Antigenic Variation of *Giardia lamblia* In Vivo

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A single *Giardia lamblia* trophozoite can give rise in vitro to *G. lamblia* with varying surface antigens. To determine whether antigenic variation also occurs in vivo, gerbils were inoculated with defined *G. lamblia* clones and the surface antigens of the intestinal trophozoites were studied at different times during the infection. The proportion of monoclonal antibody 6E7-reacting trophozoites from WB C1-6E7S-inoculated gerbils had decreased significantly by day 3 postinoculation, indicating the presence of a heterogeneous population. On day 7, the 170-kilodalton antigen was no longer present and was replaced by a variety of antigens, including a major protein of 92 kilodaltons. With the exception of isolates from gerbils inoculated with WB A6-6E7S, the banding patterns of *G. lamblia* isolated from gerbils on day 7 or later were the same regardless of the clones used for inoculation. These studies show that *G. lamblia* changes its surface antigen(s) in vivo within 7 days following inoculation and appears to maintain the same set of surface antigens during the course of infection.

*Giardia lamblia* is a flagellated protozoan which resides in the small intestine of humans and other animals. Although disease manifestations vary from asymptomatic carriage to fulminant diarrhea and malabsorption (16, 17), the mechanism of variable clinical symptomatology is not fully understood.

Earlier studies showed a large diversity among *Giardia* isolates, particularly the surface antigens (8–10, 12, 13). Recently, in vitro studies (T. E. Nash, A. Aggarwal, and R. D. Adam, submitted for publication) showed that clones of *G. lamblia* were able to generate organisms with varying antigens. When clones of the WB isolate which possess a 170-kilodalton (kDa) surface antigen were exposed to a 170-kDa-antigen-specific cytotoxic monoclonal antibody (MAb), all but a few *G. lamblia* organisms were killed. The surviving *Giardia* isolates no longer possessed the 170-kDa antigen, and analysis of these surviving populations revealed new surface antigens. Exposure of one of these cultures to a cytotoxic MAb specific to one of the new antigens resulted in the survival of *G. lamblia* possessing another set of antigens. Although loss and gain of antigens are clearly established in vitro, their biological relevance and whether these changes occur in vivo are uncertain. Antigenic variation in vivo may contribute to prolonged infection or to changes in virulence or immunogenicity. *G. lamblia* isolates from humans can infect gerbils, and this model has previously been used to study the course of infection by different human isolates (1, 3). To determine whether surface antigens change during the course of infection, gerbils were infected with characterized clones of the WB isolate, and the surface antigens were analyzed over the course of infection.

MATERIALS AND METHODS

Isolates. WB A6-6E7S and WB C1-6E7S are clones derived from isolate WB. They possess a major 170-kDa antigen on their surface which is recognized by MAb 6E7 (Table 1). In addition, this MAb is cytotoxic to *Giardia* isolates possessing this antigen. In vitro, when these clones are exposed to MAb 6E7, all but a few organisms are killed. The surviving *Giardia* clones, A6-6E7R and C1-6E7R, no longer possess the 170-kDa antigen and are resistant to MAb 6E7. The loss of this antigen and replacement by another antigen(s) was estimated to occur at a frequency of about 1/1,000 (Nash et al., submitted).

Culture. *G. lamblia* was maintained in TYI-S-33 medium with antibiotics as previously described (5).

Experimental procedures. Six- to eight-week-old Mongolian gerbils were infected as mentioned elsewhere (1). Briefly, gerbils were pretreated with 60 mg of metronidazole per day for 3 days and, 1 week later, inoculated by gavage with trophozoites from defined WB clones (Nash et al., submitted). *Giardia* trophozoites were harvested from the intestines at different times postinoculation (p.i.), axenized in TYI-S-33 with antibiotics, and maintained in medium from overnight to 4 days before surface labeling.

(i) Experiment 1. Groups 1a, 1b, and 1c consisted of groups of 10 gerbils each inoculated with 2 × 10⁸ trophozoites of WB C1-6E7S, WB C1-6E7R, or WB A6-6E7R, respectively. Trophozoites were harvested from the intestines at weekly intervals and radiolabeled after 3 or 4 days in culture.

(ii) Experiment 2. Fifteen gerbils were inoculated with 2 × 10⁹ trophozoites of WB C1-6E7S and sacrificed 3, 4, 5, and 6 days later. Recovered trophozoites were maintained in TYI-S-33 medium for 3 or 4 days and then cloned by limiting dilution (Nash et al., submitted). Clones of trophozoites possessing or not possessing the 170-kDa antigen were selected by immunofluorescence assay (IFA) with MAb 6E7. One of the IFA-positive clones and one negative clone were radiolabeled and further analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

(iii) Experiment 3. Antigenic change in vitro occurred at a frequency of about 1/1,000; therefore, gerbils inoculated with 2 × 10⁹ *Giardia* trophozoites may have been inoculated with about 2,000 *Giardia* trophozoites whose surface antigens varied. Three groups of five gerbils were each inoculated with 1 (group 3a), 10 (group 3b), or 100 (group 3c) trophozoites of clone WB A6-6E7S to rule out the possibility that the inoculum contained a heterogeneous population of *G. lamblia*. Recovered trophozoites from infected gerbils were studied after maintenance in medium overnight. At the time gerbils were inoculated, culture tubes were also inocu-
labeled with the same number of WB A6-6E7S and compared to isolates obtained from gerbils.

**Other procedures.** Trophozoites were tested for the presence of a 170-kDa antigen by previously established procedures with indirect immunofluorescence using MAb 6E7 (1) and by surface labeling (8). Acrylamide electrophoresis without mercaptoethanol was performed as described by Laemmli (6).

**RESULTS**

Loss of the 170-kDa antigen or banding pattern changes or both were demonstrated in all three gerbil experiments. Group 1a gerbils were inoculated with the 170-kDa-antigen possessing WB C1-6E7S clone. By day 7 p.i., the 170-kDa antigen was no longer detected on the surface of *G. lamblia* isolated from gerbil intestines and was replaced by a series of new bands. A major band was detected at 92 kDa (Fig. 1). The antigens from *G. lamblia* isolated on day 28 p.i. were the same as those from day 7 (Fig. 1). Group 1b gerbils were inoculated with WB C1-6E7R, which lacked the 170-kDa antigen. The surface antigens of trophozoites isolated on day 7 p.i. varied from the inoculum WB C1-6E7R, but no change in the banding patterns was seen between trophozoites on days 7 and 28 p.i. The surface antigens of trophozoites isolated from groups 1a and 1b on day 7 or later were similar despite the fact that the original inocula differed.

Trophozoites isolated on days 7 and 28 p.i. from gerbils inoculated with WB A6-6E7R did not show a 64-kDa antigen as seen in the original inoculum. Surprisingly, the antigen pattern was similar to that of trophozoites isolated from gerbils inoculated with either WB C1-6E7S or WB C1-6E7R (Fig. 1). The 170-kDa antigen was not detected on any of the *Giardia* trophozoites isolated from the groups of gerbils inoculated with *G. lamblia* possessing or not possessing the 170-kDa antigen. Therefore, reversion to 170-kDa-antigen positive *G. lamblia* did not occur during the infection. In other experiments, 170-kDa-antigen-negative *G. lamblia* did not revert to 170-kDa antigen positive even after 13 sequential infections in gerbils or mice (data not shown).

To determine how quickly loss of the 170-kDa antigen and replacement with other surface components occurred, *Giardia* trophozoites were isolated from gerbils 3 to 6 days p.i. with WB C1-6E7S. On day 3, the 170-kDa antigen was the only major antigen present (Fig. 2, lane 1). Analysis by IFA revealed that most of the *Giardia* trophozoites were 170-kDa antigen positive, but some were clearly negative, which indicates the loss of the 170-kDa antigen from some of the trophozoites and a resulting heterogeneous population. On days 4, 5, and 6 p.i., other antigens were radiolabeled in addition to the 170-kDa antigen (Fig. 2, lanes 2 through 4). The relative proportion of organisms with the 170-kDa antigen decreased, and there was an increase in the number of trophozoites with no 170-kDa antigen detected by IFA on days 4, 5, and 6 p.i. On day 6, analysis of cultures by IFA revealed that most of the *G. lamblia* were 170-kDa antigen negative, but a few organisms still possessed the 170-kDa antigen. Although multiple bands were seen when cultures were radiolabeled, randomly selected IFA-positive and -negative clones derived from these cultures tended to possess one major antigen, further suggesting that the complex banding pattern seen on the surface of *G. lamblia* isolated on individual days is due to the presence of a heterogeneous population (Fig. 3).

Group 3 gerbils were inoculated with 1, 10, and 100 trophozoites from WB A6-6E7S. In the previous experiments, because the inocula were relatively large, it was likely that gerbils were inoculated with a heterogeneous population. Of the 15 gerbils inoculated, only 1 gerbil ino-

![FIG. 1. Autoradiograph of SDS-PAGE analysis of radiolabeled trophozoites isolated from gerbils on different days p.i. with various clones of the WB *Giardia* isolate. Lanes: 1, WB C1-6E7S inoculum; 2 and 3, trophozoites isolated on days 7 and 28 p.i., respectively, from WB C1-6E7S-infected gerbils; 4 and 5, trophozoites isolated on days 7 and 28 p.i., respectively, from gerbils infected with WB C1-6E7R; 6 and 7, trophozoites isolated on days 7 and 28 p.i., respectively, from gerbils infected with WB A6-6E7R; 8, in vitro-derived WB C1-6E7R inoculum. Numbers on the right indicate molecular size standards (in kilodaltons).](http://iai.asm.org/)

![FIG. 2. Autoradiograph of SDS-PAGE analysis of 125I-labeled trophozoites isolated from gerbils infected with WB C1-6E7S (groups). Lanes: 1, day 3 p.i.; 2, day 4 p.i.; 3, day 5 p.i.; 4, day 6 p.i.; 5, in vitro-derived WB C1-6E7S inoculum. Numbers on the left are molecular size standards (in kilodaltons).](http://iai.asm.org/)
ulated with 100 trophozoites was infected. Analysis of this culture revealed the loss of the 170-kDa antigen, which was replaced by a major band at about 64 kDa (Fig. 4). In contrast, an in vitro culture initiated with the same inoculum and radiolabeled at the same time as the gerbils still possessed only the 170-kDa antigen. Interestingly, a WB A6-6E7R clone produced in vitro after exposure to cytotoxic MAb 6E7 appeared to have the same major surface antigen as that of intestinal isolated trophozoites (Nash et al., submitted).

DISCUSSION

The present studies document that antigenic variation is not solely an in vitro phenomenon, but that the surface antigens of *G. lambia* change in vivo as well. Previous in vitro studies demonstrated the ability of clones of *G. lambia* to generate organisms with varying surface antigens. In these in vitro studies, *Giardia* clones WB C1-6E7S and WB A6-6E7S, which possess a 170-kDa surface antigen, were exposed to cytotoxic MAb 6E7, which is specific to the 170-kDa antigen. The survivors, WB A6-6E7R and WB C1-6E7R, were resistant to the effects of MAb 6E7, and the 170-kDa antigen was replaced by a 64-kDa antigen in WB A6-6E7R and by a 170-kDa disperse antigen in WB C1-6E7R (Fig. 5). A clone of WB A6-6E7R was exposed to another cytotoxic MAb, 5C1, which reacted specifically with the 64-kDa surface antigen. The surviving *G. lambia* showed other antigens which no longer reacted with MAb 5C1.

In the present studies, the surface antigens of the inoculating clones changed during the course of infection. The 170-kDa antigen was lost from two separate 170-kDa-antigen possessing clones, and the surface antigens of two 6E7-resistant clones which did not possess the 170-kDa antigen also changed. Furthermore, there was no reappearance of the original 170-kDa antigen in gerbils inoculated with the 6E7-resistant clones.

**FIG. 3.** Heterogeneous populations of *G. lambia* isolated from WB C1-6E7S-infected gerbils on days 3 to 6 p.i. were cloned by limiting dilution. Clones were selected by IFA with MAB 6E7. SDS-PAGE of radiolabeled IFA-positive and -negative clones was done. Lanes: 1, in vitro-derived inoculum WB C1-6E7S; 2 and 3, IFA-positive and -negative clones, respectively, on day 3 p.i.; 4 and 5, IFA-positive and -negative clones, respectively, on day 4 p.i.; 6 and 7, IFA-positive and -negative clones, respectively, on day 5 p.i.; 8, IFA-negative clone isolated on day 6 p.i. Numbers on the left are molecular size standards (in kilodaltons).

**FIG. 4.** Autoradiograph of radiolabeled trophozoites analyzed by SDS-PAGE. Lanes: 1, in vitro-derived WB A6-6E7S inoculum; 2, trophozoites isolated from gerbils infected with WB A6-6E7S; 3, trophozoites from culture tube inoculated with WB A6-6E7S parallel to gerbil inoculation; 4, in vitro-derived WB A6-6E7R. Each lane shows the banding pattern of trophozoites isolated from an individual gerbil. Numbers on the left are molecular size standards (in kilodaltons).

Although there was antigenic variation in vivo, it differed from in vitro variation. Surface antigen patterns of radiolabeled trophozoites isolated from WB C1-6E7S-infected gerbils did not resemble those of in vitro-derived WB C1-6E7R; however, trophozoites isolated from WB A6-6E7S-inoculated gerbils possessed an antigen which was the same size as a 64-kDa antigen found in in vitro-derived WB A6-6E7R. Trophozoites isolated from all groups of gerbils except those inoculated with WB A6-6E7S showed almost the same...
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antigenic pattern, regardless of the inoculating clone (Fig. 5), indicating a nonrandom selection of antigens in vivo compared with the sequential selection of different antigens in vitro. Once the antigens had been selected in vivo, they remained unchanged, since we were not able to detect any major variation between trophozoites on days 7 and 28 p.i. Since clones of trophozoites isolated from the intestine yielded trophozoites with fewer bands, this pattern probably represents a heterogeneous population of organisms in which each trophozoite expresses a limited number of surface antigens.

In previous experiments, gerbils infected with uncloned WB self-cured after 4 to 5 weeks (1). Our present studies show no change in surface antigens from weeks 1 to 4. The failure of WB to undergo further antigenic change could be an important reason why gerbils infected with WB are able to self-cure and do not develop chronic infections. Given the ability of G. lamblia to change in vitro, it is not clear why further surface antigen changes did not occur in vivo. The course of WB infection in gerbils should be contrasted with that in gerbils infected with GS/M, which develop a chronic infection (1). From preliminary results analyzing GS/M clones in vitro, GS/M surface antigens seem to change in vitro much more rapidly than WB surface antigens. If this antigenic variation also occurs in vivo, it may be the reason for the development of chronic infections.

Antigenic variation has been studied in a number of other protozoa and bacteria. The attack phases of Trypanosoma congolense (11) and Borrelia spp. (4) possess heterogeneous populations, each possessing a single antigen on the surface. Variation of these proteins allowed the organisms to elude the host immune mechanisms, thus causing cycles of parasitemia or bacteremia. A heterogeneous population of trophozoites was present from day 3 p.i. onwards in gerbils infected with G. lamblia. Unlike Borrelia spp. and T. congolense, G. lamblia did not produce cycles of bacteremia or parasitemia. Major changes in the antigen patterns were not detected in Giardia populations on day 7 or 28 p.i.

In vitro studies suggest that immune pressure is one of the mechanisms capable of selecting G. lamblia with varying surface antigens. However, this may not be the case in vivo. Gerbils inoculated with two WB 6E7S clones, each possessing the 170-kDa antigen, were later found to be infected with G. lamblia possessing different surface antigens. This change was apparent by day 3, and the 170-kDa antigen was no longer detected on day 7. The early disappearance of the 170-kDa antigen indicates that the antibody is probably not an important factor in selecting for the presence or absence of certain G. lamblia, particularly in the early infection; however, formal studies are needed to prove this. Once G. lamblia produces a heterogeneous population in vivo, it would not be surprising if some antigen(s) possesses certain biological characteristics which convey advantage to one isolate of G. lamblia and not to another. In addition, antigenic variation under nonimmune conditions has been reported to occur in Trypanosoma brucei (7).

One potential mechanism which is associated with other changes is the presence of double-stranded RNA viruses which have been described in Trichomonas vaginalis and G. lamblia. Trichomonas vaginalis is able to alternate between two different phenotypes (2), an ability which is associated only with the presence of a double-stranded RNA virus (2, 15). Although some of the Giardia isolates possess double-stranded RNA virus (14), the WB isolate which we used for the present studies does not possess double-stranded RNA virus (C. C. Wang, personal communication).

Giardia infections in humans are characterized by a wide range of clinical courses and symptoms. Chronic infections clearly occur, and antigenic change may be responsible for prolonged infection, as well as for different clinical manifestations.

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