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Giardia lamblia Infections in B-Cell-Deficient Transgenic Mice

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In the present study, we infected B-cell (and antibody-)-deficient transgenic mice with the *Giardia lamblia* clone GS/M-83-H7. These animals were inhibited in intestinal anti-*Giardia* immunoglobulin A (IgA) production and could not resolve the parasite infection, and antigenic diversification within the respective parasite populations occurred in an unusually slow manner. These findings indicate an important immunological function of local IgA antibodies which promotes antigenic variation of the parasite and is involved in control of the parasite infection.

Giardia lamblia is a zoonotic protozoan parasite which resides in the small intestines of humans and various animals. Manifestations of *G. lamblia* infections vary from asymptomatic carriage to severe diarrhea and maladsorption (1, 2). Many studies have suggested a central role for the immune system in the determination of the outcome of the parasite infection (reviewed in references 6 and 7). Experimental infections in different animal models have revealed that the immunological host-parasite relationship is strongly influenced by the parasite's capacity to undergo antigenic variation (7, 13). This antigenic variability of the parasite is generated by a continuous change of the major surface antigen, named variant surface protein (VSP). A number of studies (reviewed in reference 13) have shown that the antigenic surface of *Giardia* varies between different isolates and that surface antigenic variation may even occur within an isolate.

In previous studies, experimental *G. lamblia* infections were performed in neonatal athymic nude mice (8, 9) and in mice with severe combined immune deficiency (9). These in vivo experiments were based on the use of the *G. lamblia* clone GS/M-83-H7 (human isolate) (2), which as yet represents the only characterized isolate that exhibits a significant infective potential in both neonatal (8, 9) and adult (5) mice. The investigations revealed that antigenic variation of the parasite as well as control of the parasite infection by the host relied on an integral functionality of the immune system. In the present paper, we describe a comparative study of *G. lamblia* infections in adult (10- to 12-week-old) immunocompetent C57BL/6 mice (purchased from the Institut für Labortierkunde, Zürich, Switzerland) and B-cell (and antibody-)-deficient (μMT) transgenic mice backcrossed on a C57BL/6 genetic background (4, 11) (kindly provided by H. Hengartner, Institute for Experimental Immunology, Zürich, Switzerland, with the permission of K. Rajewsky and W. Müller, Institute for Genetics, Cologne, Germany). In contrast to nude mice (12) or those with severe combined immune deficiency (3), μMT transgenic mice possess an intact T-cell network but are blocked in B-cell differentiation at the pre-B-cell stage (11). Due to the defect, the mutant mouse strain is incapable of generating primary and secondary antibody responses. These immunological characteristics made our selected mouse system an ideal experimental animal model in which to specifically investigate the potential influence of anti-*Giardia* antibodies, and in particular intestinal anti-*Giardia* immunoglobulin A (IgA) antibodies, on the antigenic dynamics within the infecting *G. lamblia* population and the course of the parasite infection.

In our study, animals were kept under specific pathogen-free conditions according to Swiss regulations governing animal experimentation and rules for animal protection, which restrict the use of experimental animals to a minimum. Experimental infections were done with 10^6 trophozoites (suspended in 200 μl of a 0.3 M $NaHCO_3$ solution) of *G. lamblia* GS/M-83-H7 by using a blunt needle for peroral inoculation. Clone GS/M-83-H7 expresses the variant-specific surface antigen VSP H7, which is defined by its reactivity to monoclonal antibody (MAb) G10/4 (2). Antigenic diversification within the intestinal parasite populations of infected mice was tested as described earlier by performing an immunofluorescence assay to monitor the switch of the trophozoite population from MAb G10/4 (VSP H7)-positive to -negative (9). For determination of the local anti-*Giardia* IgA concentration, we applied the procedure of Gottstein and coworkers (8), which is suitable for the extraction of IgA from the intestinal epithelium and lamina propria. Immunoreactivity of antibodies was tested as described previously (17) by using purified recombinant VSP H7 (MBP/VSP H7 fusion protein expressed in *Escherichia coli*) and/or a total protein extract from *G. lamblia* GS/M-83-H7 as antigenic reagents for enzyme-linked immunosorbent assays (ELISAs) (Fig. 1) and/or Western blots (Fig. 2), respectively. Time points for sample collection suitable for monitoring both in vivo antigenic variation of the parasite and regression of the infection were selected according to the results of a pilot experimental infection of C57BL/6 mice with the parasite (data not shown).

In immunocompetent control mice, complete antigenic switch of trophozoites from MAb G10/4 (VSP H7)-positive to -negative occurred between days 7 and 10 postinfection (p.i.). In B-cell (and antibody-)-deficient animals, however, antigenic variation was visible for the first time at 48 days p.i. and only part (about 60 to 75%) of the intestinal parasite population was affected by this diversification process by day 64 p.i., when the experiment was terminated. The ELISA shown in Fig. 1 revealed that, in control animals, production of local anti-*Giardia* and anti-VSP H7 IgA antibodies increased during the course of infection and reached a peak at 48 days p.i. As expected, in mutant mice no anti-*Giardia* IgA was detectable during the entire period of infection. Moreover, an ELISA-based investigation of the intestinal samples with respect to their anti-*Giardia* and anti-VSP H7 IgM antibody content gave negative results for both experimental animal groups (data not shown). The Western blot experiment (Fig. 2) demonstrated

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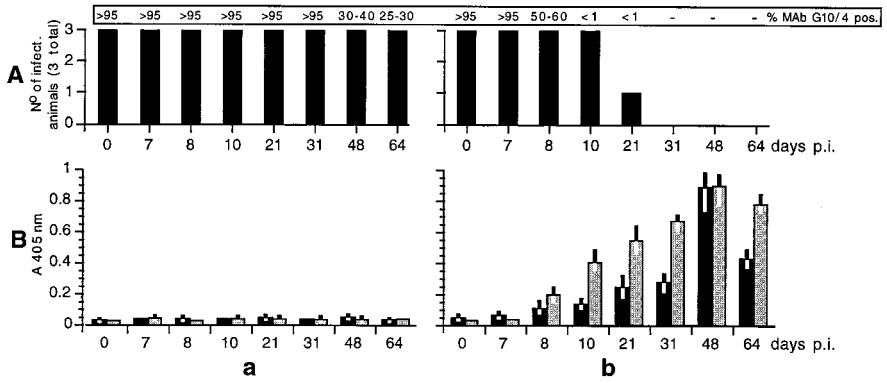

FIG. 1. Follow-up analysis of *G. lamblia* GS/M-83-H7-infected B-cell-deficient transgenic mice (graphs a) and immunocompetent control mice (graphs b) in terms of detectability of intestinal parasites (monitored by microscopical examination and 6-day cultivation of adherent trophozoites from the intestinal contents) (A) and production of intestinal IgA against total *Giardia* protein (black bars) or purified recombinant VSP H7 protein (grey bars) as determined by ELISA (B). Three mice from each group were sacrificed on the indicated day after infection, and intestinal IgA was analyzed. Mean values as well as ranges are shown. The numbers above the columns in panel A indicate percentages of intestinal parasites with a MAb G10/4 (VSP H7)-positive variant antigen type.

that local IgA antibodies, emerging early during infection in immunocompetent mice, were preferentially directed against the MAb G10/4-reactive VSP H7. VSP H7 appeared as a 72-kDa major band and an additional complex banding pattern probably reflecting different folding stages and/or degradation products of the major surface antigen. Interestingly, this relatively monospecific antibody population emerged around the time point of infection (8 days p.i.) when antigenic variation of the parasite was initiated. These findings indicated that, in immunocompetent animals, local anti-VSP H7 antibodies promoted the switch of the variant antigen type of the parasite population from MAb G10/4 (VSP H7)-positive to -negative, whereas retarded antigenic variation in B-cell- (and antibody-) deficient mice must have occurred in an antibody-independent fashion. This is in agreement with earlier observations suggesting that antigenic variation can occur spontaneously (15) and that immunological (15) or physiological (16) factors can select for, or against, different variant antigen types.

In a further analysis, we determined the course of the *G. lamblia* infection within the two mouse strains. For this purpose, the parasite burden in infected animals was quantified by using a previously described method based on the microscopical examination of adherent trophozoites from intestinal washes and by additionally confirming the presence of intestinal parasites through a 6-day proliferation of such adherent trophozoites in TYI-S-33 culture medium containing antibiotics (5). A follow-up study of the intestinal parasite burden in B-cell (and antibody-)-deficient mice and immunocompetent control mice revealed that the intensity of infection was generally very low, allowing detection of a maximum of 5 to 10 parasites per microscopical field. In B-cell (and antibody-)-deficient animals, trophozoites were detected throughout the experimental time period (Fig. 1A, graph a) and could even be directly demonstrated (by microscopical examination) at day 64 p.i. In contrast, analysis of the intestines from antibody-producing control mice did not result in direct or highly sensitive indirect (in 6-day cultures) detection of the parasites after day 21 p.i. (Fig. 1A, graph b). As can be seen in the Western blot in Fig. 2, regression of the infection went along not only with a high level of anti-VSP H7 IgA production but also with a lower level of synthesis of local IgA antibodies against 52- and 23-kDa proteins of the parasite. These two minor antigens will have to be investigated in terms of their target function for the antibody-mediated immune attack of the murine host.

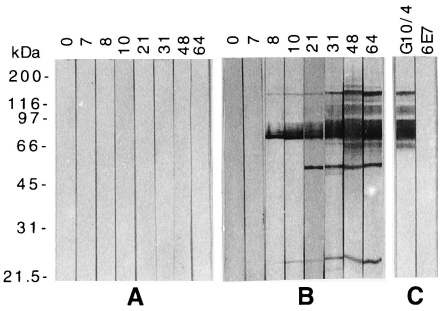

FIG. 2. Western blot analysis of parasite-specific intestinal IgA production in *G. lamblia* GS/M-83-H7-infected B-cell-deficient mice (A) and immunocompetent control animals (B). Sodium dodecyl sulfate-10% polyacrylamide gel-fractionated and blotted total protein from *G. lamblia* GS/M-83-H7 was incubated with intestinal IgA preparations sampled from mice at different time points (indicated above the strips as days p.i.) during infection. Reference blots incubated with VSP H7-reactive MAb G10/4 and the negative control, MAb 6E7, are shown in panel C. Sizes of the protein markers are on the left.

Taken together, the experimental infections of B-cell (and antibody-)-deficient mice and the appropriate antibody-producing control animals with *G. lamblia* GS/M-83-H7 demonstrate the key role of local anti-*Giardia* IgA antibodies in those immunological processes that both modulate (rapid) antigenic variation of the parasite and influence the outcome of the parasite infection. Our further studies will concentrate on exploring in more detail the particular effector mechanisms by which the antibodies control antigenic diversification and proliferation of the parasites in the murine host. In particular, we will tackle three important questions. (i) Are local IgA antibodies the only immunological effectors which influence antigenic variation of the parasite and the course of the parasite infection? (ii) What are the antigenic targets of the parasite that stimulate the local IgA antibody response involved in elimination of the parasite from its intestinal habitat? (iii) Do local IgA antibodies cause indirect parasiticidal effects, or do they directly interfere in the colonization process of the intestinal parasite population through cytotoxic effects on the trophozoites? Direct parasiticidal effects have already been observed in previous in vitro cytotoxicity assays with monoclonal

anti-VSP H7 antibodies (2, 10, 14, 17). Accordingly, it is possible that killing of *G. lamblia* trophozoites by direct interaction of cytotoxic intestinal IgA antibodies with the parasite represents a key mechanism of the murine immune defense to combat *G. lamblia* infections.

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REFERENCES

1. **Adam, R. D., A. Aggarwal, A. A. Lal, V. F. de la Cruz, T. McCutchan, and T. E. Nash.** 1988. Antigenic variation of a cysteine-rich protein in *Giardia lamblia*. J. Exp. Med. **167:**109–118.
2. **Aggarwal, A., J. W. Merritt, and T. E. Nash.** 1989. Cysteine-rich variant surface proteins of *Giardia lamblia*. Mol. Biochem. Parasitol. **32:**39–48.
3. **Bosma, G. C., R. P. Custer, and M. J. Bosma.** 1983. A severe combined immunodeficiency mutation in the mouse. Nature **301:**527–530.
4. **Bründler, M. A., P. Aichele, M. Bachmann, D. Kitamura, K. Rajewsky, and R. M. Zinkernagel.** 1996. Immunity to viruses in B cell-deficient mice: influence of antibodies on virus persistence and on T cell memory. Eur. J. Immunol. **26:**2257–2262.
5. **Byrd, L. G., J. T. Conrad, and T. E. Nash.** 1994. *Giardia lamblia* infections in adult mice. Infect. Immun. **62:**3583–3585.
6. **Farthing, M. J. G.** 1995. *Giardia lamblia*, p. 1081–1105. *In* M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), Infections of the gastrointestinal tract. Raven Press, New York, N.Y.
7. **Faubert, G. M.** 1996. The immune response to *Giardia*. Parasitol. Today **12:**140–145.
8. **Gottstein, B., P. Deplazes, and I. Tanner.** 1993. In vitro synthesized immunoglobulin A from nu/+ and reconstituted nu/nu mice against a dominant surface antigen of *Giardia lamblia*. Parasitol. Res. **79:**644–648.
9. **Gottstein, B., and T. E. Nash.** 1991. Antigenic variation in *Giardia lamblia*: infection of congenitally athymic nude and *scid* mice. Parasite Immunol. **13:**649–659.
10. **Hemphill, A., S. Stäger, B. Gottstein, and N. Müller.** 1996. Electron microscopical investigation of surface alterations on *Giardia lamblia* trophozoites after exposure to a cytotoxic monoclonal antibody. Parasitol. Res. **82:**206–210.
11. **Kitamura, K., J. Roes, R. Kühn, and K. Rajewsky.** 1991. A B-cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. Nature **350:**423–426.
12. **Milich, D. R., and M. E. Gershwin.** 1977. T cell differentiation and the congenitally athymic (nude) mouse. Dev. Comp. Immunol. **1:**289–298.
13. **Nash, T.** 1992. Surface antigen variability and variation in *Giardia lamblia*. Parasitol. Today **8:**229–234.
14. **Nash, T. E., and A. Aggarwal.** 1986. Cytotoxicity of monoclonal antibodies to a subset of *Giardia* isolates. J. Immunol. **136:**2628–2632.
15. **Nash, T. E., D. A. Herrington, M. M. Levine, J. H. Conrad, and J. W. Merritt.** 1990. Antigenic variation of *Giardia lamblia* in experimental human infections. J. Immunol. **144:**4362–4369.
16. **Nash, T. E., J. W. Merritt, Jr., and J. T. Conrad.** 1991. Isolate and epitope variability in susceptibility of *Giardia lamblia* to intestinal proteases. Infect. Immun. **59:**1334–1340.
17. **Stäger, S., R. Felleisen, B. Gottstein, and N. Müller.** 1997. *Giardia lamblia* variant surface protein (VSP) H7 stimulates a heterogeneous repertoire of antibodies displaying differential cytological effects on the parasite. Mol. Biochem. Parasitol. **85:**113–124.

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