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Identification and Typing of Herpes Simplex Viruses with Monoclonal Antibodies

N. BALACHANDRAN,[1] BARBARA FRAME,[1] MAX CHERNESKY,[1] EDMUNDO KRAISELBURD,[2] YAMIL KOURI,[2] DORIS GARCIA,[2] CAROL LAVERY,[1] AND W. E. RAWLS[1]*

Faculty of Health Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada,[1] and Department of Microbiology, University of Puerto Rico, and Latin American Center for Sexually Transmitted Diseases, San Juan, Puerto Rico 00936[2]

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Monoclonal antibodies which reacted with type-specific antigens of herpes simplex virus type 2 or with antigens shared by herpes simplex virus types 1 and 2 were used in an indirect immunofluorescence assay to type virus isolates and to detect viral antigens in cells obtained from herpetic lesions. Complete concordance was obtained for 42 isolates typed by endonuclease restriction analysis of viral DNA and by indirect immunofluorescence with monoclonal antibodies. Examination of a limited number of ulcerative lesions revealed that indirect immunofluorescence and virus isolation were comparable in detecting herpes simplex virus. The results indicate that monoclonal antibodies can be used to accurately identify and type isolates of herpes simplex virus.

There are two types of herpes simplex viruses which can be distinguished by a number of biological, biochemical, and immunological methods (21). Herpes simplex virus type 1 (HSV-1) is usually associated with lesions of the oral cavity, eye, and cerebrum, whereas herpes simplex virus type 2 (HSV-2) is usually associated with genital lesions in adults and systemic infections in newborns (21). Presently, effective therapy for herpetic lesions is not available. However, the application of successful treatment modalities will require methods for rapid and accurate diagnosis. To this end, hybridomas secreting antibodies to antigens of HSV-2 have been developed and characterized (3, 15). The use of these monoclonal antibodies in an indirect immunofluorescence assay to detect viral antigens in cells obtained from ulcerative lesions and to type virus isolates is described in this paper.

The production of hybridomas secreting antibodies to HSV-2 antigens has been previously described (15), and the antigens to which the antibodies reacted were characterized by the methods detailed elsewhere (3). Monoclonal antibodies secreted by three hybridomas were used. Hybridoma 18βB3 secreted immunoglobulin G1 (IgG1) antibodies which reacted to an antigen present on glycoprotein D (gD) of both HSV-1 and HSV-2. Hybridoma 17αA2 secreted IgG2a antibodies which reacted with a type-specific antigen present on gE of HSV-2, and hybridoma 17βA3 secreted IgG1 antibodies which reacted with a type-specific antigen on gD of HSV-2. Large quantities of monoclonal antibodies from these hybridomas were prepared by injecting cells intraperitoneally into pristane-treated mice and harvesting the ascitic fluid.

Specimens of vesicle fluid or the exudate from lesions were placed in 2 ml of transport medium and submitted to the laboratory for virus isolation. At the laboratory in Hamilton, Canada, primary tube cultures of grivet monkey kidney cells, rhesus monkey kidney cells, and human foreskin fibroblasts were inoculated with 0.25 ml of the specimen-containing transport medium, and the tubes were incubated in roller drums at 37°C. The cultures were observed daily for the appearance of a cytopathic effect for up to 10 days. At the laboratory in San Juan, Puerto Rico, 0.1 ml of the samples was inoculated within 4 h of collection onto monolayers of Vero cells grown in tissue culture vessels. The monolayers were washed once with Eagle minimal essential medium after a 1-h adsorption and incubated at 37°C in the minimal medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 2% fetal bovine serum. The monolayers were observed for cytopathic effects, and cultures were considered negative if none was observed after at least 6 days of incubation (26). Positive cell cultures were harvested and stored at −70°C. For typing the HSV isolates, monolayers of Vero cells were inoculated with virus and observed until 80 to 100% of the cells showed a viral cytopathic effect. The cells were then scraped from the flask, washed in phosphate-buffered saline, placed onto four spots on microscope slides, air dried, and fixed for 10 min with cold acetone. The fixed cells

were then reacted for 30 min at 37°C with appropriate dilutions of monoclonal antibodies (predetermined by titration with cells infected with standard virus), washed three times with phosphate-buffered saline, and then reacted for 30 min at 37°C with goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Cappel Laboratories). The slides were washed again, mounted with cover slips, and examined with a Leitz fluorescence microscope. Monoclonal antibodies from the three hybridomas were each reacted with one spot of the cells; normal mouse serum was reacted with the fourth spot. Slides containing cells infected with HSV-1 (KOS strain) or HSV-2 (333 strain) were included in each test as controls. When the infected cells were stained only by monoclonal antibodies from hybridoma 18βA3 the virus was considered to be type 1, and when the cells were stained by the monoclonal antibodies from all three hybridomas the virus was considered to by type 2. In some cases, cells from the base of the herpetic lesions were obtained with a scalpel blade and smeared onto two spots on a microscope slide. The cells were dried, fixed with acetone, and tested for viral antigen by indirect immunofluorescence with monoclonal antibodies from hybridomas 18βA3 and 17αA2 or with normal mouse serum. Selected virus isolates were typed by restriction endonuclease digestion and gel electrophoresis of the viral DNA by a modification of the technique of Lonsdale (16).

Material from 39 patients in San Juan was examined for infectious virus or viral antigen by indirect immunofluorescence. Some of the specimens from all patients were not processed by both techniques. The results are shown in Table 1. Both methods were specific in that no virus was isolated from ulcers which had been clinically determined not to be herpetic, nor were viral antigens detected in cells obtained from these ulcers. Virus was isolated from 13 of 21 (62%) herpetic lesions, and viral antigens were detected in the scrapings of 14 of 22 (64%) of these lesions. Virus was isolated from 13 of 14 (93%) lesions which had not crusted, and antigen was detected by indirect immunofluorescence in cells from 12 to 15 (80%) of these lesions. The background fluorescence of smears stained with monoclonal antibodies was low, permitting ready identification of antigen-positive cells. The slides were assessed independently by two observers, and in 33 of 36 instances both observers agreed upon the presence or absence of antigen-containing cells. There was 82% agreement between the results obtained by virus isolation and by indirect immunofluorescence.

The validity of typing HSV isolates by indirect immunofluorescence with monoclonal antibody was assessed by examining the DNA restriction patterns of selected isolates typed with monoclonal antibodies. A total of 42 isolates were examined, and there was complete agreement between the results obtained by the two methods (data not shown). Included in the analysis were 20 isolates from genital lesions, of which 12 were typed as HSV-2 and 8 as HSV-1. Thus, typing with the monoclonal antibodies yielded the same information as did endonuclease digestion of viral DNA, regardless of the site from which the isolate was obtained.

The applicability of monoclonal antibody typing was further assessed by examining 147 isolates. In all but one instance, the isolates were readily typed as either HSV-1 or HSV-2 (Table 2). The single exception had the restriction endonuclease pattern of HSV-1 but reacted with monoclonal antibodies from hybridoma 17βA3. All of the oral isolates were typed as HSV-1; however, 22 of 67 isolates from genital sites

TABLE 1. Comparison of indirect immunofluorescence and virus isolation methods in diagnosing HSV in ulcerative lesions

Clinical diagnosis	HSV[a] detected by:	
	Immunofluorescence	Virus isolation
Herpetic lesion		
Primary[b]	12/15	13/14
Recurrent[c]	2/7	0/7
Other[d]	0/14	0/15

[a] Number positive for HSV over number tested.
[b] Patients diagnosed as having primary herpetic lesions or recurrent lesions less than 4 days old.
[c] Patients diagnosed as having recurrent herpetic lesions 4 or more days old or lesions possibly caused by HSV.
[d] Other lesion sites and isolates examined included lung, cerebrospinal fluid, urine, eye, and finger.

TABLE 2. Typing of HSV isolates by indirect immunofluorescence

Source of isolate	Location[a]	No. of isolates	No. typed as:	
			HSV-1	HSV-2
Lesion				
Oral region	Hamilton	52	52	0
	San Juan	3	3	0
Genital region	Hamilton	55	21	34
	San Juan	12	1	11
Other[b]	Hamilton	25	21	4

[a] Isolates were obtained from specimens submitted to the Regional Virology Diagnostic Laboratory, St. Joseph's Hospital, Hamilton, Ontario, Canada, by physicians in the region or from patients at the Latin American Center for Sexually Transmitted Diseases in San Juan, Puerto Rico.
[b] Other isolates were obtained from the lung, cerebrospinal fluid, urine, eye, and finger.

were also HSV-1. The proportion of HSV-1 isolates from genital sites was significantly different between the specimens obtained in Hamilton and those obtained in San Juan. Of 25 isolates from other sites, 21 were HSV-1.

The feasibility of rapidly diagnosing herpes simplex virus infections by an immunofluorescent assay was first suggested in 1959, when it was reported that material from 8 of 15 patients was stained by virus-specific fluorescent antibody and that virus could be isolated from the same 8 patients (5). The following year, Kaufman (13) reported finding virus by isolation and viral antigen by immunofluorescence in material from 8 of 17 patients with corneal disease. In both of these studies, material without detectable virus did not have viral antigens detectable by immunofluorescence in the cells. Gardner and coworkers (8) examined samples from 34 patients and found evidence of HSV by both immunofluorescence and virus isolation in 21 samples and by virus isolation only in 1 sample, and found no HSV by either method in 12 samples. Thus, in these three studies there was agreement between the virus isolation and immunofluorescence methods in 65 to 66 (98%) samples examined. Subsequently, agreement between these two diagnostic methods was found in a number of studies: in 163 of 183 (89%) (19), 70 of 81 (86%) (28), 40 of 43 (93%) (32), 36 of 39 (92%) (34), 37 of 43 (86%) (10), 7 of 8 (88%) (24), and 110 of 148 (74%) (18) samples.

We found agreement between the virus isolation and indirect immunofluorescence methods with monoclonal antibodies in 27 of 33 (82%) samples examined. As in other studies, we found that both virus isolation and immunofluorescence were highly specific, as neither test was positive for samples taken from lesions not thought to be herpetic. The sensitivity of the methods depended on the efficiency of sample collection and processing and on the age of the lesion. A major limitation of the immunofluorescence technique was the requirement for an adequate sample of cells taken from the base of the lesion. In our study, two samples that were positive by the virus isolation method were negative by the immunofluorescence method because there were insufficient cells for analysis. The rate of virus isolation from lesions clinically diagnosed as herpetic has varied from 45 to 84% (1, 10, 12, 14, 18, 20, 22, 27, 29–31, 33). Virus was most readily isolated from primary lesions and recurrent lesions in the vesicular phase (9, 14, 20, 27, 29). Our results are in accord with these observations and suggest that immunofluorescence with monoclonal antibody and virus isolation are of comparable sensitivity in diagnosing such lesions.

Moseley et al. (18) reported examining material from herpetic lesions by direct immunofluorescence with commercially available antisera to HSV-1 and HSV-2. They observed that 37 of 54 (69%) specimens reacted with HSV-1 conjugate and 46 of 54 (85%) specimens reacted with HSV-2 conjugate, suggesting that the conjugates were not strictly type specific. We examined a limited number of smears from lesions by indirect immunofluorescence with an HSV-2-specific monoclonal antibody and a monoclonal antibody which reacted with both HSV-1 and HSV-2. In three of three instances in which viral antigen was detected, the virus type indicated by immunofluorescence of the smear was the same as the type isolated from the lesion. Thus, it may be possible to both identify and type the virus directly from smears of the lesions by immunofluorescence with monoclonal antibodies.

A number of techniques for differentiating HSV-1 from HSV-2 strains have been described, and those recommended for typing isolates are based on biological differences between the types (17, 23), differences in reactivity to homologous and heterologous antisera raised against prototype viruses (2, 4, 7, 11, 19, 25), and biochemical differences (6, 16). A consistently reproducible difference between types has been found in their endonuclease restriction cleavage patterns (6, 16). This technique, however, requires equipment and expertise which are not available in many diagnostic laboratories. Techniques based upon serological identification are hampered by the cross-reactivity of antisera prepared against either HSV-1 or HSV-2; also, each antiserum must be preadsorbed or used at certain dilutions to obtain specificity, and the appropriate dilution varies with each antiserum preparation. We found that monoclonal antibodies with type specificity for HSV-2 coupled with monoclonal antibodies which reacted to both HSV-1 and HSV-2 accurately typed isolates. Only 1 of 147 isolates reacted aberrantly with the monoclonal antibodies selected for this study; thus, the technique was more than 99% accurate. By using infected cells from the monolayers used to isolate the viruses, isolates could be quickly and cheaply typed by indirect immunofluorescence.

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