JOURNAL OF CLINICAL MICROBIOLOGY, May 1987, p. 958–959
0095-1137/87/050958-02$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 25, No. 5

Detection of Herpes Simplex Virus in Direct Specimens by Immunofluorescence Assay Using a Monoclonal Antibody

P. POULETTY,[1]†* J. J. CHOMEL,[2] D. THOUVENOT,[2] F. CATALAN,[3] V. RABILLON,[1] AND J. KADOUCHE[1]

Unité d'Immunologie, Clonatec, 75012 Paris,[1] Laboratoire de Virologie, Faculté de Médecine, 69373 Lyon Cedex 08,[2] and Institut Fournier, 75680 Paris Cedex 14,[3] France

Received 29 September 1986/Accepted 28 January 1987

A monoclonal antibody (MAb), designated CHA 437, was developed against herpes simplex virus (HSV). This MAb (isotype, immunoglobulin G2b K) reacted with HSV type 1 and HSV type 2. It showed no cross-reactivity with varicella-zoster virus, cytomegalovirus, or Epstein-Barr virus. Direct detection of HSV antigen in clinical specimens using indirect immunofluorescence with this MAb was compared with tissue culture isolation. For the 682 specimens tested, the direct specimen test gave a sensitivity of 84.6% and a specificity of 95.7%.

Herpes simplex virus (HSV) is involved in various infections, such as genital, ocular, oral, cutaneous, and neonatal infections, and may cause severe diseases in immunocompromised patients. Diagnosis is based on isolation of virus using tissue culture (TC) (10) and typing using type-specific monoclonal antibodies (MAbs) (1, 9). Such techniques require more than 24 h. Few studies have reported the reliability of MAbs for detection of HSV antigen in direct specimens (1, 3–5). In this report, we demonstrate high sensitivity and specificity of a single anti-HSV type-common MAb in a direct specimen test (DST) when compared with TC.

Cell fusion using the SP20 parental cell line was performed after immunization of BALB/c mice with purified infected Vero cell extracts (Ratcliffe strain) according to published procedures (9). Hybrid supernatants were screened by indirect immunofluorescence using infected Vero cells. MAb isotype was determined on concentrated supernatant by double immunodiffusion using specific antisera (Biosoft, Paris, France). MAb was produced either from cell culture grown in RPMI 1640 with 10% fetal bovine serum or from pristane-primed BALB/c mice (7). Specificity of the selected MAb was evaluated by indirect immunofluorescence on HSV type 1 (HSV-1; Ratcliffe strain), HSV type 2 (HSV-2; Roizman strain), cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, and adenovirus using TC. A total of 723 specimens submitted to the Institut Fournier in Paris or to the virology laboratory of the Faculte de Médecine in Lyon were processed in a prospective study for HSV detection. Of these, 682 specimens were considered adequate for DST. Of the 41 specimens (5.7%) excluded from the study, 7 were positive by TC. Of the 682 adequate specimens, 351 were from genital sites, 5 were from rectal sites, 29 were from skin lesions, 35 were from oral sites, 250 were from bronchoalveolar fluid, 4 were from lung sites, and 8 were from other sites.

Specimen collection was performed after exposing the base of the lesion by firm scraping with a Dacron swab. The specimen was then suspended in 300 μl of viral transport medium containing antibiotics. A 50-μl sample of the cell suspension was spotted onto a slide for immunofluorescence tests, air dried, and fixed in cold acetone. Bronchoalveolar fluid samples were first centrifuged at 900 × *g* for 8 min. The cell pellet was washed three times with phosphate-buffered saline (PBS), suspended in PBS, and applied onto a slide to obtain cellular confluence.

DST was performed as follows. The smear was covered with an appropriate amount of anti-HSV MAb CHA 437 (undiluted supernatant) and incubated for 30 min at 37°C in a moist chamber. After three washes with PBS, fluorescein-conjugated goat anti-mouse immunoglobulin G antibody (Biosoft, Paris, France) was added and incubated for another 30 min. After being washed for 1 min with PBS containing Evans blue (diluted 10,000-fold) and twice with PBS, the slides were dried and examined with a Leitz fluorescence microscope at a magnification of ×400. Positive and negative controls consisting of slides containing HSV-infected and uninfected cells were done with each DST series. Specimens were considered adequate for DST evaluation if an average of five or more epithelial cells per field were observed.

Each specimen collected was also tested by TC using permissive Vero and MRC-5 cells according to previously described procedures (10). Identification was done when cytopathic effect was observed according to Chomel et al. (2) using direct immunofluorescence with type-specific MAbs (Herpes test IF; Biotrol and Clonatec, Paris, France) or indirect immunofluorescence (Diagnostic Pasteur kit; Marnes la Coquette, France).

One MAb, designated CHA 437, was selected. Its isotype was immunoglobulin G2b K. It reacted by indirect immunofluorescence with HSV-1- and HSV-2-infected cells (hybrid supernatant endpoint titer, 320 for HSV-1 and HSV-2; ascitic fluid titer, 10^5 for HSV-1 and HSV-2). The pattern of fluorescence staining observed was predominantly nuclear or nuclear and cytoplasmic, depending on the stage of infection. Specific reactivity was not observed with uninfected cells or with cells infected with varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, or adenovirus.

DST using MAb CHA 437 was compared with TC for 682 specimens (Table 1). Infected cells in the DST usually showed a very bright staining and could be clearly distinguished from the counterstained uninfected or nonepithelial cells. A single slide with an adequate number of intact epithelial cells could be read in about 1 min. The sensitivity of the DST was 84.6%. Its specificity was 95.7%. The positive predictive value was 81.3%, and the negative predictive value was 96.6%. Of the 104 specimens positive by

* Corresponding author.

† Present address: Department of Medical Microbiology, Stanford University School of Medicine, Stanford, CA 94305.

TABLE 1. Comparison of DST using MAb CHA 437 with TC isolation

DST result	No. of TC isolates		
	Positive	Negative	Total
Positive	104	24	128
Negative	19	535	554
Total	123	559	682

both assays, 32 were HSV-1 and 72 were HSV-2. Of the 19 specimens negative by DST and positive by TC, 6 were HSV-1 and 13 were HSV-2. Of the 535 specimens negative by both assays, 12 were positive for another virus (varicella-zoster virus, 4 specimens; cytomegalovirus, 6 specimens; parainfluenza virus 3, 2 specimens). No significant difference in sensitivity or specificity was observed with respect to the type of HSV or to the site of specimen collection (Table 2).

We selected an MAb reacting with an antigen expressed by HSV-1 and HSV-2. It was used for direct detection of HSV antigen in clinical specimens. We obtained high sensitivity and specificity compared with TC for HSV-1 and HSV-2 and for genital and nongenital specimens. The sensitivity of DST for HSV was similar to that reported for respiratory syncytial virus and *Chlamydia trachomatis* (6, 11). Specimens positive by TC which were negative by DST were not mutant HSV strains not recognized by MAb CHA 437, because specific staining was obtained in all cases using the same MAb after TC. They rather lacked specific inclusion-containing cells on the smear. Testing several specimens collected from various vesicles could greatly increase the sensitivity, as suggested with one of our patients for whom only one of three smears was positive. It is clear that the quality of specimen collection is critical, as has been seen for other direct assays (6). Nevertheless, without excluding any specimen from the study, the overall sensitivity of DST would have still been 80%, higher than that of other reports (3, 5). The specificity of DST and its positive predictive value may be higher than those obtained, because the 24 specimens positive by DST and negative by TC were collected from patients with clinical herpetic lesions. HSV may be detected when virus is not viable. As previously observed for other infections (such as respiratory syncytial virus or chlamydial infections), direct antigen detection or nucleic acid spot hybridization can be positive when TC is negative (6, 8, 11).

The importance of rapid diagnosis for HSV infections has increased because specific treatment is now available

TABLE 2. Comparison of DST with TC isolation with respect to HSV type and site of specimen collection

Specimen or site	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
HSV-1	84.2			
HSV-2	84.7			
Genital sites	84.9	93.8	83.2	94.5
Nongenital sites	83.3	97.3	75.8	98.3

(acyclovir). Herpes infection must be especially quickly identified during pregnancy so that an informed decision concerning delivery can be made. A DST using an MAb such as MAb CHA 437 offers a reliable technique for the diagnosis of HSV infection. In more than 80% of clinical cases, the herpetic infection can be confirmed in less than 2 h. Whether DST using MAb CHA 437 is as sensitive with cervical specimens and could be used for routine surveillance of pregnant women has to be further evaluated. In preliminary results obtained at the Institut Fournier (unpublished data), of 92 cervical swabs, 13 specimens were positive by DST and TC, 77 were negative by both techniques, 2 were positive by DST and negative by TC, and no specimen positive by TC was negative by DST. Nevertheless, because the overall sensitivity of DST is less than 100%, all negative or inadequate specimens should be verified by TC.

The expert assistance of A. M. Chevrinais, E. Ouizman, and B. Cornet is gratefully acknowledged.

LITERATURE CITED

1. **Balachandran, N., B. Franme, M. Chernesky, E. Kraiselburd, Y. Kouri, D. Garcia, C. Lavery, and W. E. Rawls.** 1982. Identification and typing of herpes simplex viruses with monoclonal antibodies. J. Clin. Microbiol. **16:**205–208.
2. **Chomel, J. J., D. Thouvenot, and M. Aymard.** 1984. Immunofluorescence indirecte appliquée a l'identification du virus cytomégalique. Feuill. Biol. **136:**62–65.
3. **Fung, J. C., J. Shanley, and R. C. Tilton.** 1985. Comparison of the detection of herpes simplex virus in direct clinical specimens with herpes simplex virus-specific DNA probes and monoclonal antibodies. J. Clin. Microbiol. **22:**748–753.
4. **Goldstein, L. C., L. Corey, J. K. McDougall, E. Tolentino, and R. C. Nowinski.** 1983. Monoclonal antibodies to herpes simplex virus: use in antigenic typing and rapid diagnosis. J. Infect. Dis. **147:**829–837.
5. **Hoffmann, B. E., D. L. Jungkind, G. J. Haller, R. Sharrar, R. A. Baker, and M. Weisberg.** 1985. Evaluation of two rapid methods for the detection of herpes simplex virus antigen in patient specimens. Ann. Clin. Lab. Sci. **15:**418–427.
6. **Kim, H. W., R. G. Wyatt, B. F. Fernie, C. D. Brandt, J. O. Arrobio, B. C. Jeffries, and R. H. Parrott.** 1983. Respiratory syncytial virus detection by immunofluorescence in nasal secretions with monoclonal antibodies against selected surface and internal proteins. J. Clin. Microbiol. **18:**1399–1404.
7. **Oi, V. T., and L. A. Herzenberg.** 1980. Immunoglobulin producing hybrid cell lines, p. 351–371. *In* B. B. Mishell and S. M. Shiigi (ed.), Selected methods in cellular immunology. W. H. Freeman & Co., San Francisco.
8. **Palva, A.** 1985. Nucleic acid spot hybridization for detection of Chlamydia trachomatis. FEMS Microbiol. Lett. **28:**85–91.
9. **Pereira, L., D. V. Dondero, D. Gallo, V. Devlin, and J. D. Woodie.** 1982. Serological analysis of herpes simplex virus types 1 and 2 with monoclonal antibodies. Infect. Immun. **35:**363–367.
10. **Rawls, W. E.** 1979. Herpes simplex virus type 1 and 2, and herpes virus simiae, p. 309–360. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
11. **Stamm, W. E., H. R. Harrison, E. R. Alexander, L. D. Cles, M. R. Spence, and T. C. Quinn.** 1984. Diagnosis of Chlamydia trachomatis infections by direct immunofluorescence staining of genital secretions. Ann. Intern. Med. **101:**638–641.