INFECTION AND IMMUNITY, May 1982, p. 714–719
0019-9567/82/050714-06$02.00/0

Vol. 36, No. 2

Antigenic Analysis of *Giardia lamblia* from Afghanistan, Puerto Rico, Ecuador, and Oregon

PHILLIP D. SMITH,†* FRANCES D. GILLIN, NUZHAT A. KAUSHAL, AND THEODORE E. NASH

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

Received 13 October 1981/Accepted 14 December 1981

The trophozoite antigens of *Giardia lamblia* to which host humoral and cellular immune responses are directed have not been identified. Therefore, we initiated studies to characterize these antigens in strains of *G. lamblia* from Afghanistan, Oregon, Ecuador, and Puerto Rico. By polyacrylamide gel electrophoresis, the electrophoretic mobility patterns of proteins of the four strains were similar; molecular weights of protein bands ranged between 12,000 and 140,000. The antigens which reacted with rabbit anti-*G. lamblia* antisera by immunoelectrophoresis were also similar for the four strains. However, comparison by crossed immunoelectrophoresis showed the Oregon strain, which has been the longest in culture, lacked a set of anodic antigens and the single neutral antigen which were present in the other three strains. In addition, other minor antigen differences between the strains were detected by this technique. When we employed trophozoites from each strain as antigen in an enzyme-linked immunosorbent assay against 10 human antisera of various titers, we also detected some differences between the strains. Although polyacrylamide gel electrophoresis and immunoelectrophoresis revealed gross similarity among *G. lamblia* from widely differing geographic locations, subtle differences detected by crossed electrophoresis and enzyme-linked immunosorbent assay suggest the existence of potentially important antigenic differences among these strains.

Host response to *Giardia lamblia*, a common enteric protozoan parasite of humans, may be modulated by immune factors. This was initially suggested by observations that prior exposure to *G. lamblia* may increase resistance to a second infection (9) and that immunoglobulin-deficient patients may have increased susceptibility to the infection (6). Circulating anti-*G. lamblia* antibodies (14, 18) and cytotoxic monocytes (13a) may participate in this modulation. Although these and other mechanisms of host defense are under investigation, the antigenic characterization of *G. lamblia* has received little or no attention.

Recently developed techniques for isolating and culturing *G. lamblia* (16) have allowed us to initiate studies to identify and characterize the trophozoite antigens of four strains of *G. lamblia* from widely differing geographic locations. Identification of these antigens may provide important information on strain-specific antigens and allow characterization of the antigen to which immune responses are directed. This will facilitate examination of host defense mechanisms against *G. lamblia*. Therefore, employing sensitive immunochemical and electrophoretic separation techniques, we describe protein and antigen characteristics of *G. lamblia* isolated from individuals who acquired their infection in Afghanistan, Oregon, Ecuador, and Puerto Rico.

† Present address: Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20205.

MATERIALS AND METHODS

Source of *G. lamblia*. The following strains of *G. lamblia* trophozoites were studied: WB (American Type Culture Collection 30957) isolated in this laboratory from a 29-year-old male who became symptomatic in Afghanistan (P. D. Smith, F. G. Gillin, W. M. Spira, and T. E. Nash, submitted for publication); PO (American Type Culture Collection 30888), isolated from a 36-year-old symptomatic female from Portland, Oregon (10), obtained from G. S. Visvesvara; and RS, from a 43-year-old asymptomatic female from Ecuador, and LT, from a 17-year-old symptomatic male from Puerto Rico, obtained from M. Wittner. Symptoms in persons infected with WB, PO, and LT included diarrhea and abdominal cramps which resolved upon eradication of the parasite. All strains of *G. lamblia* trophozoites were obtained from the patient's proximal intestinal fluid by either intubation or Enterotest capsule (Hedico, Palo Alto, Calif.).

***G. lamblia* culture.** Organisms were subcultured twice per week in filter-sterilized TYI-S-33 (3) medium with vitamins, 10% heat-inactivated adult bovine se-

rum, penicillin (50 U/ml), and streptomycin (50 μg/ml). Morphological features and growth curves for organisms from the four strains were similar.

Antigen preparation. Intact and solubilized *G. lamblia* trophozoites were used as antigen. Intact organisms were prepared by pooling viable log phase cultures and washing four times in phosphate-buffered saline (pH 7.4). Soluble antigen was prepared by sonicating washed organisms (15) and then removing debris by centrifugation at 250 × *g* for 30 min. Protein content of the solubilized trophozoites was determined by the method of Lowry et al. (8).

Rabbit immunization. Four New Zealand white male rabbits weighing 3 to 4 kg each were immunized with *G. lamblia* WB trophozoites. The antigen emulsion contained equal portions of a suspension of trophozoites (WB) and adjuvant. The initial immunization with Freund complete adjuvant was administered in the hind paw; all subsequent boosts employed Freund incomplete adjuvant and were administered in several subcutaneous sites. A total of approximately 100×10^6 trophozoites were administered over a 4-week period at weekly intervals; the animals were rested for at least 4 weeks, and the series of subcutaneous injections was repeated two times, after which precipitating antibody in the immune rabbit serum was detected by immunodiffusion in 1% agarose in 0.075 M barbital buffer, pH 8.6. Sera were pooled for the experiments employing rabbit antiserum.

PAGE. The protein constituents of the four strains were separated and compared by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (7). Briefly, soluble antigen (4.5 mg of protein per ml), prepared by boiling in sample buffer containing SDS and 2-mercaptoethanol, was electrophoresed on a vertical 5 to 15% polyacrylamide gradient slab gel at 40 V for 16 h. High- and low-molecular-weight protein standards (Bio-Rad Laboratories, Richmond, Calif.) were run simultaneously. Gels were washed, dried, and stained for protein with 0.125% Coomassie brilliant blue. The molecular weights of *G. lamblia* trophozoite proteins were determined by comparing their electrophoretic mobility with that of the standard proteins.

To determine whether soluble antigen was representative of intact organisms, the protein pattern of intact organisms (WB) was compared by SDS-PAGE with that of the supernatant fractions of sonicated trophozoites (WB).

IEP. The antigens in the four strains were first identified by immunoelectrophoresis (IEP). Ten microliters of soluble antigen (4.5 mg of protein per ml) was electrophoresed in 1% agarose at 6 V/cm for 45 min in 0.05 M barbital buffer (pH 8.6) and then reacted with rabbit anti-*G. lamblia* WB serum. Plates were washed, dried, and stained with Coomassie brilliant blue.

CIE. To enhance identification and comparison of the antigens, crossed immunoelectrophoresis (CIE) was performed (1). Ten microliters of soluble antigen was electrophoresed in the first dimension in a 1% agarose gel (0.15 by 10 by 10 cm) at 10 V/cm for 40 min. A longitudinal 1- by 7-cm-wide gel strip containing the separated antigens was transferred to a second plate containing agar with 20% rabbit anti-*G. lamblia* serum. The separated antigens were further electrophoresed, at right angles to the first dimension, for 18 h at 2 V/cm. The dimensions for the antibody-containing anodic gel were 0.11 by 5 by 7 cm and for the antibody-containing cathodic gel were 0.11 by 3 by 7 cm. An intermediate gel (0.13 by 1.0 by 7 cm) containing no antiserum was poured between the antigen strip and the anodic gel. Plates were washed, dried, and stained with Coomassie brilliant blue.

ELISA. The capacity of each *G. lamblia* strain to serve as antigen for human immunoglobulin G anti-*G. lamblia* antibody was compared in an enzyme-linked immunosorbent assay (ELISA) as previously described (14). Briefly, equivalent numbers of trophozoites from the four strains employed as antigen in parallel ELISAs were tested in duplicate with 10 human sera (dilution 1:64) shown previously (14) to contain immunoglobulin G anti-*G. lamblia* antibody at various titers. Optical densities were read at 30 and 45 min, and the optical densities of duplicate determinations were averaged. The resulting 80 determinations (2 readings × 10 sera × 4 antigens) were subjected to an analysis of variance.

RESULTS

PAGE. The trophozoite protein patterns of soluble antigen of the four *G. lamblia* strains were analyzed by SDS-PAGE. The electrophoretic migration patterns (Fig. 1) showed 26 protein bands with molecular weights ranging from

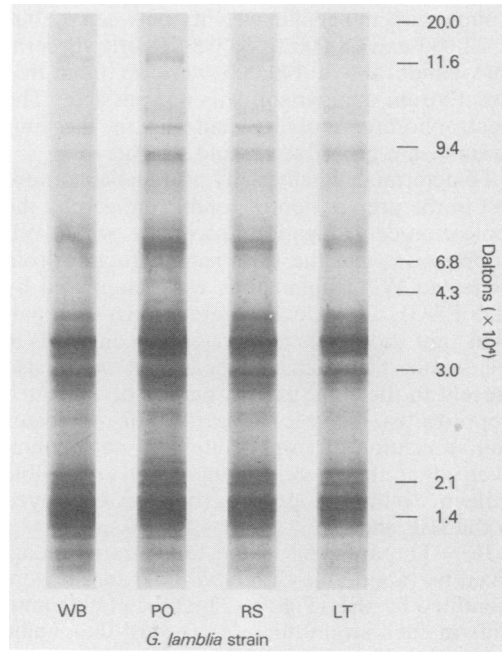

FIG. 1. SDS-PAGE of four strains of *G. lamblia*. Twenty microliters of soluble antigen (4.5 mg of protein per ml) from the WB (Afghanistan), PO (Oregon), RS (Ecuador), and LT (Puerto Rico) strains was electrophoresed as described in the text, and the protein bands were stained with Coomassie brilliant blue.

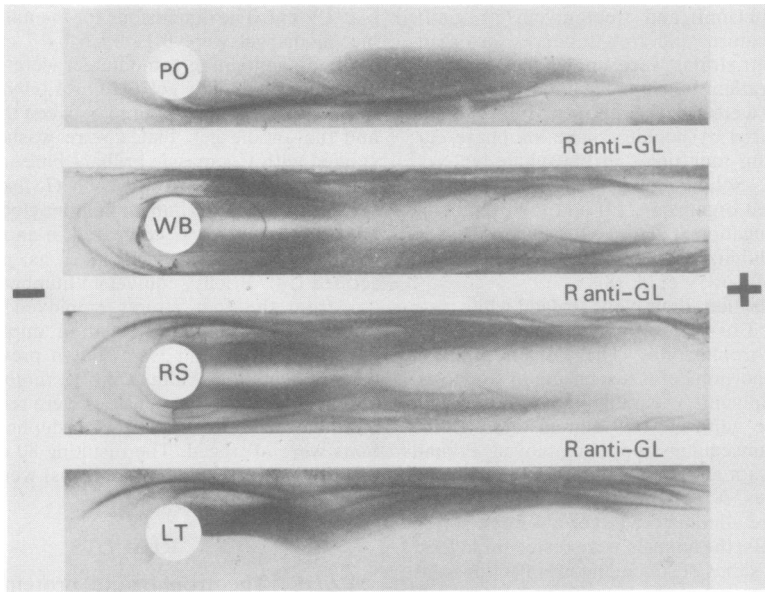

FIG. 2. Immunoelectrophoresis of *G. lamblia*. Ten microliters of soluble antigen (4.5 mg of protein per ml) from each strain was electrophoresed as described in the text and then reacted with rabbit anti-*G. lamblia* WB serum. Gels were stained with Coomassie brilliant blue.

10,000 to 140,000; the majority fell into two groups with molecular weights between 12,000 to 22,000 and 28,000 to 38,000. Poorly discernable bands above 140,000 were so faint that direct strain comparison was not possible. The electrophoretic mobility patterns of the four strains were reproducible and similar.

To determine whether any protein bands were lost in the preparation of soluble antigen by the sonication-centrifugation procedure, whole WB trophozoites and the supernatant fraction from sonicated WB trophozoites were compared by SDS-PAGE. The electrophoretic migration patterns (not shown) demonstrated that all bands in the whole trophozoite preparation were also present in the supernatant fraction of sonicated trophozoites (soluble antigen). This indicates that sonication of trophozoites did not qualitatively alter the protein composition of soluble antigen. Soluble antigen was therefore employed in the IEP and CIE.

IEP. The antigens in the four strains recognized by rabbit anti-*G. lamblia* WB serum were identified by IEP (Fig. 2). The precipitable antigens in each strain migrated toward the anodic electrode. The antigens appeared similar, but overlap made clear differentiation of each precipitin band difficult.

CIE. Precipitable antigens were therefore compared by CIE employing rabbit anti-*G. lamblia* WB serum in the second-dimension gel. The CIE profiles for the four strains (Fig. 3) demonstrate remarkable overall similarity in the antigens. The more densely staining precipitin arcs correspond to major antigens, and the less densely staining arcs correspond to minor antigens as detected by the rabbit antisera. Strain WB, against which the antiserum was raised, had the most complete set of antigens in this system: 37 anodic antigens and 1 neutral antigen. However, subtle but reproducible antigenic differences were observed. A schematic representation (Fig. 4) of the CIE profile from each strain further identifies these differences: Strain PO lacked the prominent major anodic antigen 33, the minor anodic antigen 32, and the single minor neutral antigen 38 which were present in the other strains; strain RS lacked minor antigens 15 and 21; strains RS and LT lacked minor antigens 7 and 29; strains RS, LT, and PO lacked minor antigen 23. Quantitative differences in other antigens (Fig. 3A and B), reflected by differences in the height of corresponding precipitin arcs, were also present.

ELISA. Sufficient cross-reactivity existed among the strains to allow trophozoites from each strain to be used for detecting anti-*G. lamblia* antibodies by ELISA. In addition, employing trophozoites from each strain as antigen in parallel ELISAs against 10 human antisera revealed significant strain differences when compared by analysis of variance. An analysis of variance showed that the largest source of variation was that among sera (Table 1). In the

remaining sources of variability there was a significant antigen-serum interaction ($F = 6.46$; $df = 27,27$; $P < 0.001$), that is, individual sera failed to respond in a parallel fashion to the various antigens. In particular, six sera (no. 1, 2, 4, 5, 8, and 10) showed marked deviation from the expected optical density (Table 2). Thus, the ELISA is a sensitive test which detects subtle antigenic differences in the four strains.

DISCUSSION

The present study demonstrates the complexity of soluble trophozoite extracts of four strains of *G. lamblia* from widely differing geographic locations. The PAGE, IEP, and CIE results emphasize a remarkable overall similarity in the trophozoite proteins and antigens. However, CIE, which detected the antigens reactive with rabbit anti-*G. lamblia* serum, more clearly revealed qualitative and quantitative antigenic differences. Most notably, the PO strain lacked a group of anodic antigens (major antigen 32 and minor antigen 33) and the single neutral antigen (minor antigen 38) which were present in the other strains. Interestingly, the PO strain has been in culture longer (approximately 6 years) than the other strains; this raises the possibility

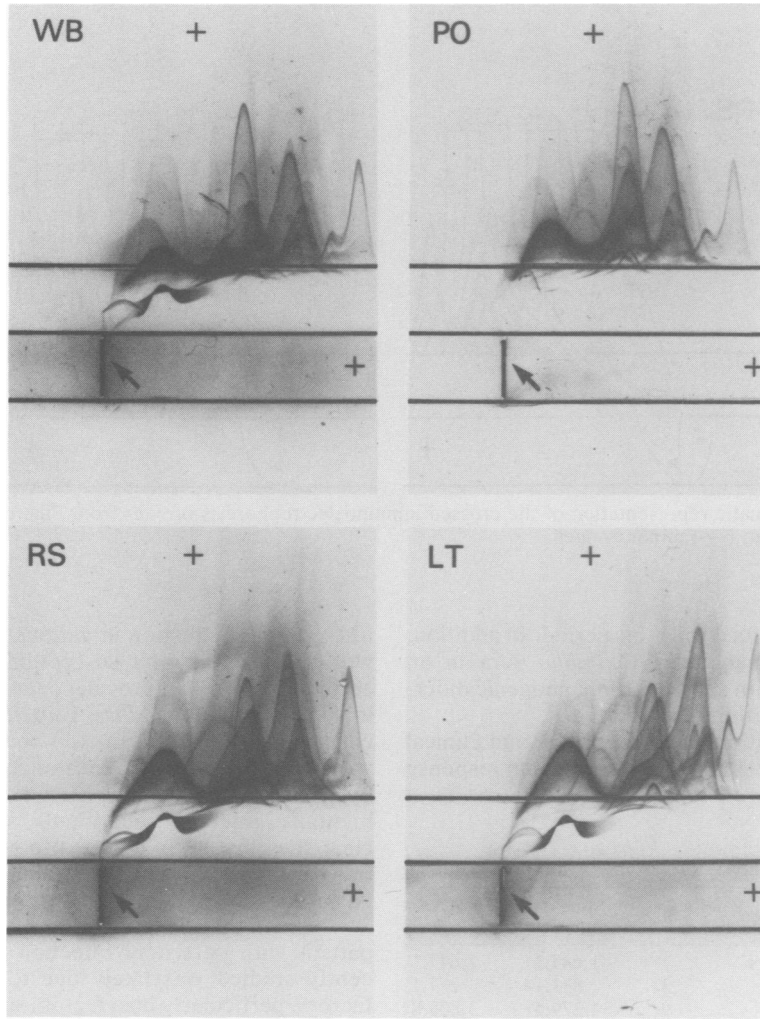

FIG. 3. Crossed immunoelectrophoresis profile of *G. lamblia*. Ten microliters of soluble antigen from each of the four strains was electrophoresed in the first dimension in a nonantibody agarose gel and then in the second dimension in a 20% antibody (rabbit anti-*G. lamblia* WB serum) agarose gel, at right angles to the first dimension, under the conditions described in the text. An intermediate gel containing no antiserum separated the antigen strip and the anodic gel. Gels were stained with Coomassie brilliant blue. +, Anode; ↖, point of application of sample.

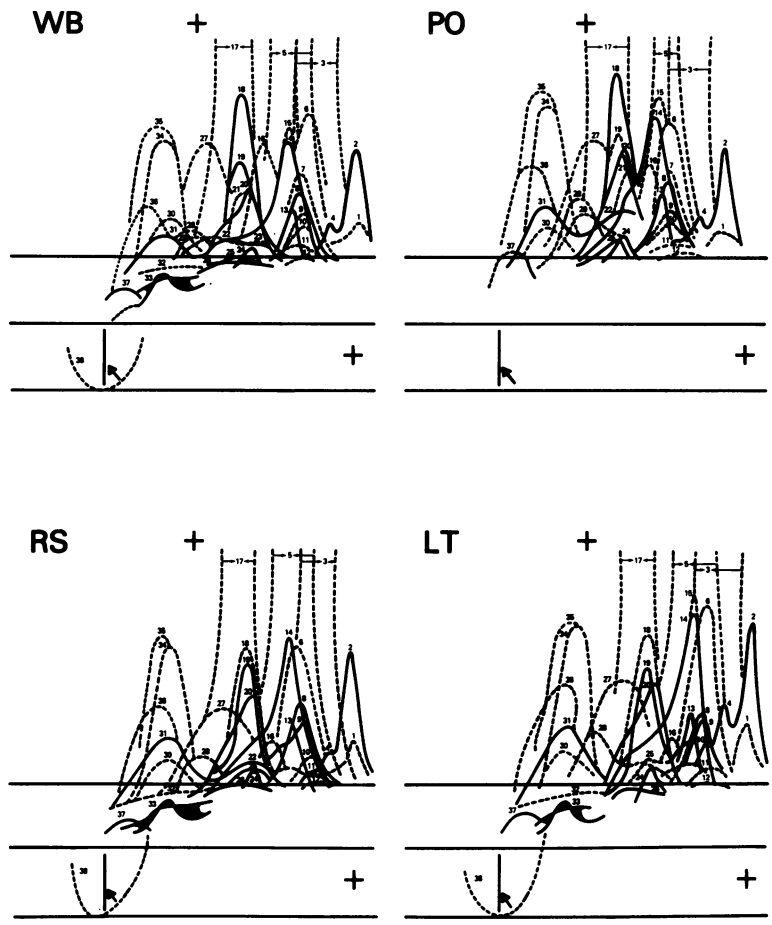

FIG. 4. Schematic representation of the crossed immunoelectrophoresis profiles from Figure 3A. Symbols: (—) major antigen; (----) minor antigen.

of antigenic shifts during this period. In addition, employing human anti-*G. lamblia* sera in an ELISA, we again showed subtle antigenic differences between the four strains.

A wide variation in susceptibility and clinical manifestation characterizes the human response to *G. lamblia* infection in various regions of the world. The variation in susceptibility is suggested by differences in prevalence of infection: 3% in some populations of the United States (19) in contrast to 23% of tourists to Leningrad (2). The variation in clinical manifestation is suggested by an earlier observation (12) that 86% of experimentally infected individuals spontaneously clear the parasite in contrast to recent descriptions of American (17), Scandinavian (11), and Australian (5) patients with chronic giardiasis. We believe that the chronicity of giardiasis in a patient with persistent infection whom we recently studied was likely due to a number of factors, particularly host factors such as reduced cytotoxicity for the organism and not drug resistance or toxin production.

The variation in susceptibility and clinical manifestation in giardiasis patients may be related to the immune response of specific host defense mechanisms. These mechanisms ulti-

TABLE 1. Analysis of variance of ELISA results

Source of variation	df	Sum of squares	Mean square
Between readings	1	1,647.11	1,647.1
Among antigens	3	831.14	277.1
Among sera	9	34,279.51	3,808.8
Reading × antigens	3	436.64	145.6
Reading × serum	9	161.01	17.9
Antigen × serum	27	1,919.24	71.1
Residual	27	297.74	11.0
Total	79	39,572.39	5,978.6

TABLE 2. Differences between expected and observed ELISA optical density for 10 human antisera with four antigens

Serum	Antigen (*G. lamblia* strain)			
	WB	PO	RS	LT
1	6.975	−1.125	−14.425	8.575
2	3.975	−10.125	−2.425	8.575
3	5.225	−4.875	−1.175	0.825
4	3.475	−1.625	−11.925	10.075
5	−0.025	−21.125	16.575	4.575
6	−0.525	8.375	2.075	−9.025
7	4.725	−5.375	−5.675	6.325
8	−7.275	25.625	4.325	−22.675
9	−2.275	−2.375	−0.675	5.325
10	−14.275	12.625	13.325	−11.675

mately must be defined in terms of selective recognition of the relevant parasite antigens. The antigenic differences among strains of *G. lamblia* which we have demonstrated may contribute to the variation in host responses to this parasite. The significance of antigenic variation in other protozoan parasites has recently been emphasized. Antigenic variation in salivarian trypanosomes (4) appears to account for their ability to evade host defense mechanisms. Antigenic variation, along with other factors, also may be important in host defense against *Plasmodium knowlesi* schizonts (13).

In this report subtle but potentially important antigenic differences in *G. lamblia* from differing geographic locations are described. We suggest that these differences may be important in the host immune response to *G. lamblia* and that the strain therefore must be considered when comparing immunological investigations of host responses to *G. lamblia*.

ACKNOWLEDGMENT

We thank David W. Alling for assistance with the statistical evaluation.

LITERATURE CITED

1. **Axelsen, N. H., J. Kroll, and B. Weeke.** 1973. A manual of quantitative immunoelectrophoresis, methods and application. Scand. J. Immunol. **2**:47–56.
2. **Brodsky, R. E., H. C. Spencer, and M. G. Schultz.** 1974. Giardiasis in American travelers to the Soviet Union. J. Infect. Dis. **130**:319–323.
3. **Diamond L. S., D. R. 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.
4. **Gray, A. R., and A. G. Luckins.** 1976. Antigenic variation in Salivarian trypanosomes, p. 493–542. *In* W. H. R. Lumsden and D. A. Evans (ed.), Biology of the kinetoplastida, vol. 1. Academic Press, Inc., London.
5. **Heap, T.** 1974. Giardiasis: a common cause of prolonged diarrhea in adults. Med. J. Aust. **2**:592–595.
6. **Hughes, W. S., J. J. Cerda, P. Hotlzapple, and F. P. Brooks.** 1971. Primary hypogammaglobulinenima and malabsorption. Ann. Intern. Med. **74**:903–910.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the head of bacteriophage T4. Nature (London) **227**:680–685.
8. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. **193**:265–275.
9. **Moore, G. T., W. M. Cross, D. McGuire, C. S. Mollohan, N. N. Gleason, G. R. Healy, and L. H. Newton.** 1969. Epidemic giardiasis at a ski resort. N. Engl. J. Med. **281**:402–407.
10. **Myer, E. A.** 1976. *Giardia lamblia*: isolation and axenic cultivation. Exp. Parasitol. **39**:101–105.
11. **Peterson, H.** 1972. Giardiasis (lambliasis). Scand. J. Gastroenterol. **7**(Suppl.):1–44.
12. **Rendtorff, R. C.** 1954. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. Am. J. Hyg. **59**:209–220.
13. **Schmidt-Ulrich, R., D. F. H. Wallach, and J. Lightholder.** 1979. Two plasmodium knowlesi-specific antigens on the surface of schizont-infected Rhesus monkey erythrocytes induce antibody production in immune hosts. J. Exp. Med. **150**:86–99.
13a. **Smith, P. D., C. O. Elson, D. B. Keister, and T. E. Wash.** 1982. Human host response to *Giardia lamblia*. I. Spontaneous killing by mononuclear leukocytes in vitro. J. Immunol. **128**:1372–1376.
14. **Smith, P. D., F. D. Gillin, W. R. Brown, and T. E. Nash.** 1981. IgG antibody to *Giardia lamblia* detected by enzyme-linked immunosorbent assay. Gastroenterology **80**:1476–1480.
15. **Smith, P. D., C. R. Horsburgh, and W. R. Brown.** 1981. *In vitro* studies on bile acid deconjugation and lipolysis inhibition by *Giardia lamblia*. Dig. Dis. Sci. **26**:700–704.
16. **Visvesvara, G. S.** 1980. Axenic growth of *Giardia lamblia* in Diamond's TPS-1 medium. Trans. R. Soc. Trop. Med. Hyg. **74**:213–215.
17. **Visvesvara, G. S., and G. R. Healy.** 1979. The possible use of an indirect immunofluorescent test using axenically grown *Giardia lamblia* antigens in diagnosing Giardiasis, p. 53–63. *In* W. Jakubowski and J. C. Hoff (ed.), Proceedings, symposium on waterborne transmission of giardiasis. U.S. Environmental Protection Agency, Cincinnati.
18. **Visvesvara, G. S., P. D. Smith, G. R. Healy, and W. R. Brown.** 1980. An immunofluorescence test to detect serum antibodies to *Giardia lamblia*. Ann. Intern. Med. **93**:802–805.
19. **Wright, R. A., H. C. Spencer, R. E. Brodsky, and T. M. Vernon.** 1977. Giardiasis in Colorado: an epidemiologic study. Am. J. Epidemiol. **705**:330–336.