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Localization of a Type-Specific Antigenic Site on Herpes Simplex Virus Type 2 Glycoprotein D

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A herpes simplex virus type 1 strain isolated from a recurrent lesion of the nose reacted with monoclonal antibodies recognizing a type 2-specific site on glycoprotein D but not with monoclonal antibodies recognizing other type 2-specific sites. DNA sequence analysis of the glycoprotein D gene of the isolate revealed a single nucleotide alteration which changed the codon for asparagine to one encoding histidine at amino acid 97 in the protein. Histidine is located at this position in glycoprotein of herpes simplex virus type 2; thus, the monoclonal antibody 17βA3 recognizes an epitope located at this region of the protein.

Herpes simplex virus isolates were initially grouped into two serotypes on the basis of antigenic differences detected by neutralization tests. By the use of hyperimmune sera (10, 13, 14) and monoclonal antibodies (2, 4, 6, 11, 17), herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) were shown to contain both type-specific and type-common antigenic sites. In most instances both type-common and type-specific sites were found on the same polypeptide. In a survey of isolates from patients we found an HSV-1 strain that reacted with a monoclonal antibody which was specific for prototype HSV-2 glycoprotein D (gD). Analysis of the DNA sequence of the gD gene of this isolate revealed a base change which changed the codon for asparagine present in the prototype HSV-1 strain to one encoding histidine present in the prototype HSV-2 strain.

The production and characterization of monoclonal antibodies to HSV-2 have been detailed elsewhere (2, 7, 8). The specificity of these antibodies was tested by indirect immunofluorescence, using cells infected with the KOS strain of HSV-1 or the 333 strain of HSV-2, and by immunoprecipitation of extracts of HSV-infected cells which had been labeled with [^{35}S]methionine. The antibodies used in this study are shown in Table 1. Monoclonal antibody C-7 was a gift of Joseph Glorioso, University of Michigan, Ann Arbor. As previously reported, 152 isolates obtained from oral, genital, or other sites were typed by immunofluorescence, using monoclonal antibodies 17αA2, 17βA3, and 18ββB3 which recognized, respectively, type 2-specific epitopes on gC and gD and a type-common epitope on gD (1). A total of 54 isolates typed as HSV-2, and 97 isolates typed as HSV-1. One isolate, SP140, which was isolated from the nose of a 38-year-old woman who experienced a recurrent herpetic infection, displayed an anomalous reaction pattern. Cells infected with SP140 reacted with antibodies recognizing the type-common and the type 2 site on gD but not the type 2 site on gC. To determine whether the isolate was a variant of HSV-2 which did not produce gC or lacked the type 2 site on gC, cells infected with the isolate were reacted with monoclonal antibodies recognizing a type 1-specific gC epitope as well as type 2-specific sites on other HSV polypeptides (Table 1). None of the monoclonal antibodies recognized type 2-specific sites on SP140 glycoproteins except for 17βA3, and the isolate reacted with the type 1 gC-specific monoclonal antibody.

The possibility that the isolate was a mixed population was excluded by demonstrating the same reaction patterns by using plaque-purified progeny (data not presented). Analysis of the restriction endonuclease cleavage patterns of DNA from SP140, KOS, and 333 revealed SP140 to be an HSV-1 strain, and it was concluded that the virus probably possessed an anomalous antigenic site on gD.

To identify the antigenic site of 17βA3 monoclonal antibodies, the DNA sequence of the gD gene of SP140 virus was compared with those of the Patton strain of HSV-1 (16) and strain G of HSV-2 (14a). The 17βA3 antibodies were found to immunoprecipitate gD of strain G but not gD of the Patton strain (Fig. 1). A *Hin*dIII-*Nru*I fragment of SP140 DNA containing the gD gene was cloned, using plasmid

TABLE 1. Reactivity of strain SP140 and known HSV-1 and HSV-2 strains with monoclonal antibodies in indirect immunofluorescence test

Monoclonal antibody	Specificity	Reactivity by immunofluorescence of[a]:		
		SP140	HSV-1 (KOS)	HSV-2 (333)
17αA2	Type 2 gC[b]	−	−	+
17βA3	Type 2 gD	+	−	+
18ββB3	Type 2 + 1 gD	+	+	+
17αC1	Type 2 gC[b]	−	−	+
17βC2	Type 2 gE	−	−	+
18αA5	Type 2 g?[b]	−	−	+
13αA3	Type 2 Na[c]	−	−	+
C-7	Type 1 gC	+	+	−
None (normal mouse serum)		−	−	−

[a] Vero cells in 60-mm petri dishes were inoculated with virus, and when the cytopathic effect was 4+, cells were harvested, spotted as dots on cover slides, fixed with acetone, and reacted with antibody and fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G. −, No reactivity; +, intense fluorescence in the majority of the cells.

[b] The glycoproteins of HSV-2 recognized by 17αA2 and 17αC1 were previously designated gF but, by convention, are now called gC, and the glycoprotein recognized by 18αA5 was previously called gC but presently remains unassigned to a letter designation.

[c] Na represents a HSV-2-specific antigen which has not been fully characterized.

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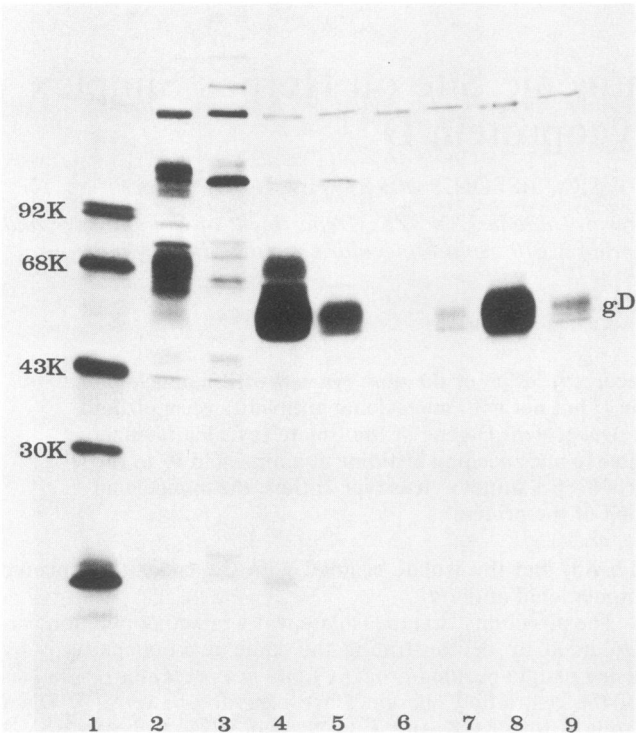

FIG. 1. Autoradiograph of [^{35}S]methionine-labeled proteins which were immunoprecipitated from extracts of infected cells and separated by electrophoresis. Extracts of cells infected with the Patton strain of HSV-1 (lanes 2, 4, 6, and 8) or with strain G of HSV-2 (lanes 3, 5, 7, and 9) were reacted with rabbit serum collected before (lanes 2 and 3) and after (lanes 4 and 5) immunization with a synthetic peptide of gD of HSV-1. The extracts were also reacted with monoclonal antibodies 17βA3 (lanes 6 and 7) and 18βB3 (lanes 8 and 9). Molecular weight markers are shown in lane 1.

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SP140     A[1]          signal peptide
Patton    MGGTAARLGAVILFVVIVGLHGVRGKYALADASLKMADPNRFRGKDLPVLDQLTDPPGVR    60
  G       --RLTSGV-TAA-L--A---RV-CA------P------------N------------K

SP140                                                  H[2]
Patton    RVYHIQAGLPDPFQPPSLPITVYYAVLERACRSVLLNAPSEAPQIVRGASEDVRKQPYNL   120
  G       ------PS-E-------T------------------H------------DEA--TH---

SP140                                        L[3]
Patton    TIAWFRMGGNCAIPITVMEYTECSYNKSLGACPIRTQPRWNYYDSFSAVSEDNLGFLMHA   180
  G       ----Y---D-------------P------V---------S------------------

SP140
Patton    PAFETAGTYLRLVKINDWTEITQFILEHRAKGSCKYALPLRIPPSACLSPQAYQQGVTVD   240
  G       ----------------------------RA-----------A---TSK---------

SP140
Patton    SIGMLPRFIPENQRTVAVYSLKIAGWHGPKAPYTSTLLPPELSETPNATQPELAPEDPED   300
  G       ----------------L-----------P-----------D-T-------V------

SP140
Patton    SALLEDPVGTVAPQIPPNWHIPSIQDAATPYHPPATPNNMGLIAGAVGGSLLAALVICGI   360
  G       -------A---SS-------------V- -H-A--A-S-P---I--LA--T------G--   359

SP140
Patton    VYWMHRRTRKAPKRIRLPHIREDDQPSSHQPLFY    394
  G       AF-VR--AQM----L------D--A-P-------    393
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FIG. 2. Comparison of amino acid sequences of the gD proteins of the Patton strain of HSV-1, G strain of HSV-2, and strain SP140. Superscripts: 1, change from ACT to GCT; 2, change from AAC to CAC; 3, change from CTG to CTT. Positions at which the Patton strain and G strain amino acids are identical are represented by a dash. All amino acids were identical for the Patton strain and SP140 strain except where indicated.

pBR322 (16), and regions of the DNA insert were sequenced by the chemical cleavage method (9, 15). The DNA sequence confirmed that the SP140 gD gene was of HSV-1 origin, despite its reactivity with the 17βA3 antibody. As compared with the sequence of the gD gene of the Patton strain of HSV-1 (16), three base changes were found in the gD gene of SP140. An A-to-G transition was observed at nucleotide 249, an A-to-C transversion was observed at nucleotide 589, and a G-to-T transversion was observed at nucleotide 747. These three changes were represented in the regions coding for the first 150 amino acids of the protein. The deduced amino acid sequences are shown in Fig. 2. The base change at nucleotide 249 would produce a change from threonine to alanine in the signal peptide (which is removed on maturation of gD), whereas the change at nucleotide 747 would be silent. However, the nucleotide change at 589 would change asparagine to histidine. Therefore, the only difference between the mature gD of the Patton strain and that of SP140 is the substitution of histidine for asparagine at this position. Since histidine also occurs at this position in the G strain of HSV-2, it can be concluded that the epitope for the antibody involves this region of the protein.

Considerable variation has been found among HSV-1 and HSV-2 isolates by a number of methods. Variation in antigenic determinants as detected by monoclonal antibodies appears to be fairly common. Pereira and co-workers (11) tested 36 isolates and found anomalous reactions with 8 of 18 different monoclonal antibody preparations. By using reaction patterns to prototype HSV-1 and HSV-2 to define type-specific and type-common specificities, two anomalous patterns could be discerned. One pattern was the loss of reactivity to a type-specific or a type-common monoclonal antibody. The second pattern was similar to that observed for SP140, namely, the reaction of a heterologous virus type with a type-specific monoclonal antibody. Where observed, 12 to 39% of variants lost reactivity, whereas 11 to 16% cross-reacted. The antigenic sites of the cross-reacting variants were on gC and gE (11), whereas in our study the variant site of SP140 was on gD.

A number of antigenic sites on gD of HSV-1 and HSV-2 have been identified, and the nature of the amino acids reacting with a type-common site has been defined (3, 4). These and other observations have suggested that a single base change in the gD gene could alter the peptide from a type-specific to a type-common moiety (5). The results of our investigation confirm this postulate since the HSV-2-specific site of gD recognized by 17βA3 monoclonal antibody became type common with respect to strain SP140 of HSV-1. The frequency with which such mutations occur at other antigenic sites of gD is not known. It is of interest that in the DNA sequences of gD of the two HSV-1 strains compared, only three base differences were found and only one would produce an amino acid change in the antigenic region of the molecule. Furthermore, this change was only detected in 1 of 98 HSV-1 strains tested. However, the monoclonal antibody detecting this site did not neutralize the virus, and selection pressures enhancing the survival of mutants at this site may not exist. Analysis of additional variant viruses will be needed to delineate the antigenic sites of the herpes simplex virus glycoproteins. Such information will be needed before the origin and significance of this variation are appreciated.

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