

Giardia lamblia infection induces different secretory and systemic antibody responses in mice

C. VELAZQUEZ,¹ M. BELTRAN,² N. ONTIVEROS,² L. RASCON,¹ D. C. FIGUEROA,¹ A. J. GRANADOS,¹ J. HERNANDEZ-MARTINEZ,³ J. HERNANDEZ² & H. ASTIAZARAN-GARCIA²

¹Department of Chemistry-Biology, University of Sonora, Hermosillo, Sonora, México, ²Department of Nutrition, Centro de Investigación en Alimentación y Desarrollo A.C., Hermosillo, Sonora, México, ³Laboratory of Biopolymers, Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, México

SUMMARY

The adult mouse model of *Giardia lamblia* infection serves as an excellent animal model to understand the immunological mechanisms involved in the control and clearance of *Giardia* infection. Little is known about the *G. lamblia*-specific antigens that stimulate the humoral immune response in this model of giardiasis. We analysed the secretory and systemic antibody responses to *G. lamblia* during primary and secondary infection in C3H/HeJ adult mice. Faecal IgA and Serum IgG anti-*G. lamblia* antibodies were observed at week 2 post-infection. Serum IgG responses remained constant over the next several weeks, whereas faecal IgA titres continued to rise from weeks 2–6 post-infection. Western blot analysis revealed that intestinal IgA and serum IgG antibody responses were directed toward several distinct proteins of *G. lamblia*. Certain proteins appeared to be recognized by both faecal IgA and serum IgG, whereas other antigens were specific for either the secretory or systemic antibody responses. *G. lamblia* primary and secondary infections were associated with differences in the antibody recognition pattern. The biochemical and immunological characterization of these antigens will help us to better understand the immunobiology of the *G. lamblia*–host interaction.

Keywords antibody response, *Giardia lamblia*, giardiasis

RESEARCH NOTE

The protozoan parasite *Giardia lamblia* is an intestinal parasite of humans, which the World Health Organization estimates to have infected 250 million people worldwide (1). Clinical manifestations of *G. lamblia* infections vary from the asymptomatic carrier state to severe diarrhoea, abdominal pain, nausea, malabsorption and weight loss (2,3). *Giardia* infections are usually self-limiting in immunocompetent individuals, indicating the presence of effective host defence mechanisms against this intestinal parasite (2–5). Despite the high incidence and clinical importance of *G. lamblia* infections, the immunological mechanisms that play a role in giardiasis are poorly understood.

The mouse model of giardiasis is a powerful tool to study the immune effector mechanisms that occur during *Giardia* infections, and has considerable advantages over other animal models (6–9). The immune system of the mouse is well characterized, an extensive variety of reagents and technologies exist for the study of the mouse immune system, and immunologically well-defined inbred strains of mice are available. Byrd *et al.* reported the development of a *G. lamblia*–adult mouse model that uses a well-characterized clone of *G. lamblia* (GS/M-83-H7) (6,10). The adult mouse model of *G. lamblia* infection has been used to better understand the immunological mechanisms active in giardiasis as well as the antigenic variation in *G. lamblia* (5,10–14). Additionally, the mouse model has helped to gain new insight into the key elements of the immune response that play a role in giardiasis. Several studies have shown that T and B lymphocytes are important to control *Giardia* infection in mice (5,10,11,14). However, our knowledge about the specific antigens of *G. lamblia*, which induce a humoral and cellular immune response, remains limited.

In the present study, we evaluated the secretory and systemic antibody response during the course of a primary and secondary *G. lamblia* infection in C3H/HeJ adult mice. Immunogenic antigens of *G. lamblia* were identified that induce mucosal

Correspondence: Carlos Velazquez, Department of Chemistry-Biology, University of Sonora. Blvd. Luis Encinas y Rosales s/n. Hermosillo, Sonora 83000, Mexico (e-mail: velaz@guayacan.uson.mx).
Humberto Astiazaran-Garcia, Department of Nutrition, Centro de Investigación en Alimentación y Desarrollo A.C. P.O. Box 1735 Hermosillo, Sonora 83000, México (e-mail: hastiazaran@cascabel.ciad.mx).

Received: 29 April 2005

Accepted for publication: 01 August 2005

and serum antibody responses. Our data demonstrate differences in antigen recognition between secretory and systemic antibody responses, and have implications for future research on giardiasis.

C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). This strain of mice is susceptible to infection with the *G. lamblia* clone GS/M-83-H7 (11). Trophozoites from the *G. lamblia* clone GS/M-83-H7 were obtained from the American Type Culture collection (ATCC 50581). Axenic *G. lamblia* cultures were maintained in the TYI-S-33 medium with antibiotics.

Soluble *G. lamblia* trophozoite antigens were obtained by using the method described by Gottstein *et al.* (15) with slight modifications. Briefly, *G. lamblia* trophozoites from confluent cultures were harvested during log-phase by chilling on ice for 30 min. One hundred million trophozoites were washed three times with sterile phosphate buffer saline (PBS), resuspended in 1.5 mL of PBS, frozen (liquid nitrogen) and thawed (room temperature) three times, and then sonicated (30 cycles for 2 min [Brandon sonifier 250, Shelton, CT, USA] in the presence of protease inhibitor cocktail [23 mM/L 4-(2-aminoethyl) benzenesulphonyl fluoride (AEBSF)], 0.3 mM/L pepstatin A, 0.3 mM/L E-64, 2 mM/L bestatin, and 100 mM/L sodium EDTA [Sigma, St. Louis, MO, USA]). Cell debris was removed by centrifugation (10 000 *g* for 30 min). The protein concentration of the soluble antigen preparation was determined by the Bradford method (Bio-Rad, Hercules, CA, USA).

Eight- to 10-week-old male C3H/HeJ mice were infected with 5×10^6 trophozoites of the *G. lamblia* clone GS/M-83-H7 by using a sterile animal feeding needle for peroral inoculation. The *G. lamblia* inoculum was prepared by washing *in vitro* cultivated trophozoites three times with ice-cold sterile PBS and resuspending them in 200 μ L of sterile PBS. Primary infection occurred on day 0, with secondary challenge taking place on day 45.

To evaluate the antibody responses during a primary and secondary *G. lamblia* infection in mice, we did at least four different experiments (at least four animals/experiment). The animal experiments shown in the paper are representative experiments and are consistent with all the experiments performed during this study.

Blood and faecal sampling of mice began (day 0) prior to *G. lamblia* infection and was performed weekly for 6 weeks after primary infection and again for 6 weeks after secondary challenge. Mice were bled from the tail vein and serum was recovered and stored at -30°C . To analyse the intestinal anti-*G. lamblia* (IgA) antibodies, faecal extracts were prepared as follows: two to three faecal pellets of each mouse were collected in a microcentrifuge tube containing 0.5 mL of PBS-1% bovine serum albumin (Sigma) and 1 mM phenylmethanesulphonyl fluoride (PMSF). Tubes were incubated overnight at 4°C . The suspension was vortexed vigorously

for 10 s and centrifuged at 10 000 *g* at 4°C for 10 min to remove insoluble material. Supernatant was collected and stored at -30°C (12).

Total faecal IgA was quantified using a sandwich enzyme-linked immunosorbent assay (ELISA). Ninety-six-well plates (Corning, Corning, NY, USA) were coated overnight at 4°C with 125 ng (50 μ L) of sheep anti-mouse IgA (α -chain specific) (Sigma). Wells were blocked with PBS containing 1% BSA (PBS-1% BSA) for 1 h at room temperature. After washing, 50 μ L of faecal extracts (diluted 1 : 100 in PBS-1% BSA) were added to triplicate wells and incubated for 90 min at room temperature. After washing, 50 μ L of peroxidase goat anti-mouse IgA (Zymed, San Francisco, CA, USA) diluted 1 : 1000 in PBS-1% BSA was added to each well for 1 h at room temperature. Plates were washed and developed with 1 mM 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABT-S; Boehringer Mannheim GmbH, Mannheim, Germany) in citrate buffer with 0.03% H_2O_2 . Optical density (at 415 nm) was read at 30 min with an ELISA reader (Benchmark Microplate Reader, Bio-Rad, Hercules, CA, USA). For each ELISA plate, a standard curve was constructed using purified mouse IgA (Sigma).

To evaluate serum anti-*G. lamblia* IgG and intestinal anti-*G. lamblia* IgA of infected mice, an ELISA was carried out using standard techniques. Briefly, 96-well plates (Corning) were coated with 50 μ L (2.5 μ g) of soluble *G. lamblia* antigen in 0.1 M sodium bicarbonate buffer pH 9.6. After overnight incubation at 4°C , plates were washed with PBS-0.05% Tween 20 (PBST), and blocked with PBS-1% BSA for 1 h at room temperature and washed. Faecal extracts (15 ng of total intestinal IgA) and mouse serum samples (diluted 1 : 10 in PBS-1% BSA) from both infected and non-infected mice were added to triplicate wells and incubated for 1 h at room temperature. After washing with PBST, antibody binding was detected with 50 μ L of HRP-conjugated goat anti-mouse IgG (1 : 1000 diluted in PBS-1% BSA) (Sigma) or HRP-conjugated goat anti-mouse IgA (1 : 1000 diluted in PBS-1% BSA) (Zymed, San Francisco, CA, USA). After 1 h of incubation for IgG ELISA plates and 90 min of incubation for IgA ELISA plates (both at room temperature), the plates were washed, and developed with 1 mM ABT-S in citrate buffer with 0.03% H_2O_2 . Optical density was measured at 415 nm. Statistical analysis was performed using paired t-tests.

Soluble *G. lamblia* proteins were separated by SDS-PAGE (12%) under reducing conditions without boiling and subsequently stained with Coomassie Brilliant Blue (Bio-Rad) by standard methods.

In order to evaluate antibody recognition by Western blotting, 500 μ g of soluble *G. lamblia* protein was mixed with an equal volume of 2X SDS-PAGE sample buffer (4% SDS, 2% 2-mercaptoethanol, 0.125 M Tris-HCl/0.1% SDS, 20% glycerol, and 0.001% bromophenol blue) and loaded on a preparative

SDS-PAGE mini-gel (12% separating gel with a 4% stacking gel). Proteins were electrotransferred to a nitrocellulose membrane for 30 min using a semi-dry blotting system (The W.E.P. Company, Seattle, WA, USA) with 120 mAmp current. Nitrocellulose membranes were blocked with PBS containing 1% dry milk and 1% BSA for 1 h at room temperature. Blocked membranes were incubated with serum (diluted 1 : 25 with PBS-1% BSA) for 1 h at room temperature. After five washes with PBST, the membranes were incubated with HRP-conjugated goat anti-mouse IgG (diluted 1 : 5000 with PBS-1% BSA) for 1 h at room temperature. Membranes were washed and developed using a SuperSignal West Pico Chemoluminescent kit (Pierce, Rockford, IL, USA). To evaluate the antibody recognition of faecal extracts (intestinal IgA), we used a modified 2X SDS-PAGE sample buffer (0.2% SDS, 0.2% 2-mercaptoethanol, 0.125 M Tris-HCl/0.1% SDS, 20% glycerol and 0.001% bromophenol blue) to run the soluble *G. lamblia* proteins in SDS-PAGE. These modifications in the SDS-PAGE sample buffer were performed after preliminary experiments revealed that standard concentration of SDS and 2-mercaptoethanol abolished the intestinal IgA antibody recognition. Peroxidase-goat anti-mouse IgA diluted 1 : 10 000 in PBS-1% BSA was used as secondary antibody, with nitrocellulose membranes developed as described previously.

In order to evaluate the course of *G. lamblia* infection in C3H/HeJ mice, we inoculated a group of animals ($n = 12$) with 5×10^6 *G. lamblia* trophozoites, and the intestinal parasite loads were evaluated at different times post-infection (p.i.). *G. lamblia* infection occurred in all inoculated mice. The highest parasite loads were observed between days 14 ($2.5 \times 10^5 \pm 1.07 \times 10^5$) and 21 p.i. ($1.8 \times 10^5 \pm 0.24 \times 10^5$). At 28 day p.i., parasites were usually undetectable. These observations are in agreement with previous reports that have shown that *G. lamblia* infection is controlled at 3–4 weeks p.i. in immunocompetent mice (11,14).

We used an ELISA assay to measure the anti-*G. lamblia* antibody response in serum (IgG) and faecal extracts (IgA) during the course of primary and secondary *G. lamblia* infection. A group of seven C3H/HeJ mice was infected with 5×10^6 *G. lamblia* trophozoites, and the anti-*G. lamblia* antibody response was evaluated at weeks 0, 1, 2, 3, 4, 5 and 6 p.i. Figure 1 shows that both systemic (Figure 1a) and mucosal (Figure 1b) anti-*G. lamblia* antibody responses became evident at week 2 p.i. For 3–4 weeks thereafter, constant levels of serum IgG antibody were found in all of the infected mice. In contrast, secretory IgA responses continued to rise from weeks 2–6 p.i. None of the serum and faecal samples obtained from pre-infected or uninfected animals had antibodies against *G. lamblia* antigens. To evaluate the antibody response during a second challenge of *G. lamblia*, mice were reinfected at day 45 p.i. (at this time of post-

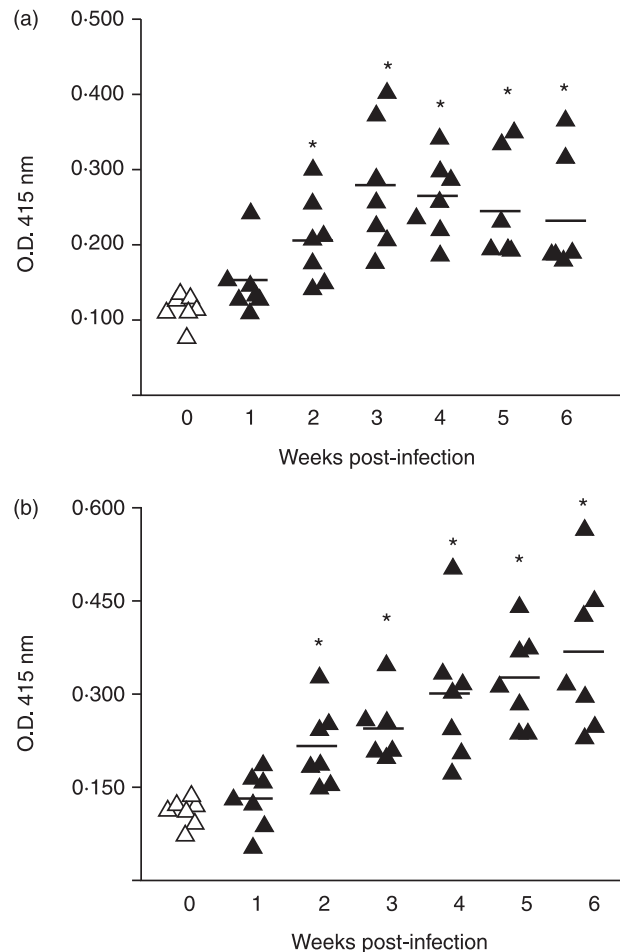


Figure 1 Time-course of anti-*G. lamblia* serum IgG (a) and intestinal IgA antibodies (b) from infected C3H/HeJ mice by ELISA. A group of mice ($n = 7$) were inoculated with 5×10^6 *G. lamblia* trophozoites. Faecal and serum samples were obtained at different times p.i. (weeks 0, 1, 2, 3, 4, 5 and 6 p.i.). Each symbol represents an individual mouse. The horizontal lines represent the arithmetic mean of the data obtained at different times post-infection. Asterisks indicate significant differences ($P < 0.001$ by paired t-test) between pre-infected (week 0) and post-infected samples.

infection, the C3H/HeJ mice had cleared the *G. lamblia* infection). The antibody response was evaluated at weeks 0, 1, 2, 3, 4, 5 and 6 post-reinfection. All the reinfected mice showed increased levels of serum IgG and faecal IgA antibodies anti-*G. lamblia* (data not shown).

In order to identify the *G. lamblia* antigens recognized by the mucosal and systemic antibody responses of infected mice, Western blots were performed with sera and faecal extracts. Serum IgG antibody responses during a primary infection were most directed against the antigenic bands of 63 and 71 kDa (Figure 2a). This antibody reactivity was strongly detected from week 3–6 p.i. The band of 71 kDa

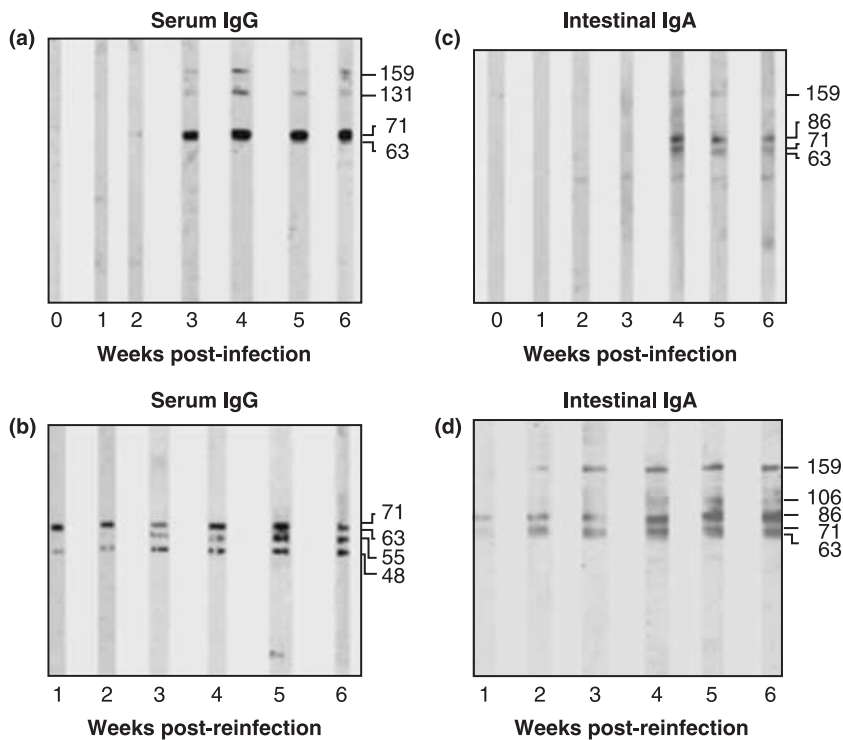


Figure 2 Western blot analysis of anti-*G. lamblia* IgG (serum [a and b]) and IgA (faecal [c and d]) antibody responses of infected (a and c) and reinfected (b and d) C3H/HeJ mice. A group of infected mice (see Figure 1 legend for details) were reinfected with 5×10^6 *G. lamblia* trophozoites at day 45 post-infection. Serum and faecal samples were obtained at different times post-infection (weeks 0, 1, 2, 3, 4, 5 and 6 p.i.) and post-reinfection (weeks 1, 2, 3, 4, 5 and 6 p.r.i.). Western blotting was performed with pools of sera and of faecal samples made by mixing equal volumes of individual sera and individual faecal extracts, respectively. Pools of sera (1 : 25) and pools of faecal extracts (1 : 10) were diluted in PBS-1% BSA.

was the first band recognized, beginning at week 2 p.i. Additional bands (159 kDa and 131 kDa) were faintly recognized from weeks 3–6 p.i. Control sera from pre-infected and uninfected mice did not exhibit any immunoreactivity. Sera collected from re-infected mice mainly recognized bands of 48, 55, 63 and 71 kDa (Figure 2b).

When the intestinal anti-*G. lamblia* IgA response was evaluated, considerable difficulty was encountered in optimizing the Western blotting conditions. Eventually, results were obtained by reducing the SDS and 2-mercaptoethanol concentrations in the sample buffer prior to SDS-PAGE. To evaluate the antibody recognition of faecal extracts (intestinal IgA), we used a modified 2X SDS-PAGE sample buffer (0.2% SDS, 0.2% 2-mercaptoethanol, 0.125 M Tris-HCl/0.1% SDS, 20% glycerol, and 0.001% bromophenol blue) to run the soluble *G. lamblia* proteins in the SDS-PAGE. These modifications in the SDS-PAGE sample buffer were performed after preliminary experiments revealed that standard concentration of SDS and 2-mercaptoethanol abolished the intestinal IgA antibody recognition. In contrast, serum IgG antibody recognition was not significantly affected by the use of standard sample buffer. Additionally, there were no appreciable differences in the electrophoretic pattern of the soluble *G. lamblia* proteins when diluted with modified or standard sample buffer (data not shown). The intestinal IgA antibody response during primary and secondary *G. lamblia* infections is shown in Figure 2 (c,d), respectively.

Western blot analysis revealed that IgA antibodies in the faecal extracts reacted mainly against the bands of 63, 71, 86 and 159 kDa (Figure 2c). This antibody recognition was clearly detected from weeks 4–6 p.i. Intestinal IgA from reinfected mice recognized the 63, 71 and 86 kDa antigens from week 1 post-reinfection to the end of the experiment (Figure 2d). The antigenic band of 159 kDa was recognized from weeks 2–6 p.r.i. An additional band of 106 kDa was faintly recognized from weeks 5–6 p.r.i. Control faecal extract from preinfected and uninfected mice exhibited no immunoreactivity.

In the present study, we have identified immunoreactive antigens of *G. lamblia* recognized by intestinal IgA and serum IgG antibodies during the course of primary and secondary *G. lamblia* infection in C3H/HeJ adult mice. Infected C3H/HeJ mice showed a significant increase in the levels of intestinal IgA and serum IgG antibodies specific to *G. lamblia* during the period of time in which intestinal trophozoite loads began to drop considerably, suggesting that the antibody response plays an important role in the clearance of *G. lamblia* infection. *Giardia* infections in humans and mice induce the production of different classes of antibodies (IgM, IgA, and IgG) and these correlates with the clearance of infection (4,11,16). Additionally, B-cell-deficient and IgA-deficient mice were unable to control *Giardia* infection, indicating a central role for B cells and IgA in host defence against this parasite (11). In contrast, another study indicated

that antibodies are not required for the control of acute *Giardia* infections (14). Additional studies need to be conducted to define the precise role of the antibody response in the adult mouse *G. lamblia* infection model.

Based on the amounts of total IgG and total IgA antibodies used in the respective ELISA plates and in the ELISA data shown in this paper, we can suggest that the relative abundance of anti-*G. lamblia* IgA antibodies in faecal extracts is much higher than serum IgG anti-*G. lamblia* antibodies. *G. lamblia*, a luminal pathogen, resides strictly in the lumen of the small intestine and does not invade through the mucosa, therefore allowing better stimulation of the mucosal immune system rather than the peripheral immune system (4). The differences in the relative abundance of specific anti-*G. lamblia* antibodies could be because of antigen availability to induce both local and systemic antibody responses.

Western blot analysis of faecal extracts and sera from infected and reinfected mice showed that the intestinal IgA and serum IgG antibody responses were directed to a limited number of protein bands (8), with molecular weights of 48, 55, 63, 71, 86, 106, 131 and 159 kDa. In the neonatal mouse model of giardiasis, using *G. lamblia* clone GS/M-83-H7, it has been reported that the serum and secretory antibody responses are almost exclusively directed against the well-characterized variable surface protein H7 (VSP H7) (15,17,18). The predicted molecular weight of this protein is 56 832 Da, but the migration of this protein in the SDS-PAGE is considerably higher (about 72 kDa). This migration of the VSP H7 varies with the acrylamide gel preparation (18). In the present study, we used the *G. lamblia* clone GS/M-83-H7, which expresses the VSP H7. This protein may be one of the main immunoreactive bands (i.e. band of 71 kDa) detected in the Western blotting assays. Further studies are required to characterize at the biochemical and molecular level these immunogenic antigens. The *G. lamblia* genome database (19), together with digestion and mass spectrometry analysis of the immunogenic antigens may help to identify these *G. lamblia* antigens (1).

There were several differences in the antibody recognition of the secretory and systemic antibody responses. The immunogenic band of 86 kDa was intensively and exclusively recognized by the intestinal IgA antibody response. In contrast, the proteins of 48 and 55 kDa were only recognized by the serum IgG antibody of reinfected animals. On the other hand, the bands of 63 and 71 kDa were detected by both intestinal IgA and serum IgG antibody responses. The observed differences between the secretory and systemic antibody responses could be because of several parasite and host factors. These include the nature and immunogenic capacity of the *G. lamblia* antigens to induce an immune response, antigen availability to induce both

local and systemic antibody responses, antigen handling by antigen presenting cells which could affect the antigen's access to the systemic immune system, as well as the well-known anatomic and functional differences between the mucosal and peripheral immune systems (20–22).

G. lamblia primary and secondary infections were associated with differences in the antibody recognition pattern. The main bands recognized by the secretory and systemic antibody responses during primary infection were the proteins of 63 kDa, 71 kDa and 86 kDa; however, during a secondary infection additional proteins were detected (bands of 48 kDa, 55 kDa, 106 kDa and 159 kDa). These changes in antibody recognition may be a consequence of the antigenic variation of the parasite. Several studies have shown that the immune response to a *G. lamblia* infection is influenced by the capability of the parasite to continuously change its surface antigens (13,15,23,24). We plan to evaluate the role of specific anti-*G. lamblia* antibodies in protection against *G. lamblia* infection.

In order to evaluate IgA antibody recognition by Western blotting, we modified the standard sample buffer used in our SDS-PAGE. The modified sample buffer had reduced SDS (20-fold less) and 2-mercaptoethanol (10-fold less) concentrations. IgA antibody recognition was only detected when *G. lamblia* proteins were diluted with modified sample buffer previous to the SDS-PAGE. This observation indicates that the denaturing and reducing conditions during SDS-PAGE completely abolished recognition, suggesting the presence of conformational epitopes present in the *G. lamblia* antigens, which are important for IgA antibody recognition. Serum IgG antibody recognition did not change under 'mild' or 'standard' SDS-PAGE conditions.

In summary, we have identified several immunogenic antigens of *G. lamblia* in the C3H/HeJ adult mouse model of giardiasis. The biochemical and immunological characterization of these proteins will provide a better understanding of the *G. lamblia*–host interaction, and will be important for developing effective control strategies against giardiasis.

ACKNOWLEDGEMENTS

This work was supported by grant I39133-N from the National Council for Science and Technology of Mexico (CONACYT) to C.V. M.B. and N.O. received scholarships from CONACYT and CIAD. We thank Brian Edelson and Boris Calderon for helpful discussions and reading of the manuscript.

REFERENCES

- 1 Palm JED, Weiland MEL, Griffiths WJ, Ljungstrom I & Svard SG. Identification of immunoreactive proteins during acute human giardiasis. *J Infect Dis* 2003; **187**: 1849–1859.

- 2 Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev* 2001; **14**: 447–475.
- 3 Eckmann L & Gillin FD. Microbes and microbial toxins: paradigms for microbial–mucosal interactions I. Pathophysiological aspects of enteric infections with the lumen-dwelling protozoan pathogen *Giardia lamblia*. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1–G6.
- 4 Eckmann L. Mucosal defences against *Giardia*. *Parasite Immunol* 2003; **25**: 259–270.
- 5 Faubert G. Immune response to *Giardia duodenalis*. *Clin Microbiol Rev* 2000; **13**: 35–54.
- 6 Belosevic M, Faubert GM, Maclean JD, Law C & Croll NA. *Giardia lamblia* infections in Mongolian gerbils – an animal model. *J Infect Dis* 1983; **147**: 222–226.
- 7 Craft JC. Experimental infection with *Giardia lamblia* in Rats. *J Infect Dis* 1982; **145**: 495–498.
- 8 Hewlett EL, Andrews JS, Ruffier J & Schaefer FW. Experimental infection of mongrel dogs with *Giardia lamblia* cysts and cultured trophozoites. *J Infect Dis* 1982; **145**: 89–93.
- 9 Yanke SJ, Ceri H, McAllister TA, Morck DW & Olson ME. Serum immune response to *Giardia duodenalis* in experimentally infected lambs. *Vet Parasitol* 1998; **75**: 9–19.
- 10 Byrd LG, Conrad JT & Nash TE. *Giardia lamblia* infections in adult mice. *Infect Immun* 1994; **62**: 3583–3585.
- 11 Langford TD, Housley MP, Boes M *et al.* Central importance of immunoglobulin A in host defense against *Giardia* spp. *Infect Immun* 2002; **70**: 11–18.
- 12 Larocque R, Nakagaki K, Lee P, Abdul-Wahid A & Faubert GM. Oral immunization of BALB/c mice with *Giardia duodenalis* recombinant cyst wall protein inhibits shedding of cysts. *Infect Immun* 2003; **71**: 5662–5669.
- 13 Nash TE. Antigenic variation in *Giardia lamblia* and the host's immune response. *Philos Trans R. Soc Lond Ser B Biol Sci* 1997; **352**: 1369–1375.
- 14 Singer SM & Nash TE. T-cell-dependent control of acute *Giardia lamblia* infections in mice. *Infect Immun* 2000; **68**: 170–175.
- 15 Gottstein B, Harriman GR, Conrad JT & Nash TE. Antigenic variation in *Giardia lamblia* – cellular and humoral immune response in a mouse model. *Parasite Immunol* 1990; **12**: 659–673.
- 16 Snider DP & Underdown BJ. Quantitative and temporal analyses of murine antibody response in serum and gut secretions to infection with *Giardia muris*. *Infect Immun* 1986; **52**: 271–278.
- 17 Gottstein B, Deplazes P & Tanner I. *In vitro* synthesized immunoglobulin-a from Nu/+ and reconstituted Nu/Nu mice against a dominant surface-antigen of *Giardia lamblia*. *Parasitol Res* 1993; **79**: 644–648.
- 18 Nash TE & Mowatt MR. Characterization of a *Giardia lamblia* variant-specific surface protein (Vsp) gene from isolate Gs/M and estimation of the Vsp gene repertoire size. *Mol Biochem Parasitol* 1992; **51**: 219–227.
- 19 McArthur AG, Morrison HG, Nixon JEJ *et al.* The *Giardia* genome project database. *FEMS Microbiol Lett* 2000; **189**: 271–273.
- 20 Macdonald TT. The mucosal immune system. *Parasite Immunol* 2003; **25**: 235–246.
- 21 McGhee JR & Kiyono H. In: Paul WD (ed): *Fundamental Immunology*, 4th edn. Philadelphia: Lippincott–Raven; 1999: 909–945.
- 22 Simecka JW. Mucosal immunity of the gastrointestinal tract and oral tolerance. *Adv Drug Deliv Rev* 1998; **34**: 235–259.
- 23 Muller N, Stager S & Gottstein B. Serological analysis of antigenic heterogeneity of *Giardia lamblia* variant surface proteins. *Infect Immun* 1996; **64**: 1385–1390.
- 24 Stager S, Felleisen R, Gottstein B & Muller N. *Giardia lamblia* variant surface protein H7 stimulates a heterogeneous repertoire of antibodies displaying differential cytological effects on the parasite. *Mol Biochem Parasitol* 1997; **85**: 113–124.