INFECTION AND IMMUNITY, Dec. 1993, p. 5394–5397
0019-9567/93/125394-04$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 61, No. 12

Antigenic Switching of TSA 417, a Trophozoite Variable Surface Protein, following Completion of the Life Cycle of *Giardia lamblia*

TZE-CHIANG MENG,[1]* MICHAEL L. HETSKO,[2] AND FRANCES D. GILLIN[2,3]

Department of Medicine, Division of Infectious Diseases,[1] Department of Pathology,[2] and Center for Molecular Genetics,[3] University of California at San Diego Medical Center, 200 West Arbor Drive, San Diego, California 92103-8416

Received 18 May 1993/Returned for modification 28 June 1993/Accepted 27 September 1993

Expression of TSA 417, the predominant cysteine-rich variable surface protein of *Giardia lamblia* WB clone C6 trophozoites, did not change during encystation in vitro. However, in vitro excystation of cysts derived in vitro or in vivo consistently produced TSA 417 nonexpressing trophozoite populations, suggesting that completion of the life cycle leads to antigenic switching.

The intestinal lumen-dwelling *Giardia lamblia* trophozoite differentiates into an infectious cyst which survives outside the host. The cysteine-rich variable surface protein (VSP) which covers the trophozoite undergoes spontaneous switching in vitro (13) and in vivo (2, 9, 14) and in response to selection by antibodies (1, 12) and intestinal proteases (16). Although *G. lamblia* occupies a different milieu, VSPs may be analogous to the variable surface glycoproteins of *Trypanosoma brucei*, which assist in evasion of host immune responses (19). As stage differentiation affects variable surface glycoprotein expression (20), we investigated whether the expression of TSA 417, the major VSP of *G. lamblia* WB (ATCC 30957) clone C6 (8) and of strain RB (isolated in our lab from a patient infected in Turkey), is similarly affected by differentiation during encystation and excystation in vitro.

G. lamblia trophozoites were cultivated and harvested as described previously (6, 7). Glutaraldehyde-fixed, nonpermeabilized trophozoites were examined by immunocytochemistry using polyclonal anti-recombinant TSA 417 antiserum (anti-rTSA 417) (8) (1:50) and protein A-horseradish peroxidase (Zymed, San Francisco, Calif.) (1:1,000) (17). About 80 to 95% of C6 (Fig. 1A) and RB (not shown) trophozoites were strongly labeled. TSA 417-nonexpressing trophozoites were selected by incubating 5×10^4 C6 trophozoites in 0.4 ml of growth medium containing antibiotics (5) with 0.1 ml of unheated anti-rTSA 417 for 45 min at 37°C. Killing was dependent on complement (not shown). A nonexpressing subclone called 1F (Fig. 1B) was derived by cloning survivors twice by limiting dilution (4). Because of frequent VSP switching (13), TSA 417-expressing trophozoites are found in nonexpressing clones and vice versa. Predominantly TSA 417-nonexpressing and -expressing subclones were also derived from untreated C6. Upon prolonged subculture, a variable tendency toward reexpression in initially nonexpressing subclones and decreased expression in predominantly expressing subclones was observed (Fig. 2).

To evaluate the effect of differentiation on TSA 417 expression, trophozoites were encysted and then excysted in vitro (5, 18) and labeled immunocytochemically, usually prior to the second subculture after excystation. Completion of the life cycle of TSA 417-expressing C6 (>75%) in vitro consistently produced trophozoites which were predominantly nonexpressing (<5%) (Table 1). Trophozoite populations derived from individual cysts obtained by cloning immediately after excystation were also TSA 417 nonexpressing. The change was not restricted to C6, as TSA 417 expression by the unrelated strain RB underwent a similar change. The characteristic ~66- and 85-kDa bands of TSA 417 (8) were not observed in immunoblots (not shown) of C6 or RB postexcystation cultures, but they were observed in those reverting to >10 to 15% TSA 417 expression, indicating that the capability of expressing the same epitope was retained.

To determine the specific stage at which TSA 417 expression changed, we monitored expression in C6 and 1F during encystation. In vitro encystation and excystation efficiency appeared to be similar in C6 and 1F, suggesting that the change did not result from more efficient encystation or excystation of TSA 417-nonexpressing trophozoites (not shown). No differences in the percentages of trophozoites expressing TSA 417 were observed between attached and nonattached trophozoite populations of C6 or 1F before or after 66 h of exposure to encystation conditions. Anti-rTSA 417 failed to label intact C6 water-resistant cysts, probably because it did not penetrate the cyst wall. While the intensity of the TSA 417 bands on immunoblots remained constant throughout encystation, it was reduced in water-resistant cysts (not shown). Whether this diminished reactivity represented a decrease in the percentage of cysts expressing TSA 417 or in the quantity of TSA 417 expressed by individual cysts could not be determined because of labeling artifacts when the cyst wall was disrupted by freeze-thawing or permeabilization with methanol. In frozen-section immunoelectron microscopy, TSA 417 was observed in plasma membranes beneath the cyst wall as well as in vacuolar membranes. In some water-resistant cysts, it was only observed in the vacuolar membranes, suggesting that these cells might have been in the process of switching (11a).

Loss of surface reactivity of an antigen could result from decreased expression of the antigen, modification or blocking of the reactive epitope(s), or failure of transport to the cell surface (3). As anti-rTSA 417 is a polyclonal antiserum against a complete recombinant protein, it is unlikely that all reactive epitopes are modified or blocked. A defect in

* Corresponding author.

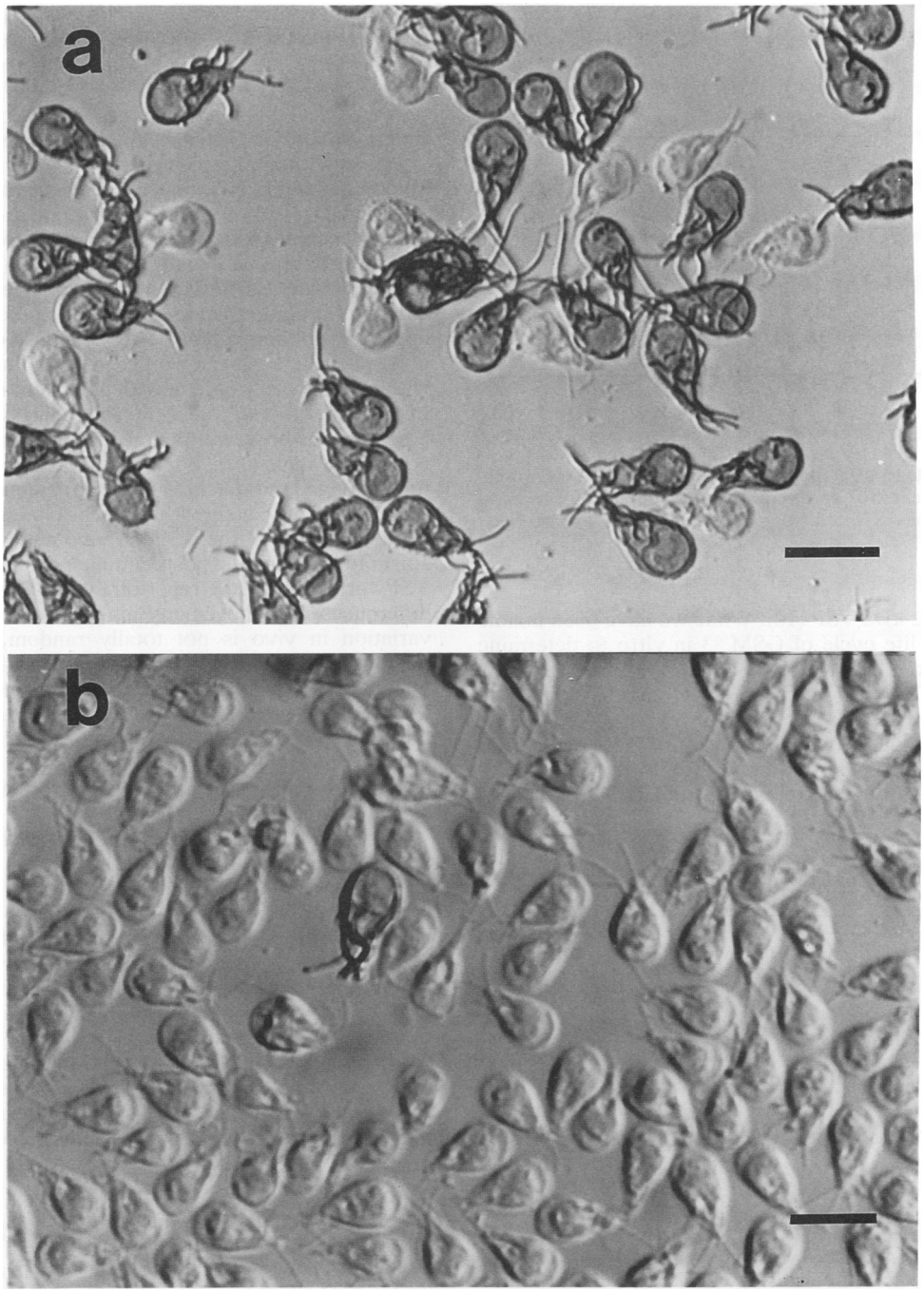

FIG. 1. (a) C6 trophozoites labeled with anti-rTSA 417. C6 is a clone of WB. Bar, 10 μM. (b) 1F trophozoites labeled with anti-rTSA 417. 1F is a subclone of C6 obtained by selection with anti-rTSA 417. A single positive cell is seen in panel b. Bar, 10 μM.

transport was ruled out by the absence of TSA 417 bands in immunoblots or immuno-electron micrographs of nonexpressing cells. Metabolic labeling of nonexpressing clones with [^{35}S]cysteine (8) revealed a variety of major bands (not shown), consistent with replacement of TSA 417 by other cysteine-rich proteins, as has been demonstrated for other VSPs (1). No change in expression was observed when trophozoites were exposed to the pH 4 induction or protease steps of the excystation procedure (5).

In contrast to our results with in vitro-derived cysts, Nash and colleagues reported that in vitro excystation of fecal cysts isolated from human volunteers early in infection with cloned strain GS/M 83, which is predominantly positive for a 72-kDa VSP, consistently produced trophozoites predominantly expressing the same VSP (14). Later, as the intestinal population shifted VSP expression, there was an accompanying loss of the 72-kDa VSP in the excysted populations. In addition, *scid* mouse mothers that acquired giardiasis from cysts shed by their nursing progeny, which had been infected with strain GS/M 83, had intestinal trophozoite populations

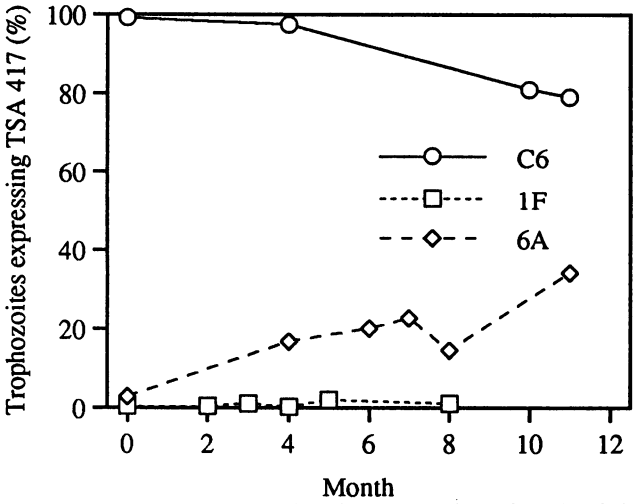

FIG. 2. Percentage of trophozoites expressing TSA 417. Subclone 6A is representative of several initially TSA 417-nonexpressing (<5%) subclones selected from C6 which tended toward reexpression of TSA 417.

expressing the 72-kDa VSP (10). While we have been unable to complete the life cycle of GSM/83 in vitro to determine whether the 72-kDa VSP behaves differently from TSA 417, we evaluated TSA 417 expression in vivo. Fecal cysts recovered from the large intestines of Swiss CF-1 suckling mice (Charles River, Wilmington, Mass.) 10 days after inoculation with TSA 417-expressing trophozoites (11) produced predominantly nonexpressing trophozoites after excystation in vitro (Table 1). The change had not occurred prior to encystation, as trophozoites recovered simultaneously from the small intestines of the mice continued to express TSA 417.

TABLE 1. TSA 417 expression in *G. lamblia* trophozoites after in vivo and in vitro encystation and in vitro excystation

Encystation type and subclone	Conditions	% Positive for TSA 417 expression[a]
In vitro encystation		
C6	Control	84.9 ± 9.0
	Postexcystation (16 cultures from 10 excystations)	3.6 ± 5.2[b]
C6	Control	96.7 ± 0.1
	Postexcystation (13 cultures from individual cysts from 1 excystation)	1.5 ± 0.8[b]
RB	Control	74.5
	Postexcystation (6 cultures from 3 excystations)	2.0 ± 1.8
1F	Control	2.8
	Postexcystation (1 culture from 1 excystation)	5.0
In vivo encystation		
C6	Intestinal trophozoites (9 cultures from 12 mice[c])	94.0 ± 1.0
	Postexcystation (9 cultures from 12 mice[c])	0.9 ± 0.8[b]

[a] Percent positivity determined by immunocytochemical labeling with anti-rTSA 417 followed by horseradish peroxidase conjugate. A minimum of 500 trophozoites were counted for each culture at ×200 or ×400 magnification. Multiple cultures were sometimes inoculated with treated cysts from a single excystation and were counted separately.

[b] $P < 0.0001$ (two-tailed unpaired t test; InStat 1.14, GraphPad Software Inc., San Diego, Calif.).

[c] Not all mice had paired cultures because of bacterial contamination.

Although their function is not understood, the VSPs are the major surface protein of the trophozoite and its principle interface with the host intestinal environment, including the immune system. Heat-inactivated anti-rTSA 417 inhibited attachment of C6 trophozoites and impaired growth in vitro (8), and complement-independent cytotoxicity was demonstrated for antibodies to other VSPs (12). Attachment-inhibiting mucosal antibodies could ameliorate giardiasis, although in limited human infection studies the presence of intestinal immunoglobulin A (IgA) against *G. lamblia* neither prevented the establishment of infection nor was associated with clearance in a patient with persistent infection (15). The importance of antibodies in antigenic variation was supported by the observation that VSPs varied in nude mice but not in *scid* mice (10). While a given VSP may predominate for prolonged periods in vitro, spontaneous variation results in heterogeneity even within recent clones (Fig. 2). Spontaneous variation of the VSPs in vivo may represent a mechanism for evading the host immune response, and the rate of VSP variation and the repertoire of VSPs may contribute to differences in pathogenicity among isolates (15). Antigenic variation in vivo is not totally random, as there was a tendency to express the same VSP when gerbils were infected with WB clones expressing different predominant VSPs (2). As changes in the VSP expression of the population in vivo occurred rapidly and earlier in infection than could be accounted for by immune selection, adaptation to host factors may also affect VSP expression and result in biased expression of certain VSPs.

Encystation is a complex process resulting in morphologic, functional, and antigenic changes. We have demonstrated that TSA 417 expression appears to switch after the completion of encystation and before subsequent regrowth after excystation in vitro. Shifting to different VSPs after excystation in a new host may allow excysted trophozoites to evade preexisting mucosal antibodies against VSPs from prior infections, especially in areas in which there is a high frequency of infection.

While these studies clearly show that completion of the life cycle of *G. lamblia* in vitro leads to switching of TSA 417, further studies are necessary to determine whether this is limited to certain VSPs and whether it occurs routinely in vivo.

We are grateful to D. S. Reiner for assistance in producing the TSA 417-nonexpressing subclones and the anti-TSA 417 antiserum, M. McCaffery for immuno-electron microscopy, and T. E. Nash for the GS/M 83 strain.

This work was supported by grants AI 19863, AI 24285, AM 35108, and HD 29275 from the National Institutes of Health. T.-C. Meng was a Merck Sharp & Dohme fellow in infectious diseases.

REFERENCES

1. **Aggarwal, A., J. W. Merritt, and T. E. Nash.** 1989. Cysteine-rich variant proteins of *Giardia lamblia*. Mol. Biol. Parasitol. **32**:39–48.
2. **Aggarwal, A., and T. E. Nash.** 1988. Antigenic variation of *Giardia lamblia* in vivo. Infect. Immun. **45**:700–707.
3. **Alderete, J. F., L. Suprun-Brown, and L. Kasmala.** 1986. Monoclonal antibody to a major surface glycoprotein immunogen differentiates isolates and subpopulations of *Trichomonas vaginalis*. Infect. Immun. **52**:70–75.

4. **Baum, K. F., R. L. Berens, R. H. Jones, and J. J. Marr.** 1988. A new method for cloning Giardia lamblia, with a discussion of the statistical considerations of limiting dilution. J. Parasitol. **64:** 267–269.
5. **Boucher, S. E. M., and F. D. Gillin.** 1990. Excystation of in vitro-derived *Giardia lamblia* cysts. Infect. Immun. **58:**3516–3522.
6. **Diamond, L. S., D. Harlow, and C. C. Cunnick.** 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans. R. Soc. Trop. Med. Hyg. **72:**431–432.
7. **Gillin, F. D., S. E. Boucher, and D. S. Reiner.** 1989. *Giardia lamblia*: the roles of bile, lactic acid, and pH in completion of the life cycle in vitro. Exp. Parasitol. 164–174.
8. **Gillin, F. D., P. Hagblom, J. Harwood, S. B. Aley, D. S. Reiner, M. McCaffery, M. So, and D. G. Guiney.** 1990. Isolation and expression of the gene for a major surface protein of *Giardia lamblia*. Proc. Natl. Acad. Sci. USA **87:**4463–4467.
9. **Gottstein, B., G. R. Harriman, J. T. Conrad, and T. E. Nash.** 1990. Antigenic variation in *Giardia lamblia*: cellular and humoral immune response in a mouse model. Parasite Immunol. **12:**659–673.
10. **Gottstein, D., and T. E. Nash.** 1991. Antigenic variation in *Giardia lamblia*: infection of congenitally athymic nude and *scid* mice. Parasite Immunol. **13:**649–659.
11. **Hill, D. R., R. L. Guerrant, R. D. Pearson, and E. L. Hewlett.** 1983. *Giardia lamblia* infection of suckling mice. J. Infect. Dis. **147:**217–221.
11a. **McCaffery, M.** Unpublished observations.
12. **Nash, T., and A. Aggarwal.** 1986. Cytotoxicity of monoclonal antibodies to a subset of Giardia isolates. J. Immunol. **136:**2628–2632.
13. **Nash, T. E., Conrad, J. T., and J. W. Merritt.** 1990. Variant specific epitopes of *Giardia lamblia*. Mol. Biochem. Parasitol. **42:**125–132.
14. **Nash, T. E., D. A. Herrington, M. M. Levine, J. T. Conrad, and J. W. Merritt.** 1990. Antigenic variation of *Giardia lamblia* in experimental human infections. J. Immunol. **144:**4362–4369.
15. **Nash, T. E., D. A. Herrington, G. A. Losonsky, and M. M. Levine.** 1987. Experimental human infections with *Giardia lamblia*. J. Infect. Dis. **156:**974–984.
16. **Nash, T. E., J. W. Merritt, and J. T. Conrad.** 1991. Isolate and epitope variability in susceptibility of *Giardia lamblia* to intestinal proteases. Infect. Immun. **59:**1334–1340.
17. **Reiner, D. S., H. Douglas, and F. D. Gillin.** 1989. Identification and localization of cyst-specific antigens of *Giardia lamblia*. Infect. Immun. **57:**963–968.
18. **Rice, E. W., and F. W. Schaefer III.** 1981. Improved in vitro excystation procedure for *Giardia lamblia* cysts. J. Clin. Microbiol. **14:**709–710.
19. **Vickerman, K.** 1989. Trypanosome sociology and antigenic variation. Parasitology **99:**S37–S47.
20. **Zeigelbauer, K., and P. Overath.** 1990. Surface antigen change during differentiation of *Trypanosoma brucei*. Biochem. Soc. Trans. **18:**731–733.