Serologic Type Conversion of a Herpes Simplex Virus Type 1 (HSV-1) to an HSV-2 Epitope Caused by a Single Amino Acid Substitution in Glycoprotein C

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A monoclonal antibody to herpes simplex virus type 2 glycoprotein C (gC-2) did not recognize wild-type herpes simplex virus type 1 gC (gC-1) but did recognize a mutant gC-1 molecule. This conversion from a type 1 to a type 2 epitope was shown to be due to a single amino acid substitution in gC-1.

Herpes simplex virus (HSV) glycoprotein C (gC) is a highly antigenic glycoprotein which is expressed on the surface of infected cells as well as being present in the virion envelope (17). A comparison of the sequences of HSV type 1 gC (gC-1) and HSV type 2 gC (gC-2) shows that these proteins exhibit approximately 65% amino acid sequence homology (2, 18) and exhibit a high degree of structural homology on the basis of hydropathy analysis (2). Despite the sequence similarities of the two molecules, antibodies elicited by gC-1 are largely type specific in that most do not cross-react with gC-2 (5, 13-16, 23). Type specificity is also characteristic of many anti-gC-2 antibodies. This is in marked contrast to the two other major antigenic glycoproteins of HSV, gB and gD, each of which shares approximately 85% amino acid sequence homology with its heterotypic counterpart (1, 20, 21) and elicits largely cross-reactive antibodies between the two serotypes (3, 6, 11, 13). The molecular basis of type specificity is not understood although the antigenic structure of gC-1 has been studied extensively by using genetic, immunologic, and biochemical techniques. In early studies, panels of monoclonal antibodies (MAbs) were generated against gC-1 and used to select neutralization or MAb-resistant (*mar*) mutants of HSV-1 (5, 7, 12). On the basis of reactivity patterns of the MAbs against the *mar* mutants, two antigenic sites emerged, each of which contained several distinct epitopes. The two antigenic sites were then shown to be topographically distinct by competitive binding assays using combinations of MAbs and by the ability of single MAbs to immunoprecipitate various gC truncation mutants (12). Recently, marker rescue analysis and sequencing of 17 *mar* mutants revealed point mutations in the gC-1 gene that caused single amino acid substitutions at positions previously predicted by immunologic mapping (22). On the basis of the extensive sequence and structural homology of gC-1 and gC-2, it was reasoned that the two glycoproteins would have colinear antigenic maps and, therefore, a mutation in gC-1 that caused a loss in recognition by a gC-1 specific MAb might result in a gain in recognition by a gC-2-specific MAb. This hypothesis was tested by producing MAbs to HSV-2 and screening them for

reactivity to the panel of gC-1 *mar* mutants that have been described in detail in previous publications (7, 12).

The gC-2-specific MAbs were produced by repeated immunization of BALB/c mice with a Formalin-inactivated preparation of HSV-2 (strain 186) grown in BALB/c 3T3 cells. Immune spleen cells were fused to the P3-X63-Ag8.653 myeloma line by established methods, and resulting hybridoma supernatants were screened for the ability to neutralize HSV-2 (strain 333) but not HSV-1 (strain KOS). Positive supernatants were tested by immunoprecipitation to identify MAbs specific for gC-2. Anti-gC-2 MAbs were then tested for reactivity to gC-1 *mar* mutants in 50% plaque reduction assays (7). As expected, most of the MAbs did not neutralize

FIG. 1. Immunoprecipitation of gC from cells infected with wild-type and *mar* mutant viruses. Nonidet P-40 extracts of [³⁵S]methionine-labeled infected cells were incubated with MAbs to gC-1 or gC-2 and then immunoprecipitated with protein A-Sepharose. Immunoprecipitates were electrophoresed on a sodium dodecyl sulfate-10% polyacrylamide gel. The *mar* mutants and gC-1-specific MAbs used in these experiments were produced in our laboratory and have been described in detail (7, 12). MAbs C11 and C16 were used to select the *mar*C11.1 and *mar*C16.1 *mar* mutants, respectively. MAb C2-3 was produced by the same immunization procedure as MAb C2-5 and is a type-specific anti-gC-2 MAb (unpublished results and this paper). None of the MAbs precipitated a glycoprotein band from uninfected cells or from cells infected with gC-deletion mutant viruses of either serotype (data not shown).

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FIG. 2. Surface antigen expression of gC on cells infected with wild-type or mutant virus. L cells infected 18 to 24 h previously were incubated on ice with the test MAb and then with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Sigma Chemical Co.). Cells were fixed in 0.5% paraformaldehyde and analyzed for membrane fluorescence by using an EPICS C 985 (Coulter Electronics Corp.). The gD-specific MAbs D3, D6, D7, and D8 constituted the gD pool, which was used as a positive control (6). All MAbs were of the immunoglobulin G2a isotype, and the negative control (.....) was an isotype-matched irrelevant MAb.

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