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Typing of Clinical Herpes Simplex Virus Isolates with Mouse Monoclonal Antibodies to Herpes Simplex Virus Types 1 and 2: Comparison with Type-Specific Rabbit Antisera and Restriction Endonuclease Analysis of Viral DNA

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A total of 122 clinical isolates of herpes simplex virus (HSV) from 107 patients were typed by using an indirect immunoperoxidase technique with commercially available type-specific rabbit antisera, recently developed mouse monoclonal antibodies to HSV types 1 and 2, and restriction endonuclease analysis of viral DNA. With the commercially available type-specific rabbit antisera, 34% of clinical HSV isolates were of indeterminate type; 63% of them were typed as HSV type 1 and 37% as HSV type 2 by using monoclonal antibody and restriction enzyme typing systems. Typing by immunofluorescence assay with the monoclonal antibodies gave identical results to those obtained by restriction enzyme analysis. Simultaneous infection with both HSV types was demonstrated by monoclonal antibody typing in five isolates from three patients. These findings were subsequently confirmed by plaque purification and restriction endonuclease analysis of viral DNA. Monoclonal antibodies were as sensitive as restriction enzyme analysis for the typing of clinical HSV isolates. Because of their simplicity, they are more amenable to use in clinical laboratories than is restriction endonuclease analysis.

Mucocutaneous herpes simplex virus (HSV) infections are among the most frequent viral infections of humans. At the University of Washington Clinical Virology Laboratory, Seattle, 685 of 866 (79%) viral isolates obtained during 1980 were HSV. The advent of antiviral chemotherapy has made the rapid and prompt diagnosis of HSV of clinical importance. Whereas prior studies have indicated that HSV type 1 (HSV-1) is an infrequent cause of infections "below the waist," recent studies suggest an increasing prevalence of genital HSV-1 infections (7, 16, 17). Similarly, HSV-2 pharyngitis appears to be increasingly common (L. Corey, H. G. Adams, Z. A. Brown, and K. K. Holmes, Ann. Intern. Med., in press). The subsequent recurrence rate of genital HSV-1 infection appears to differ from that of genital HSV-2 disease (16). In addition, antiviral activity of many chemotherapeutic agents, for example, E-5-(2-bromovinyl)-2'-deoxyuridine, differs between the two HSV types (5, 6). As such, the typing of clinical HSV isolates may, in many clinical situations, be of prognostic and direct therapeutic importance.

Several typing systems for distinguishing HSV-1 from HSV-2 have been described. These include biological assays, such as growth of the virus on chorioallantoic membrane (10), serological methods using antisera to prototypes HSV-1 and HSV-2 (2, 11), polypeptide comparisons (12, 15, 18), and analysis of viral DNA by either restriction endonuclease or DNA hybridization techniques (3, 4, 9). In this report, we compare the typing results of an indirect immunoperoxidase (IP) assay employing rabbit hyperimmune antisera to prototypes HSV-1 and HSV-2 (2), an indirect immunofluorescence assay (IFA) method using recently developed monoclonal antibodies to HSV-1 and HSV-2, and restriction endonuclease analysis of viral DNA (4, 9).

MATERIALS AND METHODS

Specimens submitted to the University of Washington Virology Laboratory for HSV isolation from 3 October 1978 to 18 November 1981 for which typing was requested by the ordering physician were utilized. Viral isolation procedures have been described previously (19). Briefly, specimens were inoculated into duplicate tubes of diploid fibroblasts (human embryon-

ic tonsil cells) and examined three times weekly for evidence of HSV cytopathic effect. Cells were harvested from tubes exhibiting 3+ to 4+ HSV cytopathic effect, spotted onto duplicate wells of 30-well glass slides (Carlson Scientific), and fixed in acetone for 10 min at 4°C. Supernatant fluids were frozen and stored at −70°C until inoculation into baby hamster kidney cells for restriction endonuclease analysis.

Typing methods. (i) Indirect IP technique. Details of the indirect IP technique have been previously described (2). Briefly, serial twofold dilutions from 1:400 to 1:6,400 of rabbit antisera to HSV-1 (MacIntyre VR3) and HSV-2 (MS) (Dako Immunoglobulins, Copenhagen, Denmark) were made in Dulbecco phosphate-buffered saline (PBS) (pH 7.2) and incubated with the acetone-fixed infected cell culture material for 1 h at 37°C. The slides were washed twice in PBS, rinsed with distilled water, and reincubated for 1 h at 37°C with a 1:75 dilution of goat anti-rabbit horseradish peroxidase conjugate (Miles Laboratories). After being washed with PBS and distilled water and dried, the substrate (indole carbazole dimethyl formamyde) was added. Slides were then examined at 400× magnification for the presence of HSV antigen. All preparations were graded as follows: 4+, staining of 100% of the cells; 3+, staining of 75%; 2+, staining of 50%; 1+, staining of 25%. Staining of ≥2+ intensity was considered to be a positive reaction at the examined dilution. A specific HSV type was assigned to specimens in which a fourfold or greater difference in endpoint dilution between the two types was noted. Specimens in which endpoints were similar or separated by only one dilution were considered to be of "indeterminate" type.

(ii) Indirect IFA technique. Monoclonal antibodies were supplied by L.G. and R.N. Methods for the production of these monoclonal antibodies have been previously described (8, 13; L. C. Goldstein, L. Corey, J. McDougall, E. Tollentino, P. Spear, and R. C. Nowinski, manuscript submitted). For HSV-1 typing, one monoclonal antibody (3-G11), which reacts with the HSV-1-specific glycoprotein C complex (80 to 120,000 daltons), was used. Three HSV-2 type-specific monoclonal antibodies were used. These antibodies (6-A6, 6-E12, and 6-H11) react with HSV-2-specific polypeptides of 140,000, 55,000, and 38,000 molecular weight, respectively (L. C. Goldstein, L. Corey, J. McDougall, E. Tollentino, P. Spear, and R. C. Nowinski, unpublished observation). Preliminary studies revealed that both staining quality and staining characteristics were slightly better with an immunofluorescent staining technique. Thus, an indirect IFA was chosen over the indirect IP procedure. Procedures for these assays were similar to that of the indirect IP test; 1:500 and 1:1,000 dilutions of each of four HSV monoclonal antibodies were freshly made before each run, added to acetone-fixed infected cells in duplicate wells on slides, and incubated for 1 h at 37°C. Slides were washed in PBS and rinsed with distilled water, and a 1:80 dilution of fluorescein isothiocyanate conjugated to goat anti-mouse antiserum (Cappel Laboratories) was added. Slides were then reincubated for 1 h, washed, rinsed, and mounted with 90% glycerol–10% PBS (pH 8.5) with 24 by 60-mm cover slips. Slides were read under epifluorescence at 400× magnification. With each IP or IFA, cells infected with prototype HSV-1 (E115) or HSV-2 (333), as well as noninfected diploid fibroblast cells, were included. Neither the monoclonal antibodies nor the rabbit antisera demonstrated positive staining with noninfected diploid fibroblasts in any of the test runs. Staining patterns differed between the HSV-1 and HSV-2 monoclonal antibodies. The HSV-1 monoclonal antibody gave fluorescence around the rim of the infected cells, whereas the HSV-2 antibodies produced intracellular fluorescence in specific pinpoint dot patterns (Fig. 1). In the IFA, the HSV type was determined as that which gave specific fluorescent staining with the homologous antisera.

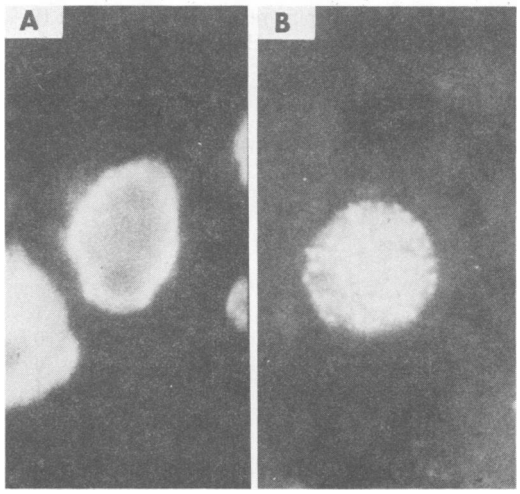

FIG. 1. Characteristic fluorescence staining patterns observed with anti-HSV monoclonal antibodies. (A) Rimming in HSV-1-infected cells stained with the anti-HSV-1 monoclonal antibody G11. (B) Cytoplasmic speckling in HSV-2-infected cells stained with the anti-HSV-2 monoclonal antibody E12.

(iii) Restriction endonuclease analysis. For restriction endonuclease digestion, viral isolates were reinoculated into baby hamster kidney (BHK-21) cells at a multiplicity of infection of one. HSV DNA was labeled with $^{32}P_i$, extracted, and prepared for restriction endonuclease analysis by a modification of the method described by Lonsdale (9). Restriction endonucleases were purchased from Bethesda Research Laboratories, and digestions were carried out under the conditions specified by the supplier. DNA fragments in the digests were separated by electrophoresis on horizontal 0.8% agarose gels (Sigma type II; M_r = 0.18) submerged in Tris-borate-EDTA buffer (50 mM Tris, 47 mM boric acid, 1.0 mM EDTA [pH 8.3]) for 17.5 h at 2.5 V/cm. After electrophoresis, the gels were dried under vacuum on Whatman 3M chromatography paper and placed on Kodak XS-1 medical X-ray film for autoradiography.

RESULTS

A total of 122 HSV isolates from 107 patients were available for analysis. Sixteen isolates were from throat swabs, lip lesions, or sputum isolates; 88 were from genital lesions; 3 were

TABLE 1. HSV typing results by the indirect IP method with rabbit anti-HSV-1 and anti-HSV-2 antisera

Site of specimen	No. of isolates		
	HSV-1	HSV-2	Indeterminate
Oropharyngeal isolates	4	2	10
Genital isolates	3	56	29
Other sites			
Brain	0	1	1
Finger	2	1	0
Buttock	0	5	0
Other mucocutaneous	2	5	1

from finger cultures; 5 were from buttock lesions, 8 were from other mucocutaneous sites; and 2 were from brain tissue. With the indirect IP technique, four of six pharyngeal isolates were typed as HSV-1. Fifty-six of 88 genital isolates, 1 of the 3 finger cultures, 1 of the brain isolates (from a neonate), all 5 of the buttock lesions, and 5 of 8 cultures from other mucocutaneous sites were typed as HSV-2. Ten of 16 pharyngeal, 29 of 88 genital, and 2 of 18 isolates from other mucocutaneous or visceral sites gave indeterminate typing results in the indirect IP assay (Table 1).

Typing of the same clinical isolates with monoclonal antibodies in the indirect IFA revealed 14 of the 16 pharyngeal isolates to be HSV-1, 69 of 88 genital isolates to be HSV-2, and 12 of 18 isolates from other sites to also be HSV-2 (Table 2). One hundred percent concordance was achieved with each of the three HSV-2 monoclonal antibodies. Occasional differences in the intensity of the fluorescence was noted, with E12 giving the most consistent quality in the immunofluorescent pattern. Of the 41 clinical HSV isolates that were typed as indeterminate by the IP assay, 23 were typed as HSV-1 by

TABLE 2. HSV typing results by indirect IFA with mouse monoclonal antibodies

Site of specimen	No. of isolates		
	HSV-1	HSV-2	Mixed HSV-1 and HSV-2
Oropharyngeal isolates	14	2	0
Genital isolates	14	69	5
Other sites			
Brain	1	1	0
Finger	2	1	0
Buttock	0	5	0
Other mucocutaneous	3	5	0

TABLE 3. Comparison of typing of HSV isolates by monoclonal antibodies and restriction endonuclease analysis of viral DNA

Type by restriction endonuclease analysis	No. of isolates by monoclonal antibody method		
	HSV-1	HSV-2	Dual HSV-1 and HSV-2
HSV-1	27	0	0
HSV-2	0	18	0
Dual HSV-1 and HSV-2 infection	0	0	5

monoclonal antibody, 13 were typed as HSV-2, and 5 specimens from three patients demonstrated characteristic staining with both the monoclonal HSV-1 and monoclonal HSV-2 antibodies, suggesting infection with both HSV types (see below). Four isolates, two HSV-1 and two HSV-2, showed opposite typing results between the monoclonal and rabbit anti-HSV antisera.

Fifty of the above clinical isolates from 40 patients were subjected to restriction endonuclease analysis with the enzymes *Bam*HI and *Hpa*I. Twenty-seven isolates were typed as HSV-1, and 19 were typed as HSV-2. Similar typing results were obtained for these isolates with the monoclonal antibody typing system (Table 3). With the rabbit anti-HSV-1 and -2 antisera, 21 of the HSV-1 and 7 of the HSV-2 isolates were indeterminate. For all four isolates with discordant results between the monoclonal and rabbit antibodies, restriction enzyme analysis corroborated the monoclonal antibody typing results.

All five of the specimens in which immunofluorescence with both the HSV-1 and HSV-2 monoclonal antibodies was seen demonstrated restriction endonuclease digestion patterns suggestive of both HSV-1 and HSV-2 DNA. To evaluate this further, each isolate was plaque purified. When plaque-purified isolates from each of the five specimens were subjected to restriction enzyme analysis with the enzymes *Bam*HI, *Hpa*I, *Sal*I, *Kpn*I, *Pvu*II, and *Bgl*II, two distinct strains were seen, one of HSV-1 and one of HSV-2 (Fig. 2). The HSV-1 and HSV-2 strains were distinct among the three patients but identical in isolates from different anatomical sites of the same patient.

DISCUSSION

Our data indicate that the monoclonal antibodies to HSV-1 and HSV-2 were highly sensitive, specific, and useful for the typing of clinical HSV isolates. Comparison of typing results with monoclonal antibodies in an indirect IFA with restriction endonuclease analysis of viral DNA indicated 100% concordance in the results. As the indirect IFA is more widely available, can be

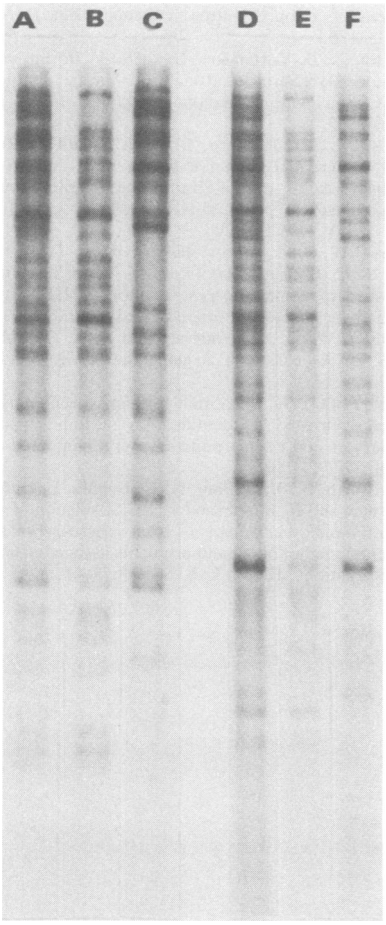

FIG. 2. Restriction endonuclease digestion of primary isolates from two patients with genital lesions demonstrating infection with both HSV types. Patient 1, lanes A to C. Lane A is the *Bam*HI digestion from the primary isolate, demonstrating fragments characteristic of both HSV-1 and HSV-2 DNA. Plaque-purified isolates from this isolate are shown in lane B (HSV-2) and lane C (HSV-1). Similar findings are shown in lanes D to F for another patient. Lane D is a primary isolate showing dual infection. Lane E is a plaque-purified HSV-2 isolate, and lane F is a plaque-purified HSV-1 isolate. The HSV-1 and HSV-2 isolates from the two patients differ in their restriction enzyme patterns.

readily performed without reinoculating the isolates, and is considerably faster and less expensive than restriction enzyme digestion, it is more amenable to widespread clinical use (1). The distinctive staining characteristics of these monoclonal antibodies allowed clear-cut differentiation between the two HSV types. Further refinements and characterization of monoclonal antibodies may also allow one to subtype and define common strains of HSV-1 and HSV-2 and hence will be useful tools in epidemiological investigations (14).

In this study, indeterminate typing results, that is, no clear-cut differences between HSV-1 and HSV-2, were noted in 34% of our clinical samples with our animal prototype antisera. This rate is about three times that seen in our previous investigations of clinical isolates of HSV infections (2, 16). The high rate of indeterminate types did not follow a discernible pattern in that analysis of indeterminate types by restriction endonuclease and monoclonal antibodies indicated 56% to be HSV-1, 32% to be HSV-2, and 12% to have mixed HSV-1 and HSV-2 infections. As our methods and patient populations have remained relatively constant throughout the years, it is likely that these results were due to lot-to-lot variation in the antisera.

It was of interest that mixed HSV-1 and HSV-2 infections were noted in five specimens from three patients. These isolates were called indeterminate with rabbit anti-HSV-1 and -2 antisera. The use of restriction enzyme analysis and monoclonal antibody typing allowed for more definitive discrimination. Plaque purification of the HSV isolates from these samples has shown that these specimens contained both HSV-1 and -2. Because of the high specificity of the monoclonal antibodies and distinct differences in the type of immunofluorescent staining patterns between the HSV-1 and HSV-2 antibodies, these reagents appear to be useful, for the typing of HSV isolates and should allow more laboratories to provide HSV typing services.

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