Clinical Investigation

Antigenic Conservation and Variation in *Giardia* Cysts From Various Vertebrate Hosts

EDWARD T. RILEY, MD, *Portland, and* HENRY H. STIBBS, PhD, *New Orleans*

Monoclonal antibodies produced against Giardia muris *cysts reacted in indirect immunofluorescence with homologous cysts and cysts from a* Giardia*-infected wild Norway rat but did not cross-react with* Giardia lamblia *cysts of human, dog, or beaver sources. Another monoclonal antibody raised against* Giardia simoni *cysts from the Norway rat reacted with homologous cysts (rat) and cross-reacted with cysts from a cow. The demonstration of antigenic differences at the cyst surfaces of* Giardia *organisms of animal and human origin suggests that it is possible to identify the animal source of* Giardia *cysts according to their pattern of reactivity with monoclonal antibodies. Such identification would have a useful application in affirming the possible zoonotic transmission of animal source* Giardia *species to humans.*

(Riley ET, Stibbs HH: Antigenic conservation and variation in *Giardia* cysts from various vertebrate hosts. West J Med 1989 Oct; 151:403-405)

Zoonotic transmission of *Giardia lamblia* cysts to humans through drinking water has been implicated as an important cause of epidemic giardiasis, an important diarrheal disease.[1,2] Identifying the source of the giardial cysts is one of the major challenges facing epidemiologists studying these outbreaks. Many different animals are hosts to *Giardia* species, and transmission experiments have revealed evidence for both cross-host-species transmission and some degree of host specificity for different species or organisms of *Giardia*.[3-6] To compound the epidemiologists' dilemma, different species of *Giardia* are to a large degree morphologically indistinguishable.[7] Consequently, determining the host reservoir—that is, cyst source—in waterborne giardiasis outbreaks and tracing cysts found while monitoring water supplies have been based on speculation and circumstantial evidence.

Clearly, to determine relatedness, a biochemical, antigenic, or genetic method of differentiating giardial cysts is needed for comparing cysts from animal sources, from drinking water, and from persons infected in outbreaks. In answer to this need, we now report the development of monoclonal antibodies that distinguish *G lamblia* (human source) from *Giardia muris* (hamster source) cysts by indirect immunofluorescence.

Materials and Methods

Giardia muris cysts were purified from the feces of experimentally infected nu nu (thymusless) mice, and *Giardia simoni* cysts were isolated from the feces of a naturally infected wild Norway rat. The original source of the *G muris* cysts was an infected golden hamster at Case Western Reserve University (Cleveland, Ohio).[8] The infected rat was trapped along a slough on the University of Washington campus that drains into Lake Washington. Cysts were purified by centrifuging crushed fecal pellets over a solution of 1 mol per liter sucrose in water (ten minutes at 500*g*) followed by centrifuging over two layers of Percoll (Sigma Chemical Company, St Louis, Missouri) of specific gravities 1.05 and 1.09 (ten minutes at 500*g*).[9] Monoclonal antibodies were prepared by immunizing BALB/c mice with the whole, live, freshly isolated, and purified *G muris* cysts through a series of four intraperitoneal injections of about 2×10^6 cysts per animal per injection over six weeks followed by one or two intravenous tail injections, four days apart, of 2×10^6 cysts in a sterile saline solution. Four or five days after the last intravenous injection, the mice were killed and the spleen cell:myeloma cell fusion was carried out. Spleen cells from the immunized mice were fused with NS-1 (P3-NS-1/1-Ag 4.1) mouse myeloma cells grown in RPMI 1640 medium (Gibco) with 15% fetal bovine serum, using 40% polyethylene glycol 1500 by established procedures.[10] Cells were plated onto 96-well culture plates, and hybridomas were selected using RPMI 1640 medium (15% fetal bovine serum) supplemented with HAT—hypoxanthine, 1×10^{-4} mol per liter; aminopterine, 4×10^{-7} mol per liter; thymidine, 1.6×10^{-5} mol per liter. Growth was enhanced by using BALB/c mouse thymus cells as feeder cells.

Hybridomas secreting antibody to the cysts were detected both by indirect immunofluorescence using the homologous cysts dried onto the bottoms of flat-bottomed, 96-well, en-

From the Department of Medicine, Emanuel Hospital, Portland (Dr Riley), and the Department of Pathobiology, University of Washington School of Public Health and Community Medicine, Seattle (Dr Stibbs). Dr Stibbs is currently with the United States-Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, Louisiana.

This research was funded by the United States Environmental Protection Agency, Office of Exploratory Research, Washington, DC, under cooperative agreement No. R811970-01 (Dr Stibbs), and a Medical Student Research Training Fellowship from the University of Washington (Dr Riley). The views and opinions expressed here do not necessarily reflect those of the Environmental Protection Agency. We gratefully acknowledge the technical assistance of Joseph Stockard in producing the hybridoma cell lines; the Department of Wildlife of the state of Washington for providing a collecting permit; J. L. Riggs of the California State Health Department for supplying his monoclonal antibody; and P. M. Wallis of the University of Calgary, J. Isaac-Renton of the University of British Columbia, Charles Hibler of Colorado State University, and Glen Clark and Robert Pacha of Central Washington University for supplying us with *Giardia* cultures and cysts.

Reprint requests to Henry H. Stibbs, PhD, US-Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, LA 70037.

ABBREVIATIONS USED IN TEXT
ELISA = enzyme-linked immunosorbent assay
PBS = phosphate-buffered saline

zyme-linked immunosorbent assay (ELISA) plates (Falcon ProBind, #3915, Becton Dickinson)—5,000 to 10,000 cysts per well—and by ELISA using 25,000 cysts per well. Stable hybridomas were cloned by limiting dilution three times. Ascitic fluid was produced in BALB/c mice pretreated with 0.3 to 0.5 ml Pristane per mouse (Sigma Chemical Company). Either culture supernatant or ascitic fluid was used in the immunofluorescence cross-testing with cysts from the various animal sources.

For the immunofluorescence cross-testing, cysts were spotted and air-dried onto eight-spot Teflon-coated slides (Bellco, Vineland, NJ) and fixed for five minutes in acetone. Cysts were then exposed to serial dilutions—ranging from 1:10 to 1:200—of monoclonal antibodies in 0.0175 mol per liter phosphate-buffered saline (PBS), pH 7.4, for 60 minutes at 37°C in a humid chamber. Afterwards the cysts were rinsed twice with PBS, then exposed to affinity-purified, fluorescein-conjugated, goat anti-mouse immunoglobulin antibody (Cappel Laboratories, West Chester, Pennsylvania) diluted 1:80 in PBS, and finally the cysts were rinsed again twice in PBS, air-dried, and mounted with PBS:glycerol (1:9) containing 0.1 mg per ml *p*-phenylenediamine to retard fading. The slides were examined under a Zeiss fluorescence microscope at 400 × magnification. An anti-*G lamblia* cyst polyclonal rabbit antiserum was used as a positive control, and preimmune ascites or NS-1 myeloma cell culture supernatant served as negative controls.

Results and Discussion

A total of four monoclonal antibodies were produced against *G muris* cysts and one against the *G simoni* cysts. When *G muris* cysts were reacted with the four monoclonal antibodies raised against *G muris* cysts, they showed a strong reaction at the location of the cyst wall (Figure 1). Cysts from the rat infected with *G simoni* also bound the anti-*G muris* monoclonal antibodies equally strongly (not shown). *Giardia lamblia* cysts from eight human patients, however, did not bind the anti-*G muris* monoclonal antibodies, nor did *Giardia* species cysts from dogs (n = 4), beavers (n = 2), muskrats (n = 3), voles (n = 3), or cattle (n = 1) react with these anti-*G muris* antibodies (Table 1). There was no difference between the four anti-*G muris* antibodies in terms of which organisms they did or did not react with.[10]

In further work with an anti-*G lamblia* monoclonal antibody developed by Riggs,[11] and with the single monoclonal antibody produced against cysts from the rat, *Giardia* species could be grouped according to their patterns of reactivity when cross-tested with the various antibodies (Table 1). The monoclonal antibodies could not differentiate human-, beaver-, or dog-source *Giardia*. Beaver-source *Giardia* has been strongly implicated in many waterborne outbreaks,[12,13] and the experimental transmission of human-source *Giardia* to dogs has been successful.[3,14] Our results suggest that using the pattern of cross-reactivity with the monoclonal antibodies predicts the ability of a *Giardia* strain to infect certain hosts.

Passing the cysts through another host species did not affect the pattern of reactivity. Human-source *Giardia* cysts passed through gerbils or rats, beaver-source cysts passed through gerbils, and *G muris* and *G simoni* cysts passed through gerbils retained their original patterns of reactivity.

One of the most notable findings of the cross-testing experiments was that the pattern of reactivity of the cyst specimens from an individual host species remained constant despite wide geographic differences in the organism's original source—that is, human cyst organisms were from Seattle and Colorado; beaver cyst organisms were from Washington, Vermont, and Alberta. Further evidence for host specificity of cyst antigens is that the wild Norway rat and one of the infected muskrats were trapped in the exact same location on our university campus within two weeks of each other, yet their giardial cysts showed completely different patterns of reactivity (Table 1).

These results are somewhat contrary to those of others who have found intrahost variation to sometimes be as great as interhost variation when trophozoite antigens, restriction endonuclease DNA fragments, and isozyme patterns were examined. These studies showed differences between *Giardia* organisms from the same host species.[15-18] Based on our findings, however, it would appear that in the cysts de-

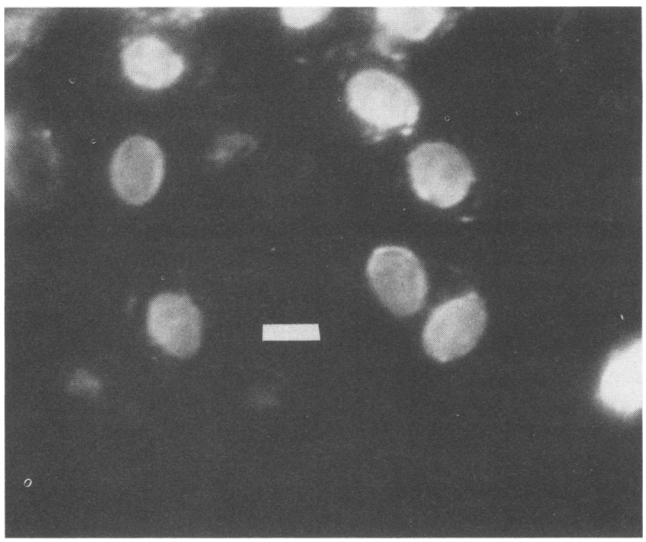

Figure 1.—*Giardia muris* cysts are shown under fluorescent microscopy after exposure to an anti-*G muris* monoclonal antibody followed by a reaction with fluorescein-conjugated goat anti-mouse immunoglobulin antibody (white bar equals 10 microns).

TABLE 1.—*Reactivity of* Giardia *Species Cysts From Various Animal Hosts With Monoclonal Antibodies Raised Against* Giardia muris *(Hamster Source),* Giardia lamblia *(Human Source), and* Giardia simoni *(Wild Norway Rat Source)*

Original Source of Giardia *Cysts*	*Antibodies*		
	*Anti-*G muris	*Anti-*G lamblia	*Anti-*G simoni
Rat, n=1	++	++	++
Beaver, n=2	–	++	–
Dog, n=4	–	++	ND
Human, n=8	–	++	–
Hamster, n=1*	++	–	–
Cow, n=1	–	–	++
Muskrat, n=3	–	–	–
Vole, n=3	–	–	–

++ = positive reaction, – = negative reaction, ND = No data available

*Source of cysts against which anti-*G muris* antibodies were produced.

rived from one host species, certain cyst-wall epitopes are conserved; monoclonal antibodies produced against them can differentiate between the giardial cysts originating naturally from different hosts but do not pick up intrahost *Giardia*-cyst variations. This suggests that the pattern of reactivity of cysts with monoclonal antibodies in immunofluorescence might be a useful criterion for establishing species of *Giardia*. The belief of Filice[19] that, based on morphologic criteria, *Giardia* should be grouped into no more than three species has become widely accepted, at least in North America. Our discovery that some degree of antigenic constancy exists among certain cyst antigens of organisms from individual host species suggests that monoclonal serotyping of cysts provides a supplementary taxonomic criterion that can be superimposed over the basic morphologic divisions Filice established. A great deal more work, however, must be done using a larger number of monoclonal antibodies produced against organisms of *Giardia* from other host species to see if this proposed immunotyping scheme will continue to show antigenic constancy of cyst epitopes within organisms. An expanded system of monoclonal typing may eventually make feasible the identification of the source of cysts found in environmental samples for water-quality monitoring. Monoclonal typing could also prove useful in studying the epidemiology of waterborne giardiasis outbreaks and could provide data regarding which animals may be sources of *Giardia* cysts during outbreaks.

REFERENCES

1. Craun GF, Jakubowski W: Status of waterborne giardiasis outbreaks and monitoring methods, *In* Tate CL Jr (Ed): International Symposium on Water-Related Health Issues. Bethesda, Md, American Water Resources Assoc, 1984, pp 167-174

2. Hughes JM, Merson MH, Craun GF, et al: Outbreaks of waterborne disease in the United States. J Infect Dis 1975; 132:336-339

3. Davies RB, Hibler CP: Animal reservoirs and cross-species transmission of *Giardia, In* Jakubowski W, Hoff JC (Eds): Waterborne Transmission of Giardiasis. Cincinnati, Ohio, Environmental Protection Agency, 1979, publication No. 600/7-79-001, pp 104-126

4. Rendtorff RC: The experimental transmission of human intestinal protozoan parasites—II. *Giardia lamblia* cysts given in capsules. Am J Hyg 1954; 59:209-220

5. Rendtorff RC, Holt CJ: The experimental transmission of human intestinal protozoan parasites—IV. Attempts to transmit *Entamoeba coli* and *Giardia lamblia* cysts by water. Am J Hyg 1954; 60:327-338

6. Grant DR, Woo PTK: Comparative studies of *Giardia* spp in small mammals in southern Ontario—II. Host specificity and infectivity of stored cysts. Can J Zool 1978; 56:1360-1366

7. Grant DR, Woo PTK: Comparative studies of *Giardia* spp in small mammals in southern Ontario—I. Prevalence and identity of the parasites with a taxonomic discussion of the genus. Can J Zool 1978; 56:1348-1359

8. Schaefer FW III, Rice EW, Hoff JC: Factors promoting *in vitro* excystation of *Giardia muris* cysts. Trans R Soc Trop Med Hyg 1984; 78:795-800

9. Stibbs HH, Riley ET, Stockard J, et al: Immunofluorescence differentiation between various animal and human source *Giardia* cysts using monoclonal antibodies. Proceedings of the 1987 Calgary *Giardia* Conference. Calgary, Alberta, University of Calgary Press, 1988, pp 159-163

10. Galfre G, Milstein C: Preparation of monoclonal antibodies: Strategies and procedures. Methods Enzymol 1981; 73:3-46

11. Riggs JL: In *Giardia* methods workshop. Denver, Colo, Water Quality Technology Conference, American Water Works Assoc, 1984, pp 495-496

12. Dykes AC, Juranek DD, Lorenz RA, et al: Municipal waterborne giardiasis: An epidemiologic investigation. Ann Intern Med 1980; 92:165-170

13. Navin TR, Juranek DD, Ford M, et al: Case-control study of waterborne giardiasis in Reno, Nevada. Am J Epidemiol 1985; 122:269-275

14. Hewlett EL, Andrews JS Jr, Ruffier J, et al: Experimental infection of mongrel dogs with *Giardia lamblia* cysts and cultured trophozoites. J Infect Dis 1982; 145:89-93

15. Bertram MA, Meyer EA, Lile JD, et al: A comparison of isozymes of five axenic *Giardia* isolates. J Parasitol 1983; 69:793-801

16. Nash TE, Keister DB: Differences in excretory-secretory products and surface antigens among 19 isolates of *Giardia*. J Infect Dis 1985; 152:1166-1171

17. Nash TE, McCutchan T, Keister D, et al: Restriction-endonuclease analysis of DNA from 15 *Giardia* isolates obtained from humans and animals. J Infect Dis 1985; 152:64-73

18. Korman SH, Le Blancq SM, Spira DT, et al: *Giardia lamblia:* Identification of different strains from man. Z Parasitenkd 1986; 72:173-180

19. Filice FP: Studies on the cytology and life history of a *Giardia* from the laboratory rat. Univ Calif Publ Zool 1952; 57:53-143