

Typing of Clinical Herpes Simplex Virus Type 1 and Type 2 Isolates with Monoclonal Antibodies

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The purpose of this study was to evaluate the performance of a herpes simplex virus (HSV) type 1-specific anti-glycoprotein C-1 monoclonal antibody (MAB) and a type 2-specific anti-glycoprotein G-2 MAB for typing of 2,400 clinical HSV-1 isolates and 2,400 clinical HSV-2 isolates, respectively, using an enzyme immunoassay. The anti-HSV-1 MAB showed sensitivity and specificity of 100%, and the anti-HSV-2 MAB showed a sensitivity of 99.46% and 100% specificity, indicating that these MABs are suitable for typing of clinical HSV isolates.

Herpes simplex virus (HSV) types 1 and 2 are ubiquitous human viruses for which a correct subtyping is essential for counseling work, epidemiological studies, and follow-up programs in vaccine trials. Virus culture, followed by antigenic detection of type-specific determinants, is often used as the “gold standard” to discriminate HSV-1 from HSV-2 (1, 16). Since several HSV-specific monoclonal antibodies (MABs) have been shown to identify type-specific epitopes (2, 5, 11, 17), these reagents have been used for type-specific antigen detection. However, the reported sensitivities and specificities for such reagents, including commercially available kits, have been found to vary among the assays in the range between 92 and 100% (4, 6, 7, 17). This discrepancy may be explained by variability in the expression of different HSV proteins or intratypic variability of different epitopes within the same protein among the isolates (11). Therefore, the selection of the type-specific MABs may be an important determinant of the sensitivity and specificity of the assay. Another explanation could be that only a restricted number of isolates (in the range between 38 to 119) have been tested in the above studies—a factor which, statistically, may significantly influence the accuracy of the test.

The purpose of this study was to investigate the sensitivity and specificity for an HSV-1-specific anti-glycoprotein C-1 (gC-1) MAB (B1.C1) and an HSV-2-specific anti-glycoprotein G-2 (gG-2) MAB (O1.C5.B2) in the typing of 2,400 clinical HSV-1 isolates and 2,400 clinical HSV-2 isolates, respectively. The MABs used were produced at our laboratory as described earlier (5, 9), and in contrast to other type-specific anti-HSV MABs used for typing for which data have been published, these have been mapped in detail. The anti HSV-1 MAB B1.C1 has been shown to bind to antigenic site II in gC-1, in which threonine₁₅₀ is crucial for binding (10). In addition, the MAB interferes with the heparan sulfate-binding activity of gC-1 (15), thereby inhibiting attachment of the virus to cells (14). The anti-gG-2 MAB epitope was recently mapped to the residues HRGGPEE, a stretch of amino acids which is localized within an immunodominant region for which all tested HSV-2-positive human sera were reactive (5).

HSV isolates from patients with clinical lesions, consecutively received at the Department of Clinical Virology in Göteborg, Sweden, were cultured on GMK-AH1 cells. Positive cul-

tures were subtyped on GMK-AH1-infected cells by an enzyme immunoassay until a total number of 2,400 HSV-1 and HSV-2 isolates each was reached. Confluent monolayers of GMK-AH1 cells were infected with each isolate in 96-well plates. When complete cytopathic effect was seen, the cells were fixed in 0.25% glutaraldehyde in phosphate-buffered saline for 30 min. The type-specific MABs and an HSV type-common anti-gE MAB (B1.E6) (2, 8) were added at a 1:100 dilution (initial concentration of 100 µg/ml) and incubated for 1 h at room temperature. Alkaline phosphatase-conjugated F(ab')₂ goat anti-mouse immunoglobulin (Jackson Immuno Research Labs) at a 1:2,000 dilution was used as conjugate, with *p*-nitrophenyl dissolved in carbonate buffer (pH 9.8) as a substrate. The reaction was read at 405 nm after 30 min. Clinical isolates with reactivity with neither of the two type-specific MABs were typed by PCR technique by using primers from the type-unique promoter region of the glycoprotein D gene (13). Clinical HSV isolates were defined as unreactive for any of the MABs if the absorbance values (optical density [OD] units) were less than 0.2 OD units above the reactivity of uninfected cells. The local HSV-2 strain B4327UR (S. Jeansson, Göteborg, Sweden) and the HSV-1 strain KOS321 were used as positive control viruses.

Of the 4,800 tested HSV isolates (2,400 of each subtype), 13 showed no reactivity with the two type-specific MABs but a clear reactivity with the type-common anti-gE MAB. These isolates were confirmed as HSV-2 positive with PCR. All isolates but three were reactive to the anti-gE MAB; one isolate was identified as HSV-1 and two isolates were identified as HSV-2 based on the reactivities of the type-specific MABs and PCR findings. In total, four isolates were reactive to all three MABs used and were confirmed to be both HSV-1 and HSV-2 by PCR and therefore judged to reflect dual infections. These findings are in agreement with those of other studies in which dual infections for the same lesion were found to be rare events (3, 7).

The anti-gC-1 MAB recognized all HSV-1 isolates, giving a sensitivity of 100%. There was a highly statistically significant difference between gC-1 and gG-2 used as target proteins ($P < 0.001$, chi-square test), indicating that the intratypic variability within these two epitopes differed among the clinical HSV isolates. Since the gC-1 protein was described earlier to be efficiently expressed at the cell surface (12), this protein has been suggested as a suitable target antigen for diagnostic MABs (17). The domain recognized by the anti-gC-1 MAB was here shown to be an ideal target for the typing of HSV-1 isolates since the epitope was found to be highly conserved

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among different clinical HSV-1 isolates. This conservation may reflect the essential function of heparan sulfate binding for the virus *in vivo*. Despite the fact that the MAb binds to a region with significant homologies to gG-2, no cross-reactivity to HSV-2 isolates was seen, indicating that gC-1 and gG-2 are structurally different within the epitope detected by the B1.C1 MAb. Although type-specific anti-gC-1 MAbs have previously been shown not to cross-react with HSV-2 isolates (7, 17), other workers (11) have described such a cross-reactivity, suggesting an intratypic variability for different epitopes within gC-1. A problem discussed earlier in a study comparing commercial kits used for typing of HSV isolates was that MAbs specific for HSV-2 were somewhat cross-reactive with HSV-1 isolates (7). One advantage of using gG-2 as the target protein for the typing of HSV-2 isolates could be that only type-specific epitopes have been described in gG-2 and that human anti-gG-2 antibodies and anti-gG-2 MAbs do not cross-react to native or denatured HSV-1 antigen as well as to homologous gG-1 peptides (5). The earlier reported HSV-2 type-specificity of the anti-gG-2 MAb (O1.C5.B2) was confirmed in this study since this antibody was unreactive to all tested clinical HSV-1 isolates. Only 13 (0.54%) of 2,400 HSV-2 isolates were unreactive to the anti-gG-2 MAb. Therefore, this epitope also seems highly conserved among clinical HSV-2 isolates.

In summary, this study describes the good performance, with both high sensitivity and high specificity, of an anti-gC-1 MAb and an anti-gG-2 MAb in the subtyping of a great number of clinical HSV isolates. These well-characterized MAbs may also be tested as reagents for rapid detection of type-specific HSV antigen directly in clinical specimens.

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