24]. Briefly, the lymphocytes from both tissue suspensions were collected

separately by teasing them in HBSS between the ends of a frosted slide and passed through GWC. The resulting filtrate was centrifuged at $250 \times g$ for 10 min at 4°C and the supernatant was discarded. The washed cells of both populations were then separately subjected to Ficoll Isopaque gradient centrifugation. The cells at the interface of the gradient were collected and finally washed and suspended in RPMI/FCS.

2.8. Cell viability

The viability of IEL, LPL as well as PP and spleen lymphocytes was checked by the 0.2% trypan blue exclusion procedure [25].

2.9. Enumeration of T cell subsets and assessment of immunoglobulin bearing cells by flow cytometry [26]

Briefly, the T/B cell markers on IEL, LPL, PP and spleen lymphocytes were labelled by incubating the individual cell preparations with FITC-labelled anti-mouse Thy 1.2, L3T4 and Lyt 2.2 monoclonal antibodies (Becton Dickinson, USA) as well as FITC-labelled goat anti-mouse F(ab)$_2$ IgG, IgA and IgM (Kirkegaard and Perry, USA) for 30 min at room temperature in the dark. After washing with PBS, the cells were fixed with 0.5% formaldehyde at 4°C for 30 min and acquired on FACScan (Becton Dickinson, USA) to assess the percentage of fluorescent cells.

2.10. Humoral immune response

The levels of anti-ESP antibodies in the serum samples collected after completion of immunisation as well as infection at various days after challenge in all group of animals were checked by standard micro-ELISA assay [27].

2.11. Statistical analysis

The statistical analysis of data was done by employing one-way analysis of variance (ANOVA) with multiple comparisons and only values < 0.01 were considered as significant. All values are expressed as mean $\pm$ S.D.
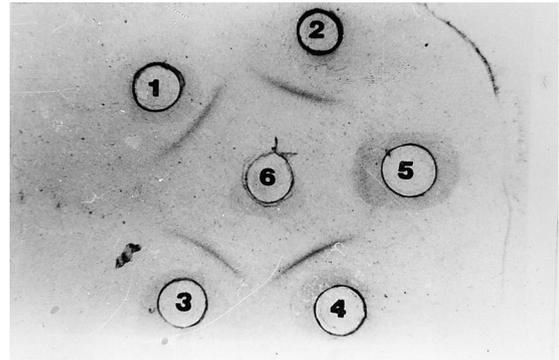
Fig. 2. Immunodiffusion pattern of the test sera of ESP-immunised mice. Wells 1, 2, 3 and 4 contain antisera from immunised mice (group 1); well 5 contains preimmune sera; well 6 contains the purified ESP.

3. Results and discussion

There has been much controversy in selecting a suitable mode of immunisation to stimulate an effective mucosal immunity. Parenteral immunisation has been the main vaccination route for infectious diseases. This route has been effective in preventing infectious diseases which have a systemic phase prior to or concomitant with their major pathological effects. However, such a route of immunisation has been at the best only marginally effective in preventing gut-dwelling infections [28–30]. The failure of the parenteral route of infection is obvious because the parenteral antigen administration fails to induce mucosal immunity [30]. However, the oral route has been found to be very effective at eliciting a specific mucosal immune response although it requires prolonged antigen administration in large amounts [20,30,31]. The oral immunisation of experimental animals with the purified ESP (as indicated by immunodiffusion, Fig. 2) was indeed found to stimulate the immune system very efficiently. The introduction of the ESP into the intestinal lumen also prevented the establishment of the *G. lamblia* trophozoites in the immunised-challenged group, whereas the infection established by 3–5 days p.i. in the intestine of infected control animals (group 3), after reaching peak levels at 9–11 days p.i., was spontaneously cleared by 17–21 days p.i. (Fig. 3).

Studies by Ferguson et al. [32] proposed that the mucosal T lymphocytes are likely to be involved in
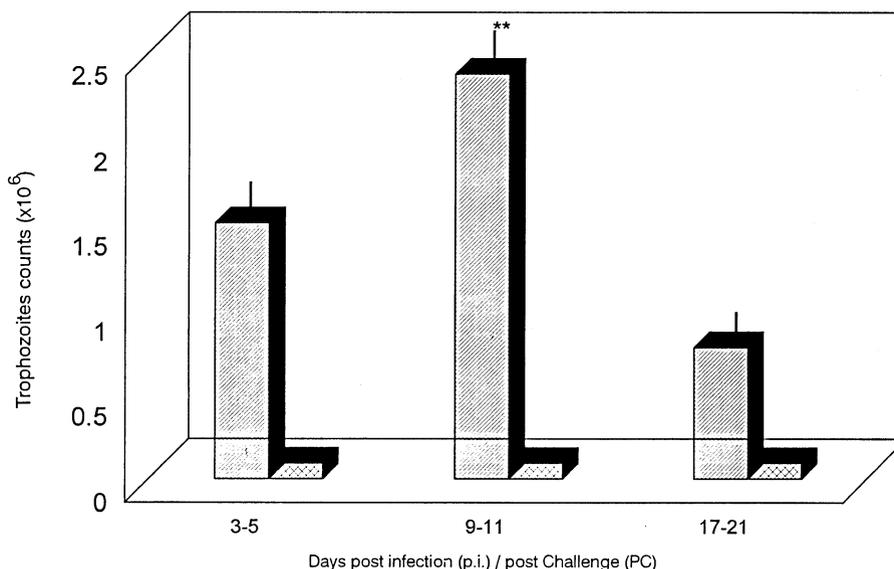
Fig. 3. Trophozoite load in the small intestine during the course of *G. lamblia* infection. hatched bars: infected group; cross-hatched bars, immunised-challenged group. Results are expressed as mean ± S.D. 3–5 days, establishment phase; 9–11 days, acute phase; 17–21 days, decline phase. **$P < 0.01$.

the immune reactions or in hypersensitivity reactions to the parasite. The data of the present study indicated that the immune responses at gut level during a primary *G. lamblia* infection involved the interactions of both intestine T cells and antibody-producing cells. The extraction of IEL and LPL using the modified method of Davies and Parrot [22] and Tagliabue et al. [23] produced high yields of both IEL and LPL per animal ($10.14 \pm 1.6 \times 10^6$ and $12.15 \pm 2.34 \times 10^6$ respectively) with $> 90\%$ viability.

Table 1
Percentage of T cell subsets and immunoglobulin-containing cells in intraepithelium (IEL)

Group	Days (p.i.;PI; PI/PC)	Total T	CD4+	CD8+	IgG	IgA	IgM
(a) Control	3–5	42.64 ± 0.99	21.86 ± 1.08	66.98 ± 2.58	2.45 ± 0.11	0.62 ± 0.43	0.62 ± 0.09
	9–11	43.56 ± 0.87	20.96 ± 1.04	67.63 ± 2.25	2.39 ± 0.23	0.61 ± 0.37	0.63 ± 0.13
	17–21	41.60 ± 0.93	22.90 ± 1.06	68.89 ± 2.63	2.42 ± 0.17	0.63 ± 0.49	0.61 ± 0.08
(b) Infected	3–5	56.66 ± 1.07**[a]	18.93 ± 0.59**[a]	78.25 ± 0.33**[a]	1.60 ± 0.08	0.62 ± 0.05	5.21 ± 0.17**[a]
	9–11	80.27 ± 0.93**[a]	35.54 ± 0.94**[a]	78.56 ± 0.95**[a]	2.73 ± 0.18	0.37 ± 0.02	1.25 ± 0.13**[a]
	17–21	74.73 ± 0.93**[a]	27.51 ± 0.93**[a]	69.76 ± 0.80	9.84 ± 0.76**[a]	6.35 ± 0.14**[a]	0.64 ± 0.44
(c) Immunised	3–5	60.72 ± 1.06**[a]	35.29 ± 1.02**[a]	62.56 ± 0.93*[a]	3.83 ± 1.09	1.21 ± 0.08	0.47 ± 0.15
	9–11	66.86 ± 0.95**[a]	38.67 ± 0.85**[a]	40.37 ± 1.18**[a]	7.72 ± 0.73**[a]	2.96 ± 0.43**[a]	1.25 ± 0.10**[a]
	17–21	70.51 ± 1.04**[a]	49.88 ± 0.90**[a]	27.44 ± 0.92**[a]	11.35 ± 0.83**[a]	7.42 ± 1.04**[a]	2.25 ± 0.10**[a]
Immunised-challenged	3–5	68.41 ± 1.01**[a,b,c]	33.56 ± 1.02**[a,c]	68.32 ± 0.98**[b,c]	4.69 ± 0.79*[a],**[b]	1.22 ± 0.36	0.59 ± 0.09**[b]
	9–11	68.73 ± 1.08**[a,b]	38.29 ± 0.98*[a],**[b]	49.64 ± 0.99**[a,b,c]	9.64 ± 0.13**[a,b,c]	2.81 ± 0.22**[a,b]	1.18 ± 0.06**[a]
	17–21	72.10 ± 0.90*[a],*[b]	52.38 ± 0.98**[a,b], *[c]	33.36 ± 0.94**[a,b,c]	13.68 ± 0.99**[a,b], *[c]	8.19 ± 0.06**[a,b]	2.60 ± 0.03**[a,b]

*$P < 0.05$; **$P < 0.01$ as compared to control (a); infected (b); immunised (c); immunised-challenged. All values are expressed as mean ± S.D. CD4+, helper/inducer T cells; CD8+, suppressor/cytotoxic T cells; p.i., post infection; PI, post immunisation; PI/PC, post immunisation/post challenge.

Table 2
Percentage of T cell subsets and immunoglobulin-containing cells in lamina propria (LPL)

Group	Days (p.i.;PI; PI/PC)	Total T	CD4+	CD8+	IgG	IgA	IgM
(a) Control	3–5	38.63 ± 0.91	25.42 ± 0.90	16.76 ± 0.93	22.19 ± 1.02	17.83 ± 0.88	3.08 ± 0.20
	9–11	39.59 ± 0.89	26.51 ± 0.79	17.81 ± 0.10	23.40 ± 1.05	16.90 ± 0.98	3.02 ± 0.15
	17–21	38.78 ± 0.98	24.91 ± 0.54	15.90 ± 0.88	21.35 ± 1.03	18.79 ± 0.77	3.05 ± 0.23
(b) Infected	3–5	60.33 ± 1.11**[a]	26.51 ± 0.53	23.40 ± 1.04**[a]	21.07 ± 0.93	17.76 ± 0.98	8.25 ± 0.36**[a]
	9–11	68.65 ± 0.85**[a]	25.49 ± 0.88	28.88 ± 1.03**[a]	23.48 ± 0.78	18.76 ± 0.35	3.53 ± 0.32*[a]
	17–21	56.50 ± 0.88**[a]	33.83 ± 0.94**[a]	16.10 ± 0.20	32.94 ± 0.86**[a]	33.06 ± 0.54**[a]	2.16 ± 0.68**[a]
(c) Immunised	3–5	80.64 ± 0.86**[a]	33.90 ± 5.32*[a]	43.65 ± 1.18**[a]	7.46 ± 0.56**[a]	4.54 ± 0.23**[a]	3.08 ± 0.28
	9–11	59.34 ± 0.69**[a]	25.84 ± 0.82	18.83 ± 0.82	10.76 ± 0.86**[a]	11.00 ± 0.89**[a]	3.03 ± 0.07
	17–21	4.97 ± 0.65**[a]	2.89 ± 0.89**[a]	1.31 ± 0.09**[a]	58.57 ± 1.14**[a]	50.33 ± 0.98**[a]	3.06 ± 0.21
Immunised-challenged	3–5	86.38 ± 0.02**[a,b,c]	37.26 ± 0.94**[a,b]	43.99 ± 1.26**[a,b]	7.96 ± 0.60**[a,b]	4.73 ± 0.70**[a,b]	3.09 ± 0.22
	9–11	60.22 ± 0.90**[a]	25.84 ± 0.84	16.73 ± 0.90**[b]	17.89 ± 0.84** [a,b,c]	15.30 ± 0.03** [a,b,c]	3.06 ± 0.10**[b]
	17–21	18.36 ± 0.89**[a,b,c]	2.97 ± 0.80**[a,b]	1.48 ± 0.41**[a,b]	53.34 ± 0.70** [a,b],*[c]	52.55 ± 0.48** [a,b],*[c]	3.08 ± 0.25

*$P < 0.05$; **$P < 0.01$ as compared to control (a); infected (b); immunised (c); immunised-challenged. All values are expressed as mean ± S.D. CD4+, helper/inducer T cells; CD8+, suppressor/cytotoxic T cells; p.i., post infection; PI, post immunisation; PI/PC, post immunisation/post challenge.

The separation of PP and splenic lymphocytes also provided a pure population of viable ($>90\%$) cells as assessed by 0.2% trypan blue exclusion. The cell count was $5.6 \pm 0.86 \times 10^7$ sp per animal and $9.3 \pm 0.34 \times 10^6$ pp per animal). The differential responses of IEL and LPL observed during the colonisation, peak and decline phases of giardial infection were considered specific to *G. lamblia* infection since the observed alterations occurred following the inoculation of trophozoites. No alterations in the

Table 3
Percentage of T cell subsets and immunoglobulin-containing cells in Peyer's patches (PP)

Group	Days (p.i.;PI; PI/PC)	Total T	CD4+	CD8+	IgG	IgA	IgM
(a) Control	3–5	28.17 ± 0.84	17.44 ± 0.75	6.20 ± 0.54	29.03 ± 0.95	4.52 ± 0.26	25.55 ± 0.87
	9–11	29.21 ± 0.86	17.99 ± 0.80	6.88 ± 0.57	29.98 ± 0.92	4.93 ± 0.70	26.39 ± 0.81
	17–21	28.31 ± 0.85	17.53 ± 1.01	6.19 ± 0.63	29.79 ± 0.91	4.52 ± 0.26	25.55 ± 0.87
(b) Infected	3–5	47.92 ± 0.94**[a]	30.72 ± 0.70**[a]	6.42 ± 0.79	29.88 ± 0.54	10.27 ± 0.66**[a]	33.64 ± 5.43**[a]
	9–11	38.97 ± 0.93**[a]	17.74 ± 0.94	6.82 ± 0.69	29.25 ± 0.65	16.66 ± 0.94**[a]	47.60 ± 0.40**[a]
	17–21	29.06 ± 0.92	17.53 ± 1.01	6.36 ± 0.84	29.28 ± 1.15	30.67 ± 0.97**[a]	32.18 ± 0.60**[a]
(c) Immunised	3–5	10.35 ± 0.85*[a]	9.07 ± 0.88**[a]	20.00 ± 0.93**[a]	28.01 ± 0.99	13.49 ± 1.17**[a]	12.34 ± 0.88**[a]
	9–11	19.44 ± 0.88*[a]	17.39 ± 0.92	9.36 ± 0.89*[a]	53.73 ± 0.89**[a]	3.76 ± 0.85	40.35 ± 0.87**[a]
	17–21	18.22 ± 0.62*[a]	12.07 ± 0.88	4.45 ± 0.95	81.65 ± 0.97**[a]	3.62 ± 0.89	48.30 ± 0.72**[a]
Immunised-challenged	3–5	16.16 ± 0.97**[a,b,c]	10.55 ± 0.80** [a,b],*[c]	21.85 ± 0.95** [a],*[c]	39.08 ± 0.69** [a],*[b]	16.53 ± 0.06**[a,b,c]	19.19 ± 0.99* [a,c],**[b]
	9–11	23.18 ± 0.91**[a,b,c]	17.76 ± 0.88	10.47 ± 0.93**[a,b]	58.38 ± 1.00** [a,c],*[b]	4.37 ± 0.10**[b]	44.62 ± 0.70**[a,b,c]
	17–21	21.62 ± 0.92**[a,b,c]	9.46 ± 0.94**[a,b,c]	5.16 ± 0.96	83.33 ± 0.90**[a,b]	10.77 ± 0.97**[a,b]	54.39 ± 0.99**[a,b,c]

*$P < 0.05$; **$P < 0.01$ as compared to control (a); infected (b); immunised (c); immunised-challenged. All values are expressed as mean ± S.D. CD4+, helper/inducer T cells; CD8+, suppressor/cytotoxic T cells; p.i., post infection; PI, post immunisation; PI/PC, post immunisation/post challenge.

lymphocyte T cell subsets or antibody-producing cells were noticed in uninfected control animals kept under identical conditions.

The establishment and acute phases of *G. lamblia* infection in the infected animals in the present study accompanied a significant influx of Lyt 2 (suppressor/cytotoxic lymphocytes) (Tables 1 and 2) and a significant decline in IgA-bearing plasma cells (Tables 1 and 2) in the gut (IEL/LPL). It has earlier been shown that the intestinal lymphocytes isolated during the course of giardial infection from NMRI mice were incapable of killing the *G. lamblia* trophozoites in vitro either in the presence or in the absence of *G. lamblia*-specific antibodies. Thus the Lyt 2 cells influxed as a result of *Giardia* infection appeared not to be cytotoxic T cells in nature but were suppressor T cells [33]. This influx of suppressor T cells during establishment and peak phases of giardial infection has been suggested by these authors to be due to the feedback suppressor pathway which might have been triggered very early or preferentially after the immune induction following *G. lamblia* infection due to the release of some ES products by the multiplying trophozoites. An early selective localisation of suppressor T cells observed in the infected animals may therefore explain the significantly low level of IgA-bearing cells in the IEL and LPL of these animals as well as in the lamina propria of immunised animals during establishment and peak phases of giardial infection. The fact that ES products signal the generation and localisation of Lyt 2 cells in the gut accounts for a significant induction of CD8+ T cells in the PP of immunised animals (Table 3), which is the main site of antigen presentation. The present study, however, indicates a significant increase in the percentage of IgA-bearing cells in the PP of immunised as well as immunised-challenged animals (Table 3), which might have been responsible for the inhibition of *G. lamblia* trophozoites to be colonised in the intestine.

Further, it was observed that the decline phase of *G. lamblia* infection in the infected as well as the immunised and immunised-challenged animals involved a selective induction of CD4+ T cells in the IEL (Table 1) and LPL (Table 2) along with a significant reduction of CD8+ T cells (Tables 1 and 2). This phase of infection was also found to be accompanied by an increased number of IgA- and IgG-

bearing cells (Tables 1 and 2 respectively). It thus appears that the inducer T cells may be responsible for the induction of antibody-dependent effector responses. It has earlier been noticed that s-IgA bound to the surface of *Giardia* trophozoites results in the clearance of the same from the gut of infected animals [34,35] while parasites with negligible surface-bound s-IgA persisted during the decline phase of infection [36,37]. The anti-giardial IgA responses have also been noticed to be altered in mice depleted of T helper lymphocytes [35]. Indeed it has earlier been observed that the clearance of *G. muris* infection in mice is regulated by induction of CD4+ T cells [35]. Based on the findings of others and ourselves, we feel that a significant enhancement of T helper/inducer activity along with a significant enhancement of IgA-bearing cells results in the clearance of the trophozoites from the intestine. At the same time, the role of IgG in the clearance of *Giardia* cannot be ignored, since a significant increase in the IgG-bearing cells during the decline phase of the experimental animals was observed, although surface-bound parasite-specific IgG antibodies on the trophozoites of *Giardia* isolated from the gut lumen of mice reported by Heyworth et al. [38] suggested a possible role of IgG in the modulation of the infection at gut level. However, the involvement of locally synthesised IgG in providing long-lasting protection against *G. lamblia* requires further investigation.

In contrast, the establishment phase of *G. lamblia* infection in the IEL, LPL as well as PP of the infected mice was accompanied by proliferation of IgM-bearing cells (Tables 1 and 2 respectively) suggesting that during the early stage of *G. lamblia* infection the immunoglobulin production was predominantly of the IgM isotype. This observation agrees well with the earlier reports in human giardiasis [39–41]. The proliferation of IgM-bearing cells during the acute phase of the disease is not affected by the feedback suppressor pathway as the initial differentiation of pre-B cells to immune IgM-bearing cells is independent of T cells or lymphokines [42].

The present data demonstrate that the prior immunisation of mice with ESP resulted in a significant stimulation of local immunity as evidenced by a significant increase in IgA- and IgG-bearing cells in the LPL-immunised animals (Table 2) which probably prevented the establishment of *G. lamblia* tropho-

Table 4
Percentage of T cell subsets and immunoglobulin-containing cells in spleen (SP)

Group	Days (p.i.;PI; PI/PC)	Total T	CD4+	CD8+	IgG	IgA	IgM
(a) Control	3–5	56.15 ± 0.90	25.10 ± 0.99	27.26 ± 0.96	52.68 ± 0.98	8.22 ± 0.34	32.71 ± 0.92
	9–11	57.17 ± 0.92	24.23 ± 0.81	28.31 ± 0.91	53.70 ± 0.91	8.99 ± 0.40	33.56 ± 0.90
	17–21	58.14 ± 0.91	24.40 ± 0.87	26.46 ± 0.94	52.93 ± 0.94	9.03 ± 0.63	34.69 ± 0.91
(b) Infected	3–5	26.38 ± 0.98**[a]	25.21 ± 0.91	6.49 ± 0.54**[a]	53.29 ± 0.98	1.96 ± 0.34**[a]	33.06 ± 0.93
	9–11	24.32 ± 0.92**[a]	7.69 ± 0.91**[a]	3.33 ± 0.07**[a]	35.53 ± 0.95**[a]	11.39 ± 0.89**[a]	13.74 ± 0.96**[a]
	17–21	50.28 ± 0.92**[a]	27.29 ± 0.92*[a]	11.49 ± 1.01**[a]	58.40 ± 0.90**[a]	2.63 ± 0.13**[a]	48.25 ± 0.95**[a]
(c) Immunised	3–5	43.56 ± 0.94**[a]	31.43 ± 0.91**[a]	5.64 ± 0.95**[a]	49.37 ± 1.01**[a]	0.89 ± 0.16**[a]	50.50 ± 0.90**[a]
	9–11	39.31 ± 0.91**[a,b,c]	27.27 ± 0.28	4.21 ± 0.28**[a]	58.24 ± 0.94**[a]	0.31 ± 0.05**[a]	34.05 ± 0.93
	17–21	32.94 ± 0.96**[a]	24.96 ± 0.94	8.60 ± 1.46**[a]	62.67 ± 0.97**[a]	0.83 ± 0.13**[a]	34.33 ± 0.93
Immunised-challenged	3–5	55.84 ± 0.95**[b,c]	23.12 ± 0.94**[c]	11.18 ± 0.98** [a,b,c]	55.39 ± 0.99*[a],** [c]	1.69 ± 0.43**[a],*[c]	39.53 ± 0.98**[a,c]
	9–11	50.91 ± 0.98**[a,b,c]	28.76 ± 0.96**[a,b]	9.98 ± 0.97** [a,b,c]	22.90 ± 0.99**[a,b,c]	0.63 ± 0.35**[a,b]	13.05 ± 0.90**[a,c]
	17–21	54.61 ± 0.99**[b,c]	32.69 ± 0.99** [a,b,c]	13.82 ± 0.96**[a,c]	2.48 ± 0.02**[a,b,c]	0.20 ± 0.10**[a,b,c]	5.66 ± 0.96**[a,b,c]

*$P < 0.05$; **$P < 0.01$ as compared to control (a); infected (b); immunised (c); immunised-challenged. All values are expressed as mean ± S.D. CD4+, helper/inducer T cells; CD8+, suppressor/cytotoxic T cells; p.i., post infection; PI, post immunisation; PI/PC, post immunisation/post challenge.

zoites in the immunised-challenged animals. Further, it was observed that the IgM-bearing cells in the LPL of the immunised animals (Table 2) and control animals were statistically identical. These findings point to the fact that the genetic switching of IgM-bearing cells to those recognising the antigen through receptors of the IgG and IgA classes has been completed during the immunisation schedule and memory IgG and IgA clones which were formed gave a booster response upon challenge in immunised animals.

A significant decline in the percentage of total T cells was observed within the PP of the immunised and immunised-challenged animals during 3–5 days PI/PC (Table 3). It appears that most of the activated T cells including CD4+ T cells might have migrated to the distal mucosal effector sites, such as the lamina propria, as the percentage of these cells was found to be significantly increased in the LPL and IEL of these animals (Tables 2 and 1 respectively) where further stimulation and terminal differentiation into antibody-producing cells takes place [43–45]. Less is known about the induction and trafficking of primed T cells after oral immunisation, particularly in man. However, immunisation of PP with keyhole limpet haemocyanin in an animal mod-

el induced a population of primed T cells which suggested within the circulation before homing specifically to the gut [46]. In the gut, immunisation by the oral or enteral route has been found to effectively stimulate a specific mucosal immune response [20,47]. The immunogen probably makes contact with immunocompetent B and T cells in the PP, and these cells, while differentiating, then migrate to the mesenteric lymph nodes and further via the thoracic duct, enter into the systemic circulation. Committed, migrating cells eventually return to the intestinal mucosa where further stimulation and terminal differentiation into antibody-producing cells takes place [43,48]. On repeated immunisation, the immunogen may also, to a lesser extent, directly stimulate antigen-specific memory cells in gut lamina propria [49,50]. It remains poorly defined whether the IgA class predominance of the gut mucosal response is already initiated in the PP or results from later influences on the B cells, i.e., while en route to or during this final differentiation in the lamina propria [51,52].

In the spleen (Table 4) of immunised animals, we found a significant increase in the percentage of CD4+ T cells. An increase in CD4+ T cells appears to be beneficial as it related very well with elimina-

tion of *G. lamblia* infection where it has been seen that when the local immunologic response is at its peak, *G. lamblia* is eliminated from the gut. We also found a sharp decline in IgA-bearing cells on the one hand and a significant increase in IgM-bearing cells on the other, in the spleen of the immunised animals during the establishment phase of the infection. It is quite likely from these findings that IgM may have taken over the functions of IgA resulting in the clearance of *G. lamblia* trophozoites. An experimental study of giardiasis utilising anti-IgM-treated mice also indicated the importance of IgM in regulation of giardial infection [14]. Farthing [53] has also reported that anti-giardial IgM potentiates the lytic effects of complement on *G. lamblia*. The role of *Giardia*-specific IgM response in regulating the disease process especially in immunocompromised hosts has also been elucidated [54].

However, in-depth investigations are required especially in view of the fact that the parasite remains confined to the intestinal lumen without invading the intestinal mucosa. It may thereby fail to elicit either a systemic inflammatory response or alterations in lymphocyte subsets in the peripheral blood [55]. The small bowel morphological changes observed during *G. lamblia* infection are believed to be determined, at least in part, by T cell-dependent activity [13]. Indeed, it is the gut-associated lymphoid tissue that plays a prominent role in the host defense against intestinal pathogens. The cells express mainly the CD4 antigen in the lamina propria whereas T cells expressing the CD8 antigen and NK cells comprise the majority of the IEL [56,57]. It remains to be determined to what degree the local intestinal immune response and changes in intestinal lymphocyte subsets are correlated by those detected in the peripheral blood [55]. Further studies are therefore needed to elucidate the changes in intestinal mucosal lymphocyte subsets occurring during *G. lamblia* infection and to correlate them with lymphocyte subset distribution in the circulation.

The present study further revealed a significant increase in the population of total T as well as CD4+ T cells in the PP of infected animals (Table 3) during the establishment phase of infection. Earlier reports on PP 'switch' T cells which possess the surface antigens and the known regulatory functions of T cells led to the hypothesis that an increase in the number of relative percentage of PP CD4+ T cells initiates subsequent events in the intestinal immune response [58]. The relative deficiency of these cells, as compared to CD8+ T cells in immunodeficient nude mice, provided indirect support for this hypothesis [59].

The cellular events within PP that follow antigen presentation are not well understood. 'Switch' T cells, which are T helper cells, have been isolated from PP and in vitro have been demonstrated to participate in the immunoglobulin isotype switching that occurs with maturation of B cells into functional plasma cells [60]. In vitro cloned PP 'switch' T cells proliferate when exposed to autologous B cells, T cells and macrophages, suggesting that cell-cell interactions are important in the generation of these 'switch' T cells [61].

Thus, from the present study, it appears that IgA antibodies act as a first line of defense for immune exclusion of the parasite at the mucosal surface, i.e., blocking the colonisation of mucosal surface by the parasite. On the other hand, the presence of circulating anti-giardial antibodies [62–64] in clinical giardiasis patients and specific serum IgG and IgA antibodies in experimental giardiasis [65] in mice indicates stimulation of the host humoral immune system. The demonstration of anti-ESP antibodies observed in the serum of immunised animals (Fig. 4) in the present study indicates that the antigen must cross the intestinal mucosa to stimulate the systemic lymphoid tissue. These antibodies may also be due to transepithelial translocation of immunoglobulins from the intestinal lumen produced by local (mucosal) antibody-producing cells stimulated by the parasite antigen [66].

The development of in vitro culture techniques for *Giardia* a decade ago has secured an adequate supply of antigen such that serum antibody responses to *Giardia* are beginning to be well characterised, most commonly by ELISA. Experimental infections in mice confirm the appearance of specific anti-*Giardia* IgA and IgG in intestinal secretions and clearance of the parasite relates closely to rising concentrations of these antibodies in the intestinal fluid.

Although circulating anti-giardial antibodies have been demonstrated in individuals suffering from giardiasis [61,64], their precise biological role has remained unclear. Vinayak et al. [33] have demon-

A

☒ [3 - 5] days ☐ [9 - 11] days ⬚ [17 - 21] days

Serum IgA

B

Serum IgM

C
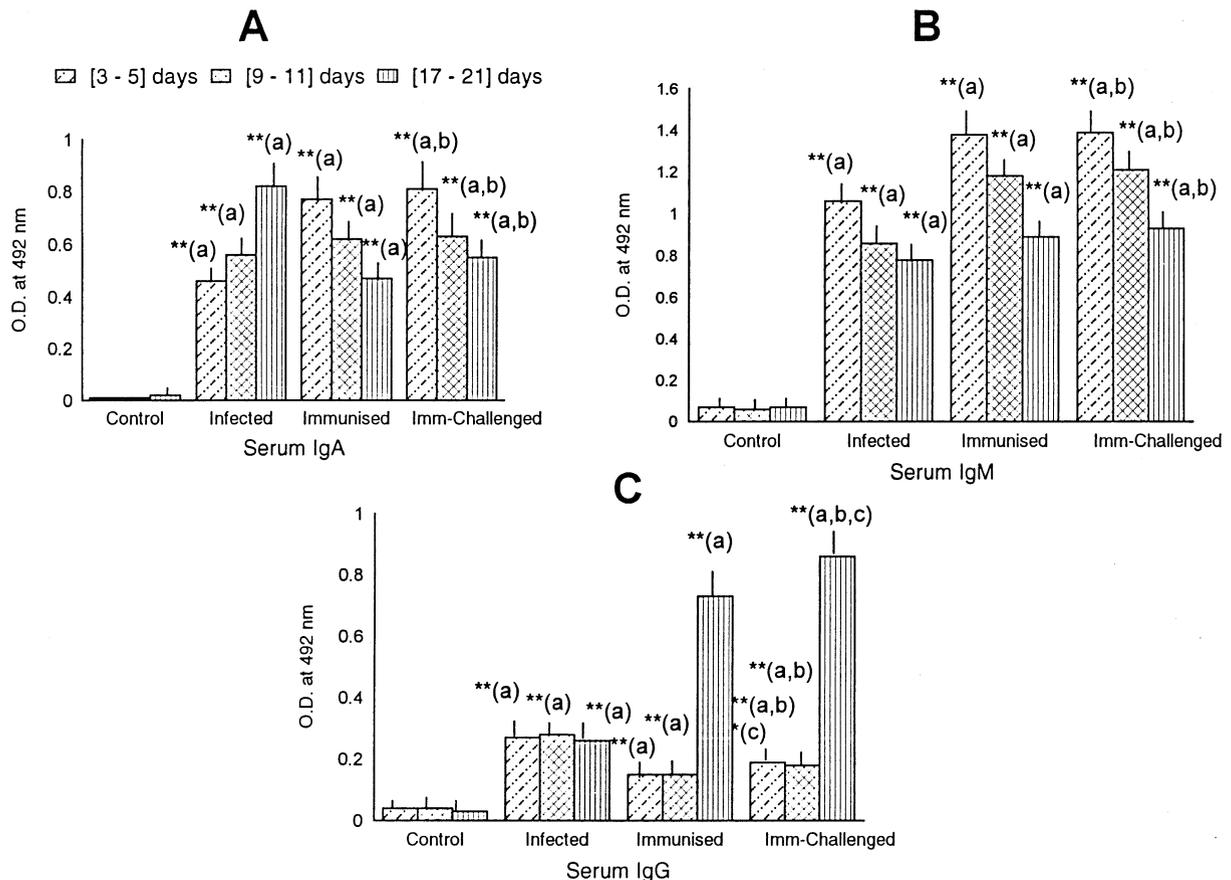
Serum IgG

Fig. 4. Humoral immune response (levels of anti-ESP antibodies in serum). A: Serum IgA. B: Serum IgM. C: Serum IgG. Bars indicate 3–5, 9–11 and 17–21 days post infection (p.i.), post immunisation/post challenge (PI/PC). *$P < 0.05$; **$P < 0.01$ as compared to control (a); infected (b); immunised (c); immunised-challenged. Results are expressed as mean ± S.D. of three separate experiments.

strated that a specific antibody response to plasma membrane (PM) and surface antigen GLSA 56 occurs in giardiasis. Comparison of groups of patients revealed low levels of antibodies against PM and GLSA 56 in persistent giardiasis, compared with non-persistent and asymptomatic cases.

In the present study, the levels of IgA, IgM as well as IgG were significantly increased in the immunised as well as immunised-challenged animals as compared to those of unimmunised control animals (Fig. 4). We observed maximum levels of IgM in the serum of the immunised as well as immunised-challenged animals (Fig. 4) which was found to be significantly higher compared to those of IgA and IgG serum levels (Fig. 4A,B respectively) at both 3–5 and 9–11 days PI/PC. But subsequently, the lev-

els of IgG in the serum increased significantly as compared to those of IgM and IgA levels at 17–21 days PI/PC. It has been reported that anti-giardial IgM is produced earlier in infection, but antibody titres decrease rapidly within 2–3 weeks after the onset of infection [67]. An experimental study of giardiasis utilising anti-IgM-treated mice has also indicated the importance of IgM in regulation of giardial infection [14]. We further observed that unimmunised (control) animals had negligible levels of anti-ESP antibodies (Fig. 4). It may, therefore, suggest that the attainment of a significant level of anti-ESP antibodies resulted in the complete elimination of the parasite. In other words, the clearance of *G. lamblia* infection correlated well with the development of anti-ESP antibodies. Therefore, the possibil-

ity exists that the ES antigen from the live tropho-zoites was absorbed across the intestinal mucosa. Alternatively there may have been an interaction of viable trophozoites with the surface epithelium resulting in activation and uptake by tissue macrophages. In support of this hypothesis is the work by Owen et al. [68], who demonstrated phagocytosis of *G. muris* by macrophages present in mouse PP. The immunisation of animals with ES antigen resulted in a significant titre of anti-ESP antibodies indicating that this protein is highly immunogenic and it seems that ESP is one of the major antigens of *G. lamblia* responsible for protection against the disease.

It is therefore pertinent to conclude that ESP of the parasite can be considered a candidate for immunoprophylactic studies. Such an agent may have a useful role in protecting vulnerable populations like children or persons from non-endemic areas travelling to endemic areas. Detailed investigations are essential to study the mechanisms by which the parasite evades the effector immune mechanisms. Nevertheless, the investigations quoted above clearly indicate that although gut-associated immune mechanisms are involved in regulating giardial infection, other effector immune mechanisms like mucosal mast cells, macrophages and other non-specific intestinal factors cannot be ignored.

References

[1] Hartong, W.A., Gourley, W.K. and Arvanitakis, C. (1979) Giardiasis in clinical spectrum and functional structural abnormalities of small intestine mucosa. Gastroenterology 77, 61–69.

[2] Char, S., Shetty, N., Narasimha, M., Elliott, E., Macaden, R. and Farthing, M.J.G. (1991) Serum antibody response in children with *Giardia lamblia* infection and identification of an immunodominant 57 kDa antigen. Parasite Immunol. 13, 329–337.

[3] Clark, J.T. and Holberton, D.V. (1986) Plasma membrane isolated from *Giardia lamblia*: identification of membrane proteins. Eur. J. Cell. Biol. 42, 200–206.

[4] Crossley, R. and Holberton, D.V. (1983) Characterisation of proteins from the cytoskeleton of *Giardia lamblia*. J. Cell. Sci. 59, 81–103.

[5] Edson, C.M., Farthing, M.J.G., Thorley-Lawson, D.A. and Keusch, G.T. (1986) An 88000 MW *Giardia lamblia* surface protein which is immunogenic in humans. Infect. Immun. 54, 621–665.

[6] Heyworth, M.F. (1990) Biological significance of *Giardia* specific antibodies. West. J. Med. 152, 293–295.

[7] Heyworth, M.F. and Vergara, J.A. (1994) *Giardia muris* trophozoite antigenic targets for mouse intestinal IgA antibody. J. Infect. Dis. 169, 395–398.

[8] Kum, K., Khanna, R., Mehta, S., Khuller, M. and Vinayak, V.K. (1987) Plasma membrane associated antigen of trophozoites of axenic *G. lamblia*. Trans. R. Soc. Trop. Med. Hyg. 82, 439–444.

[9] Rosof, J.D. and Stibbs, H.H. (1986) Isolation and identification of a *Giardia lamblia*-specific stool antigen (GSA 65) useful in coprodiagnosis of giardiasis. J. Clin. Microbiol. 23, 905–910.

[10] Vinayak, V.K., Dutt, P. and Puri, M. (1991) An immunoenzymatic dot-ELISA for the detection of *Giardia lamblia* antigen in stool eluates of clinical cases of giardiasis. J. Immunol. Methods 137, 245–251.

[11] Vinayak, V.K., Kum, K., Chandna, R., Venkateshwarlu, K. and Mehta, S. (1985) Detection of *Giardia lamblia* antigens in the faeces by counterimmunoelectrophoresis. Pediat. Infect. Dis. J. 4, 383.

[12] Ament, M.E. and Rubin, C.E. (1972) Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes. Gastroenterology 62, 216–226.

[13] Roberts-Thomson, I.C. and Mitchell, G.F. (1978) Giardiasis in mice. I. Prolonged infections in certain mouse strains and hypothymic (nude) mice. Gastroenterology 75, 57–61.

[14] Snider, D.P., Gordon, J., McDermott, M.R. and Undertown, B.J. (1985) Chronic *Giardia muris* infection in anti-IgM treated mice. Analysis of immunoglobulin and parasite specific antibody in normal and immunoglobulin deficient animals. J. Immunol. 134, 4153–4162.

[15] Stevens, D.P., Frank, D.M. and Mahmoud, A.A.F. (1978) Thymus dependency of host resistance to *Giardia muris* infection: Studies in nude mice. J. Immunol. 120, 680–682.

[16] Holberton, D.V. and Ward, A.P. (1981) Isolation of the cytoskeleton from *Giardia*. Tubulin and a low molecular weight protein associated with microribbon structures. J. Cell. Sci. 47, 139–166.

[17] Scott, M.J. and Snary, D. (1979) Protective immunisation of mice using cell surface glycoprotein from *Trypanosoma cruzi*. Nature 282, 73–74.

[18] Aggarwal, A., Sharma, G.L., Bhatia, A., Naik, S.R., Chakravarti, R.N. and Vinayak, V.K. (1980) Effects of corticosteroid and irradiation on experimental *G. lamblia* infection in mice. Ann. Trop. Med. Parasitol. 74, 361–371.

[19] Ouchterlony, O. (1962) Diffusion in gel methods for immunological analysis II. In: Progress in Allergy (Kallos and Waksman, B.H., Eds.), Vol. VI, pp. 30–154. Basel, Karger.

[20] Lange, S. and Holmgren, J. (1978) Protective antitoxic cholera immunisation in mice: influence of route and number of immunisations and mode of action of protective antibodies. Acta Pathol. Microbiol. Scand. C Immunol. 86, 145–152.

[21] Vasudeva, V., Ganguly, N.K., Anand, B.S., Radhakrishnan, V.D. and Mahajan, R.C. (1982) A study of *Giardia* infection in irradiated and thymectomised mice. J. Trop. Med. Hyg. 85, 119–122.

[22] Davies, M.D.J. and Parrott, D.M.V. (1981) Preparation and purification of lymphocytes from the epithelium and lamina propria of murine small intestine. Gut 22, 481–488.

[23] Tagliabue, A., Befus, A.D., Clark, D.A. and Bienenstock, J. (1982) Characteristics of natural killer cells in the murine intestinal epithelium and lamina propria. J. Exp. Med. 155, 1785–1796.

[24] Lycke, N., Lindholm, L. and Holmgren, J. (1983) IgA isotype restriction in the mucosal but not in the extramucosal immune response after oral immunisations with cholera toxin or cholera B subunit. Int. Arch. Allergy Appl. Immunol. 72, 119–127.

[25] Jondal, M., Holm, G. and Wigzell, H. (1972) Surface markers on human lymphocytes. J. Exp. Med. 136, 207–215.

[26] Hornquist, E., Goldschmidt, J., Holmdahl, R. and Lycke, N. (1991) Host defense against cholera toxin is strongly CD4+ T cell dependent. Infect. Immun. 59, 3630–3638.

[27] Voller, A., Bidwell, D. and Bartlett, A. (1976) Enzyme immunoassay in diagnostic medicine. Theory and Practice. Bull. WHO, 53–55.

[28] Newcomb, R.W., Ischizaka, K. and Denald, B.L. (1969) Human IgG and IgA diphtheria antitoxins in serum, nasal fluids and saliva. J. Immunol. 103, 215–219.

[29] Ogra, P. (1971). The secretory immunoglobulin system of the gastrointestinal tract. In: The Secretory Immunological System (Dayton, D.H. Jr., Small, P.A. Jr., Chanock, R.M. and Tomasi, T.B. Jr., Eds.), p. 259. US Government Printing Office, Washington, DC.

[30] Pierce, N.F. and Gowans, J. (1975) Cellular kinetics of the intestinal immune response to cholera toxoid in rats. J. Exp. Med. 142, 1550–1563.

[31] Dolezel, J. and Bienenstock, J. (1971) Gamma A and non gamma A immune response after oral and parenteral immunisation of hamster. Cell. Immunol. 2, 458–469.

[32] Ferguson, A., McClure, S.P. and Townley, R.R.W. (1976) Epithelial lymphocytes infiltration in biopsies from children with diarrhoea. Acta Pediatr. Scand. 65, 541–546.

[33] Vinayak, V.K., Kum, K. and Khanna, R. (1989) Serum antibodies to giardial surface antigens: lower titres in persistent than in non-persistent giardiasis. J. Med. Microbiol. 30, 207–212.

[34] Heyworth, M.F. (1989) Intestinal IgA response to Giardia muris depleted of helper T lymphocytes in immunocompetent mice. J. Parasitol. 75, 246–251.

[35] Waight Sharma, A. and Mayrhofer, G. (1988) Biliary antibody responses in rats infected with rodent Giardia duodenalis isolates. Parasite Immunol. 10, 181–191.

[36] Khanna, R., Joshi, K., Kum, K., Malik, A.K. and Vinayak, V.K. (1990) An ultrastructural analysis of changes in surface architecture of intestinal mucosa following Giardia lamblia infection in mice. Gastroenterol. Japon. 25, 649–658.

[37] Vinayak, V.K., Khanna, R. and Kum, K. (1990) Gut associated immune responses in clinical and experimental giardiasis: an overview. Trop. Gastroenterol. 11, 4–8.

[38] Heyworth, M.F., Kung, J.E. and Eriksson, E.C. (1986) Clearance of Giardia muris infection in mice deficient in natural killer cells. Infect. Immun. 54, 903–904.

[39] Goka, A.K.J., Rolston, D.D.K., Mathan, V.I. and Farthing, M.J.G. (1987) Human serum IgA response to Giardia lamblia. Gut 28, A1351.

[40] Ridley, M.J. and Ridley, D.S. (1976) Serum antibodies and jejunal histology in giardiasis associated with malabsorption. J. Clin. Pathol. 29, 30–34.

[41] Thompson, A., Rowland, R. and Hecker, R. (1977) Immunoglobulin bearing cells in giardiasis. J. Clin. Pathol. 30, 292–294.

[42] Abney, E.R., Cooper, M.D., Kearney, J.F., Lawton, A.R. and Parkhouse, R.M.E. (1978) Sequential expression of immunoglobulin in developing mouse B lymphocytes: a systemic survey that suggests a model for the generation of immunoglobulin class diversity. J. Immunol. 120, 2041–2049.

[43] Biewenga, J., VanRees, E.P. and Sminia, T. (1993) Induction and regulation of IgA responses in microenvironment of gut. Clin. Immunol. Immunopathol. 67, 1–7.

[44] Czerkinsky, C., Svennerholm, A.M. and Holmgren, J.F. (1993) Induction and assessment of immunity at extramucosal surfaces in humans: Implications for vaccine development. Clin. Infect. Dis. 16, 106–116.

[45] Miller, C.J., McGhee, J.R. and Gardner, M.B. (1992) Biology of disease, mucosal immunity, HIV transmission and AIDS. Lab. Invest. 68, 129–145.

[46] Dunkley, M.L. and Husband, A.J. (1986) The induction and migration of antigen-specific helper cells for IgA responses in the intestine. Immunology 15, 379–385.

[47] Crabbe, P.A., Nash, D.R., Bazin, H., Eyssen, H. and Heremans, J.F. (1967) Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunisation with ferritin. J. Exp. Med. 130, 723–744.

[48] Husband, A.J. and Gowans, J.L. (1978) The origin and antigen dependent distribution of IgA containing cells in the intestine. J. Exp. Med. 148, 1146–1160.

[49] Husband, A.J. (1982) Kinetics of extravasation and redistribution of IgA-specific antibody containing cells in the intestine. J. Immunol. 128, 1355–1359.

[50] Pierce, N.F. and Cray, W.C. (1982) Determinants of the localisation, magnitude and duration of a specific IgA plasma cell response in enterically immunised rats. J. Immunol. 128, 1311–1315.

[51] Elson, C.O., Heck, J.A. and Strober, W. (1979) T cell regulation of murine IgA synthesis. J. Exp. Med. 149, 632–643.

[52] Tseng, J. (1981) IgA allotype suppression in mice: a cellular implication for the IgA preponderance in the gut. Cell. Immunol. 65, 247–257.

[53] Farthing, M.J.G. (1993) Diarrhoeal disease: Current concepts and future challenges: Pathogenesis of giardiasis. Trans. Soc. Trop. Med. Hyg. 87, Suppl. 3, 17–21.

[54] Khanna, R., Kum, K. and Vinayak, V.K. (1990) Gut-associated immune effector responses in immunocompetent and immunocompromised mice with Giardia lamblia. FEMS Microbiol. Immunol. 2, 137–145.

[55] Schlesinger, M., Granot, E., Rabinowitz, R. and Deckelbaum, R.J. (1993) Peripheral blood lymphocyte subsets in infants with diarrhoea with and without Giardia lamblia infection. Pediatr. Res. 33, 15–18.

[56] Kelly, J., O'Farrelly, C., O'Mahoney, C., Weir, D.G. and Feighery, C. (1987) Immunoperoxidase demonstration of the cellular composition of the normal and coeliac small bowel. Clin. Exp. Immunol. 68, 177–188.

[57] Selby, W.S., Janossy, G., Bofill, M. and Jewell, D.P. (1983) Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. Clin. Exp. Immunol. 52, 219–228.

[58] Strober, W. (1982) The regulation of mucosal immune system. J. Allergy Clin. Immunol. 70, 225–230.

[59] Carlson, J.R., Heyworth, M.F. and Owen, R.L. (1985) Response of Peyer's patch T cell subpopulation to *Giardia muris* infection (abstr.) Clin. Res. 33, 32A.

[60] Kawanishi, H., Saltzman, L.E. and Strober, W. (1983) Mechanisms regulating IgA class specific immunoglobulin production in murine gut associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells in vitro. J. Exp. Med. 157, 433–450.

[61] Kawanishi, H., Ozato, K. and Strober, W. (1985) The proliferative response of cloned Peyer's patch switch T cells to syngeneic and allogeneic stimuli. J. Immunol. 134, 3586–3591.

[62] Smith, P.D., Gillin, F.D., Brown, W.R. and Nash, T.E. (1981) IgG antibody to *Giardia lamblia* detected by enzyme linked immunosorbent assay. Gastroenterology 80, 1476–1480.

[63] Smith, P.D., Gillin, F.D., Spira, W.M. and Nash, T.E. (1982) Chronic giardiasis: Studies on drug sensitivity, toxin production and host immune response. Gastroenterology 83, 797–803.

[64] Vinayak, V.K., Jain, P. and Naik, S.R. (1978) Demonstration of antibodies in Giardiasis using immunodiffusion technique with *Giardia* cysts as antigen. Ann. Trop. Med. Parasitol. 72, 581–582.

[65] Anders, R.F., Roberts-Thomson, I.C. and Mitchell, G.F. (1982) Giardiasis in mice. Analysis of humoral and cellular immune response to *Giardia muris*. Parasite Immunol. 6, 45–57.

[66] Schlamowitz, M. (1976) Membrane receptors in the specific transfer of immunoglobulins from mother to young. Immunol. Commun. 5, 481–500.

[67] Goka, A.K.J., Mathan, V.I., Rolston, D.D.K. and Farthing, M.J.G. (1986) Diagnosis of giardiasis by specific IgM antibody by enzyme linked immunosorbent assay. Lancet ii 2, 184–186.

[68] Owen, R.L., Allen, C.L. and Stevens, D.P. (1981) Phagocytosis of *Giardia muris* by macrophages in Peyer's patch epithelium in mice. Infect. Immun. 33, 591–601.