JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1980, p. 506-508 Vol. 12, No. 4
0095-1137/80/10-0506/03$02.00/0

Improved Diagnostic Accuracy Using Monoclonal Antibody to Group A Streptococcal Carbohydrate

MOON H. NAHM,†* PATRICK R. MURRAY, BRIAN L. CLEVINGER, AND JOSEPH M. DAVIE

Division of Laboratory Medicine and Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110

Monoclonal mouse antibody to streptococcal group A carbohydrate was evaluated in a diagnostic microbiology laboratory. A total of 262 isolates of beta-hemolytic streptococci were classified with commercial reagents and the monoclonal antibody to group A carbohydrate by using immunofluorescence and Lancefield precipitin tests. Both monoclonal and commercial antibodies were identically reactive by the precipitin test but significantly different by immunofluorescence. Monoclonal antibody gave the same reactivity as seen in the precipitin test, but commercial antibodies gave both false-positive and false-negative results. These results suggest that monoclonal reagents may be superior to conventional antisera.

Serological methods can be rapid, sensitive, and specific and therefore are important tools in diagnostic laboratories. However, the major limitation of conventional antisera is standardization. Most antisera are heterogeneous mixtures of antibodies differing in specificity and affinity for the antigenic determinants. Furthermore, it is probably true that no two animals produce antisera that are precisely alike. Absorption to remove unwanted specificities is another source of variability. For these reasons, comparison of results between laboratories using different reagents is difficult. The recent developments in hybridoma technology overcome these limitations by providing immortal cell lines that produce monoclonal antibody. We report in this study the clinical evaluation of a hybridoma antibody prepared against group A streptococcal carbohydrate.

MATERIALS AND METHODS

Hybridoma production. Spleen cells from an A/J mouse hyperimmunized with group A streptococcal vaccine (1) were fused to the mouse plasmacytoma cell line NSI/1-Ag 4-1 by using essentially the procedure of Galfre et al. (6). Approximately 10^8 splenocytes were fused with polyethylene glycol (Carbowax PEG 1500; Fisher Scientific Co., Fair Lawn, N.J.) to 10^7 myeloma cells. After 2 weeks of growth in selection medium (8), culture supernatants were analyzed for anti-group A carbohydrate (GAC) activity by ^{125}I-GAC binding after isoelectric focusing in acrylamide gels (10). Cells demonstrating anti-GAC activity were cloned in soft agar over 3T3 mouse fibroblast monolayers (4). About 10^7 cells from the positive clones were injected intraperitoneally into pristane-treated BALB/c mice. Within 10 to 20 days the ascites fluid contained about 5 mg of hybridoma protein per ml. The hybridoma used in this study, HGAC-1, produces an immunoglobulin G3 (K) protein, which was purified from ascitic fluid by 50% ammonium sulfate precipitation followed by euglobulin precipitation. The purified protein produced a single band by microzone electrophoresis.

Bacteria. The organisms used in these studies were recovered initially from clinical specimens and included 38 bacitracin-sensitive, beta-hemolytic streptococci from St. Louis Children's Hospital (provided by Thomas Brotherton) and 224 randomly selected beta-hemolytic streptococci from Barnes Hospital.

Lancefield precipitin test. The Rantz-Randall autoclave extraction method (11) was used to prepare the carbohydrate extract. Several isolated colonies of beta-hemolytic streptococci were inoculated into 12 ml of Todd-Hewitt broth. After overnight incubation, the beta-hemolytic streptococci were concentrated by centrifugation, suspended in 0.8 ml of sterile saline, and autoclaved for 15 min at 121°C. The samples were centrifuged for 10 min at 1,500 × g, and the supernatant fluids were analyzed for group-specific streptococcal carbohydrates. The precipitin test (7) was performed with both a 1:10 dilution of HGAC-1 ascites fluid and undiluted, commercially prepared antisera (Difco, Detroit, Mich.) to group A, B, C, D, F, and G streptococci.

Fluorescent-antibody test. Fluorescein-conjugated HGAC-1 ascites (Fl-HGAC-1) (four fluorescein groups per molecule), prepared by the dialysis method (3), and commercially prepared (Difco) group A streptococcal fluorescent-antibody reagents were used. Both reagents were initially tested against quality control strains of group A, B, C, and D streptococci, alpha-hemolytic streptococci, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Fl-HGAC-1 at 40 μg/ml reacted only with group A streptococci. The commercial antibody prep-

† Present address: Division of Laboratory Medicine, Department of Medicine and Pathology, Washington University School of Medicine, St. Louis, MO 63110.

aration reacted with group A and C streptococci and to a lesser intensity with *S. aureus*. Therefore, the commercial preparation was mixed with nonconjugated antiserum to group C streptococci. The working dilutions of Fl-HGAC-1 and the commercial antibody preparation (1:120 and 1:20, respectively) were determined by testing twofold dilutions of the antisera against the quality control organisms. In this study we used the dilution that was twice the concentration necessary to give maximum fluorescence with the group A *Streptococcus* and no cross-reactivity with the other control organisms.

Beta-hemolytic streptococci were picked from the primary isolation plate, suspended in saline, and fixed with 95% ethanol to a clean microscope slide. After being stained with fluorescent antiserum and washed with phosphate buffer to remove the unbound reagents, slides were examined under oil immersion with a Leitz SM-Lux fluorescent microscope. Results with Fl-HGAC-1 and commercial antisera were read by different technologists. Any discrepant results were confirmed by another technologist, and equivocal reactions were repeated.

RESULTS

Lancefield precipitin test. Summarized in Table 1 are the Lancefield precipitin test results for 262 beta-hemolytic streptococci, using a set of commercial typing sera and HGAC-1 ascites. The commercial reagents unambiguously typed all but five samples to only one of the groups. The HGAC-1 ascites correctly typed all isolates with no false-positive or false-negative reactions. In the positive reactions, HGAC-1 produced a more distinct precipitate consistently faster than the commercially prepared reagent (an average of 10 to 15 s versus 60 to 90 s).

Fluorescent-antibody test. Because fluorescent-antibody testing is commonly performed in clinical laboratories, purified Fl-HGAC-1 was compared with commercially prepared fluorescent antibody (Table 2). Whereas all isolates were correctly identified with Fl-HGAC-1 antibody, seven false-positive and two false-negative reactions were observed with the commercial reagent. The false-positive reactions occurred with four group C, two group F, and one group G streptococci.

DISCUSSION

In this report we demonstrate that a monoclonal GAC-binding hybridoma protein can be used to type group A streptococci. Using both precipitin and fluorescent-antibody methods, HGAC-1 matched exactly the results of the standard Lancefield precipitin method on 262 samples from two clinical laboratories. Although commercial antisera correctly identified all group A organisms in precipitin tests, occasional false-positive and false-negative results were observed with the fluorescein-conjugated antisera (2).

There were several reasons for using an anti-GAC hybridoma antibody to demonstrate the clinical usefulness of a monoclonal antibody. First and most importantly, the accurate typing of streptococci is an important and frequently used clinical test. Second, the Lancefield precipitin test could provide definitive serological results with which to compare the results of HGAC-1. Third, the antigenic determinant of the GAC is well defined and known to involve the terminal *N*-acetylglucosamine on a rhamnose core (5). HGAC-1 binds to *N*-acetylglucosamine conjugated to bovine serum albumin, substantiating its specificity for the *N*-acetylglucosamine hapten (unpublished data).

We have demonstrated here that a monoclonal reagent can be superior to conventional antisera in bacterial typing. Clearly, if this hybridoma is used in other clinical laboratories, results from these laboratories should be directly comparable. Furthermore, highly accurate and plentiful reagents should facilitate the development of other tests for earlier detection. In the past, throat swabs have been analyzed directly by immunofluorescence (9). Direct analysis of throat swabs, using enzyme-linked immunosorbent assays or immunofluorescence assays, are also feasible with these improved reagents. It is

TABLE 1. *Lancefield precipitin assay on clinical samples with conventional group-specific reagents and HGAC-1 ascites*

Lancefield group	Results of precipitin assay with conventional grouping reagents						No. of clinical specimens	No. positive with HGAC-1
	A	B	C	D	F	G		
A	+	−	−	−	−	−	113	113
B	−	+	−	−	−	−	83	0
C	−	−	+	−	−	−	22	0
F	−	−	−	−	+	−	26	0
G	−	−	−	−	−	+	13	0
Nongroupable	−	−	−	−	−	−	5	0

TABLE 2. *Fluorescent-antibody testing of beta-hemolytic streptococci*[a]

Grouped by precipitin analysis	Fl-HGAC-1		Commercial FA reagents	
	Positive	Negative	Positive	Negative
A	113	0	77	2
Non-A	0	149	7	138

[a] A total of 262 isolates were tested with Fl-HGAC-1 reagent, and 224 isolates were tested with the commercially prepared fluorescent-antibody (FA) reagent. The 38 isolates initially selected by bacitracin sensitivity were not tested with the commercial reagent.

likely that hybridoma technology will ultimately provide us with standardized antibodies for a lower cost and that future techniques will not be limited by the availability and quality of antibodies.

ACKNOWLEDGMENTS

We thank Ann Niles and Alexander Shaffer for their expert help with these studies.

This work was supported by Public Health Service grant AI-15353 from the National Institutes of Health. M.H.N. was supported by Public Health Service training grant GM-07157, and B.L.C. was supported by Public Health Service Postdoctoral Fellowship AI-05599, both from the National Institutes of Health.

LITERATURE CITED

1. **Briles, D. E., and J. M. Davie.** 1975. Clonal dominance. I. Restricted nature of the IgM antibody response to group A streptococcal carbohydrate in mice. J. Exp. Med. **141**:1291–1307.
2. **Chitwood, L. A., M. B. Jennings, and H. D. Riley, Jr.** 1969. Time, cost, and efficacy study of identifying group A streptococci with commercially available reagents. Appl. Microbiol. **18**:193–197.
3. **Clark, H. F., and C. C. Shepard.** 1963. A dialysis technique for preparing fluorescent antibody. Virology **20**: 642–644.
4. **Coffino, P., R. Baumel, R. Laskov, and M. D. Scharf.** 1972. Cloning of mouse myeloma cells and detection of rare variants. J. Cell Physiol. **79**:429–440.
5. **Coligan, J. E., T. J. Kindt, and R. M. Krause.** 1978. Structure of the streptococcal groups A, A-variant and C carbohydrates. Immunochemistry **15**:755–760.
6. **Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard.** 1977. Antibodies to major histocompatibility antigen produced by hybrid cell lines. Nature (London) **266**:550–552.
7. **Lancefield, R. C.** 1933. A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med. **57**:571–595.
8. **Littlefield, J. W.** 1964. Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. Science **145**:709–710.
9. **Moody, M. D.** 1972. Old and new techniques for rapid identification of group A streptococci, p. 177–188. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases: recognition, understanding and management. Academic Press, Inc., New York.
10. **Nicolotti, R. A., D. E. Briles, J. A. Schroer, and J. M. Davie.** 1980. Isoelectric focusing of immunoglobulins: improved methodology. J. Immunol. Methods **33**:101–115.
11. **Rantz, L. A., and E. Randall.** 1955. Use of autoclaved extracts of hemolytic streptococci for serological grouping. Stanford Med. Bull. **13**:290–291.