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Serological Analysis of Herpes Simplex Virus Types 1 and 2 with Monoclonal Antibodies

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A panel of monoclonal antibodies to herpes simplex virus glycoproteins was used for serological analysis of 130 strains. Based on specific immunological determinants, strains of each serotype clustered into subgroups. Monoclonal antibodies were suitable reagents for serotyping and have potential application to epidemiology of herpes simplex virus infections.

Herpes simplex viruses are subdivided into types 1 and 2 (HSV-1 and HSV-2) on the basis of neutralization tests with antisera to the glycoproteins in the envelope of the virus (18, 21). Although subsequent studies have shown that HSV-1 and HSV-2 share many biological properties (13), including the structural organization of their genomes (9), they were found to differ in restriction enzyme cleavage sites in their DNAs (8, 11), in electrophoretic properties of the virus-specific polypeptides (4, 12, 17, 19), and in immunological specificity of some of the virus-specific proteins (6, 20, 23, 24, 25). Although there now exist many different methods for differentiating HSV-1 and HSV-2 strains, serological tests involving antisera produced against each of the serotypes remain the major laboratory method for the differentiation of the serotypes. The problems with these assays stem from the fact that polyvalent antisera prepared against HSV-1 and HSV-2 cross-react in serological tests, and differentiation between the serotypes frequently hinges on quantitative estimates of reactivity. Antisera prepared against individual glycoproteins gA/gB, gD, and gE of HSV-1 cross-react (1, 5, 7, 14). Although antibody to HSV-1 gC has been shown to be type specific (20, 26), the corresponding antibody to HSV-2 gC has been difficult to prepare and is not generally available, and its immunological specificities are unknown.

We recently reported on the properties of monoclonal antibody to HSV-1 glycoproteins gC and gD, and we have since prepared similar reagents reactive with HSV-2 glycoproteins (15a, 16; L. Pereira, *in* J. Hurrell, ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, in press). Among these, we singled out monoclonal antibodies which react only with the homologous type to determine the serotype of clinical isolates. The question we wished to ask was whether such reagents, because of their specificity, would be less ambiguous in differentiating HSV-1 and HSV-2 isolates than are polyvalent antisera. Systematic analysis of HSV-1 and HSV-2 strains has shown considerable intratypic variability with respect to both restriction enzyme cleavage sites and electrophoretic mobility of virus-specific polypeptides (2, 3, 10, 15). Although type-specific monoclonal antibody should more readily differentiate between the serotypes, it was conceivable that some isolates would lack the antigenic determinant site necessary for reactivity with the monoclonal antibody.

A total of 130 HSV isolates were selected for serological analysis. All of the strains were clinical isolates passed once in human fetal diploid kidney cells. Isolates were serotyped by immunofluorescence with hamster immune sera to HSV-1 and HSV-2 and with monoclonal antibodies. The tests showed that one type 2-specific monoclonal antibody failed to react with some strains, which suggested that they differed with respect to this immunological determinant. The antigenic variation among strains of both serotypes was studied by serological analysis with a more extensive panel of monoclonal antibodies to viral glycoproteins.

Procedures for producing, selecting, and characterizing the clones to HSV were described previously (15a, 16; Pereira, in press). Immunological reactivities of 18 monoclonal antibodies in the panel are shown in Table 1. Clones were grouped into classes by reactivity with HSV-1(F) and HSV-2(G) in three different tests: immunofluorescence, neutralization, and immunoprecipitation. The panel was comprised of two

TABLE 1. Monoclonal antibodies grouped by reactivity in immunofluorescence (IF), neutralization (NT), and radioimmunoprecipitation (RIP) tests with HSV-1(F) and HSV-2(G)

Class	Clone	IF		NT		RIP	
		F	G	F	G	F	G
1	HC1 HC2	+	0	+	0	gC	None
2	HD3	+	+	+	0	gD	gD
3	HD1 HD2 HD4 H106 H128 H170 H351	+	+	+	+	gD	gD
4	H154	0	+	0	+	None	gD
5	H120 H233 H343	+	+	+	+	gA/gB	gA/gB
6	H368	0	+	0	+	None	gA/gB
7	H222	0	+	0	+	None	gE
8	H379	0	+	0	0	None	None
9	H105	0	+	0	0	None	gE

type 1-specific antibodies to glycoprotein gC (class 1). The neutralizing activity of antibody in class 2 was type 1 specific, but the antibody cross-reacted in immunofluorescence and immunoprecipitation tests. Seven antibodies precipitated glycoprotein gD of both serotypes (class 3); one was HSV-2 specific (class 4). Three cross-reactive antibodies precipitated glycoproteins gA and gB, which share antigenic determinants (class 5); one antibody was type 2 specific (class 6). Two type 2-specific clones reacted with glycoprotein gE (classes 7 and 9); one lacked neutralizing activity (class 9). The monoclonal antibody in class 8 was HSV-2 specific by immunofluorescence with membranes of infected cells, but failed to yield a detectable immunoprecipitate. Table 2 summarizes the results of indirect immunofluorescence tests with immune sera and monoclonal antibodies to HSV-1 and HSV-2. The experimental procedures were previously described (21). In each instance the titer of monoclonal antibodies was extremely high when compared with polyvalent sera. HC1, H222, and H368 were type specific insomuch as they reacted with high titers to homologous serotype virus only and failed to react with the heterologous serotype virus, even at high antibody concentrations.

In the second series of immunofluorescence tests, the strains were serotyped with polyvalent sera and reacted with monoclonal antibodies HC1, HD1 and H368 (Table 3). Although the immune sera to HSV-1 and HSV-2 cross-reacted, they were routinely employed by experienced laboratory personnel for serotyping based on the difference in brightness of reactions between homologous and heterologous virus-infected cells. All of the strains fluoresced with cross-reacting monoclonal antibody HD1. In tests with type 1-specific antibody HC1, all

TABLE 2. Titers of immune sera and cross-reactive and type-specific monoclonal antibodies in indirect immunofluorescence tests

Serological reagent	Titer	
	HSV-1	HSV-2
HSV-1 immune sera[a]	≥2,048	≥1,024
HSV-2 immune sera[a]	≥2,048	≥1,024
HD1 (cross-reactive)	≥32,768	≥32,768
HC1 (type 1 specific)	≥32,768	≤8
H222 (type 2 specific)	≤8	≥32,768
H368 (type 2 specific)	≤8	≥32,768

[a] Immune hamster sera fluoresced brighter at equivalent dilutions with the homologous serotype virus.

TABLE 3. Reactivity of immune sera and monoclonal antibodies to 130 HSV isolates

Serological reagent	No. positive/no. tested	
	HSV-1	HSV-2
Standard sera[a]	63/63 (100)[b]	67/67 (100)
HD1 (cross reactive)	56/56 (100)	55/55 (100)
HC1 (type 1 specific)	63/63 (100)	2/67 (3)
H222 (type 2 specific)	0/30 (0)	62/64 (97)
H368 (type 2 specific)	0/30 (0)	44/52 (85)

[a] Immune sera prepared against HSV-1 and HSV-2 were used in immunodiagnostic tests for serotyping by experienced laboratory personnel.

[b] Number in parentheses refers to percent reactive strains.

HSV-1 strains reacted. It should be noted that two strains serotyped as HSV-2 cross-reacted with HC1 antibody. The DNAs of these strains were analyzed with restriction endonuclease *Hpa*I, which yielded fragment patterns characteristic of type 2 strains (data not shown). HSV-2-specific monoclonal antibody H222 failed to react with 3% and H368 failed to react with 15% of the type 2 strains, which suggested that antigenic sites of homologous glycoproteins varied among strains. Because of the lack of reactivity with some strains and the potential for occasional cross-reactions, we concluded that artificial mixtures of type-specific antibodies would be more reliable for routine serotyping of clinical isolates.

In the third series of immunofluorescence tests, 36 strains were chosen for serological analysis with a panel of 18 monoclonal antibodies (Table 4). The data showed four patterns of reactivity; however, antibodies to different sites on the same glycoprotein were distinguished because they failed to react with some strains which lacked the determinant. The first pattern (type 1 specific) was shown by antibodies in class 1. Although positive with all type 1 strains, they cross-reacted in two instances. A second pattern (generally cross-reacting) was shown by most of the antibodies in classes 2, 3, 4, and 5. However, within these classes, antibodies H106, H154, and H351 had different specificities. Most anomalous was antibody H106, which cross-reacted with HSV-1(F) and HSV-2(G) as indicated in Table 1, but reacted by immunofluorescence with type 2 isolates only. In contrast, antibody H154, originally classified as type 2 specific (Table 1), reacted with most HSV-1 and all HSV-2 isolates tested. A third pattern (type 2 specific) was observed with antibodies in classes 6, 7, and 8. Of these, H368 and H222 failed to immunofluoresce with some type 2 strains. A fourth pattern was given by the antibody in class 9, which reacted with all type 2 strains and a small number of type 1 strains. It should be noted that two of the type 1 strains fluoresced weakly with type 2-specific antibody H368. These strains were subjected to analysis with restriction endonuclease *Hpa*I and showed DNA cleavage patterns characteristic of HSV-1 (data not shown).

Table 5 summarizes the reactivities of HSV-1 and HSV-2 strains with the panel of monoclonal antibodies. Two points emerged from this analysis. First, several type-specific antibodies were suitable for serotyping: HC1 and HC2, which detected HSV-1 strains; H222, H368, and H379, which reacted with HSV-2 strains; and H106, which proved to be specific for the type 2 isolates in this study. Second, 8 of 18 monoclonal antibodies in the panel showed variable reactivity with strains of each serotype. The data indicated that certain strains failed to express the determinant site reactive with particular monoclonal antibodies. Based on the reactivity of antibodies with different specificities, the strains were divided into subgroups. Analysis of HSV-1 strains positive with different subsets of antibodies H105, and H154, and H351 showed that four subgroups emerged, with half of the strains clustering into one subgroup. Reactivity of HSV-2 strains with different subsets of antibodies HC1, H368, H379, and H106 identified six subgroups, with the bulk segregating into two subgroups. It is of interest that three epidemiologically related HSV-2 strains fell into the same subgroup.

The data presented in this report show that monoclonal antibodies are powerful reagents for serotyping HSV strains and provide the first demonstration of intratypic antigenic variation. Furthermore, the specificity of monoclonal antibodies was vividly demonstrated by the failure of some antibodies to react with particular HSV strains due to loss or modification of an antigenic site. Serological analysis with a select panel of monoclonal antibodies indicated that intratypic variation of antigenic sites on homologous glycoproteins is not uncommon and occurs in both serotypes. The effectiveness of monoclonal antibodies as serotyping reagents will be considerably enhanced by the use of mixtures of type-specific antibodies. A limited number of discriminating antibodies showed that HSV strains can be differentiated on the basis of immunological determinants. We expect that subgroups are dependent upon the number of antibodies used for analysis and that additional groupings are likely to emerge as more hybridomas are used. Monoclonal antibodies should be readily adapted as serotyping reagents and may prove suitable for epidemiological study of HSV.

TABLE 4. Reactivity of monoclonal antibodies in immunofluorescence tests with HSV strains

Clone	HSV-1 strains																		HSV-2 strains																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
HC1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
HC2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?[a]	−	−	−	?	−	−	−	−	−	−	−	−	?	−	−	−	−
H222	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	?	+	+	+	?	+	+	+	+	+	+	+	+	+	+	+	+	+
H368	−	−	−	−	−	?	−	−	−	−	−	−	−	?	−	−	−	−	+	−	+	−	−	−	+	+	−	+	−	−	+	+	+	+	+	+
H379	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
H105	?	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H106	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
H154	?	−	+	+	+	?	+	−	+	+	+	+	+	?	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H351	+	+	+	+	+	?	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HD1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HD2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HD3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HD4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H128	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H170	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H120	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H233	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H343	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] Weak fluorescence or questionable.

TABLE 5. Percent reactive[a] strains in immunofluorescence tests with monoclonal antibodies

Clone	% Reactive strains	
	HSV-1	HSV-2
HC1	100	11
HC2	100	0
H222	0	88
H368	0	61
H379	0	88
H105	16	100
H106	0	88
H154	66	100
H351	88	100
HD1	100	100
HD2	100	100
HD3	100	100
HD4	100	100
H128	100	100
H170	100	100
H120	100	100
H233	100	100
H343	100	100

[a] Questionable reactions were counted as negative.

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