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A RELATED EPITOPE IS CONSISTENTLY PRESENT ON GLYCOPROTEIN C OF HERPES SIMPLEX VIRUS TYPE 1 AND 2

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Summary. – A monoclonal antibody (VE8) directed to glycoprotein C of herpes simplex virus type 1 (HSV-1) cross-reacted with all HSV type 2 (HSV-2) strains tested. Positive reaction was also observed with all investigated HSV-1 strains, indicating that the related epitope is consistently present in HSV-1 and HSV-2.

Key words: herpes simplex virus (HSV); glycoprotein C; type-common monoclonal antibody

Herpes simplex virus (HSV) specifies at least seven different glycoproteins (Spear, 1985; Longnecker *et al.*, 1987; McGeoch *et al.*, 1987; Fuller *et al.*, 1989). Because of their exposed location in the virion envelope and in membranes of infected cells, HSV glycoproteins are important inducers of the humoral immune response of the infected host. Resulting from high DNA homology of HSV type 1 (HSV-1) and type 2 (HSV-2), most of the viral glycoproteins have numerous type-common determinants causing extensive cross-reactivity of polyclonal sera (Schneweis and Nahmias, 1971). However, among the major glycoproteins of the virion, glycoprotein C (gC) was considered for a long time to elicit only type-specific antibodies (Vestergaard *et al.*, 1978; Ching and Lopez, 1980; Eberle and Courtney, 1981). Accordingly, more than 51 different monoclonal antibodies (MAbs) directed against gC of HSV-1 (gC-1) or HSV-2 (gC-2) and showing no cross-reactivity are described (Showalter *et al.*, 1981; Pereira *et al.*, 1982; Balachandran *et al.*, 1982; Rector *et al.*, 1982; Holland *et al.*, 1983; Para *et al.*, 1983; Goldstein *et al.*, 1983; Zezulak and Spear, 1983; Marlin *et al.*, 1985; Para *et al.*, 1985; Balachandran *et al.*, 1987; Seidel-Dugan *et al.*, 1988). Based on this type-specificity, gC-1 and gC-2 are used for identification of HSV-1 and/or HSV-2 antibodies in patient's sera (Lehtinen *et al.*, 1985).

In contrast, there are only few reports presenting data on cross-reactivity of gC-specific antibodies. First, Pereira *et al.* (1982) described a neutralizing mouse MAb against gC-1 (HC1), which cross-reacted with two of 67 HSV-2 strains in an immunofluorescence test. Zweig *et al.* (1983) presented a non-neutralizing gC-2 specific MAb (104-S), which weakly precipitated gC of

HSV-1 from both infected cell extracts and purified gC-preparations. Zezulak and Spear (1983) showed that a conventional rabbit antiserum prepared against HSV-1 virion envelope proteins immunoprecipitated gC of HSV-2.

Here we describe a non-neutralizing MAb, designated VE8, prepared against HSV-1 and specific for gC, which shows extensive cross-reactivity with gC of HSV-2. In contrast to MAb HC1 of Pereira (1982), VE8 reacts with all HSV-1 and HSV-2 strains tested, although reactivity was quantitatively different. In addition, MAb VE8 is shown to react with gC-1 and gC-2 expressed by transfected NIH 3T3 cells.

The two MAbs used in these studies, designated IIIE8 and VE8 were prepared by fusing spleen cells from BALB/c mice, which had been hyperimmunized with HSV-1 strain 342 hv (Schneweis *et al.*, 1984), with X63-Ag8.653 mouse myeloma cells. Hybrids were selected in serum-free HAT-medium. Cell-free culture fluids were concentrated by ammonium sulphate precipitation followed by dialysis. The isotype of both MAbs was IgG 2a as determined by standard double-diffusion agarose gels using rabbit anti-mouse immunoglobulin class and subclass sera. Both MAbs had no neutralizing activity against HSV-1 or HSV-2 in presence or absence of complement. Antigen specificity of the MAb was revealed by immunoblotting using viral lysates purified by sucrose gradient and a biotin-streptavidin-peroxidase detection system. In addition, glycoprotein--enriched extracts from infected cells and from cells transfected with either plasmid pMSGgC-1 or pMSGgC-2 were prepared (Kleim *et al.*, 1990) and incubated with MAb IIIE8 and VE8. pMSGgC-1 contains a 1.8-kbp insert with the gC-1 gene coding sequences 3' with respect to the mouse mammary tumour virus long terminal repeat (MMTV-LTR) residing in the cloning vector pMSG (Pharmacia). pMSGgC-2 was constructed by isolation of a 2.0-kbp *BssH*II fragment from HSV-2 G (Ejercito *et al.*, 1968) DNA and ligation of this fragment into the polylinker site of pMSG with the aid of *Sal*I linkers. pMSGgC-2 therefore contains the gC-2 translation initiation codon approximately 40bp 3' with respect to the MMTV-LTR. NIH 3T3 cells were transfected with pMSGgC-1 or pMSGgC-2 using a modification of the calcium phosphate precipitation procedure (Graham and Eb, 1973). Expression of gC was induced by adding of dexamethasone to the culture medium.

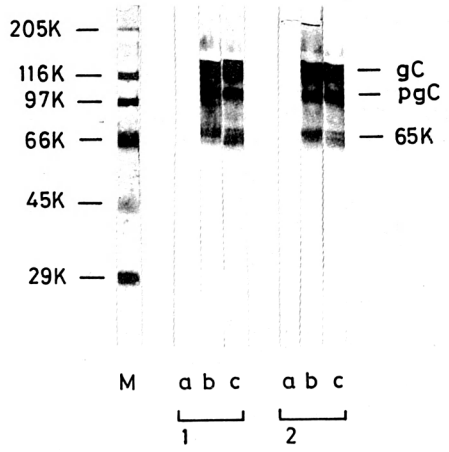

Fig. 1
Immunoblots with HSV-1 and monoclonal antibodies IIIE8 (panel 1) and VE8 (panel 2): gE$^-$/C$^-$ mutant GCI-8 (lanes a), HSV-1 gE$^-$ mutant Angelotti E3/3 (lanes b), and HSV-1 342 hv (lanes c) (virus was purified as described in the text)
The antibodies recognize glycoprotein gC (130 kD M_r), its precursor pgC, and the 65,000 M_r degradation product. Size of molecular weight markers (M) are indicated on the left.

In immunoblots, MAb III8 and VE8 were similarly reactive with HSV-1 glycoprotein gC (M_r 130,000) its precursor pgC and the 65,000 M_r degradation product (Fig. 1). To confirm the specificity, they were reacted with Western blots of electrophoretically separated polypeptides of the HSV-1 glycoprotein E minus (gE-) mutant Angelotti E3/3 (Neidhardt *et al.*, 1987) and of the HSV-1 gC-/gE- mutant GCI-8 (Schranz *et al.*, 1989). (The mutants were kindly provided by Prof. Kaerner, Heidelberg, F.R.G.) No reactivity was observed with the gC-/gE- virus mutant, whereas reaction with the gE- mutant was shown to be equivalent to that obtained with wild-type virus.

When we performed immunoblots using antigen from purified HSV-2 strain G virions and MAb VE8, bands in the range of an approximate M_r of 75,000 to 65,000 apparently gC-2 and its precursor pgC-2 became visible (Fig. 2, lane 2a). In contrast, no reaction with gC-2 was observed with the MAb IIIE8 (lane 1a). As expected, glycoprotein-enriched extracts from cells transfected with pMSGgC-1 and treated with dexamethasone for 24 hr, reacted with MAbs IIIE8 and VE8 (data not shown). To ascertain the cross-reactivity of MAb VE8, we incubated this MAb with Western blot strips containing antigen of a cell line derived from transfection of NIH 3T3 cells with pMSGgC-2. As shown in lane 2c, the same staining pattern as with HSV-2 virion antigen (lane 2b) was observed, although - due to the low expression of gC from pMSGgC-2 transfected cells (Friedman *et al.*, 1989) - only faint gC-2 specific bands were obtained.

To analyse whether the cross-reacting epitope of gC-2 is unique in HSV-2 strain G or present in numerous HSV-2 strains, we tested a panel of 31 HSV-2

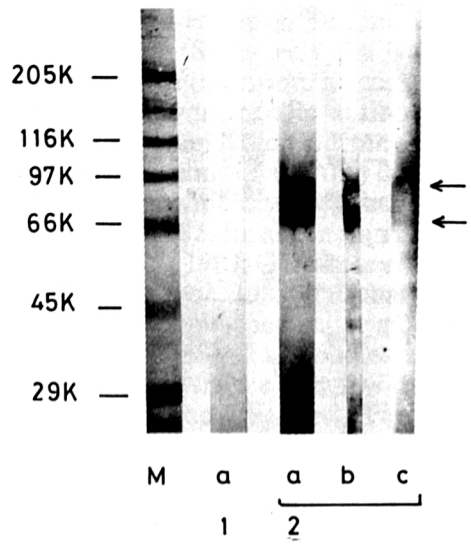

Fig. 2
Immunoblots of HSV-2 virions and gC-2 expressed in transfected cells
Sucrose gradient purified HSV-2 G virions (lanes a) and glycoprotein--enriched extracts (lane b) from infected Vero cells, and extracts from pMSGgC-2 transfected NIH 3T3 cells (lane c) were separated by SDS-PAGE and the blots reacted with monoclonal antibody IIIE8 (1) and VE8 (panel 2). Arrows in panel 2 indicate gC-2 specific bands (75 kD and 65 kD mol wt).

Table 1. Reactivity of gC-specific monoclonal antibodies IIIE8 and VE8 in immunofluorescence tests with HSV-1 and HSV-2 strains

HSV-1	monoclonal antibody IIIE8	VE8	HSV-2	monoclonal antibody IIIE8	VE8
GCI-8[1] (gC⁻/gE⁻)	-§	-	G[4]	-	++
			Haase[7]	-	+
Ang. E3/3[2] (gE⁻)	++	+++	US[8]	-	++
			US syn[9]	-	++
342hv[3]	+++	+++	1*-7	-	+
F[4]	++	++	8-12	-	++
WAL wt[5]	+++	+++	13-27	-	+++
Kit & Dubbs[6]	+++	+++			
1*-3	++	++			
4-5	++	+++			
6-9	+++	++			
10-21	+++	+++			

[1] Schranz *et al.*, 1989
[2] Neidhardt *et al.*, 1987
[3] Schneweis *et al.*, 1984
[4] Ejercito *et al.*, 1968
[5] Schröder *et al.*, 1981
[6] Schneweis, 1972
[7] Schneweis, 1962
[8] Schneweis and Nahmias, 1971
[9] Plaque-picked syncytial mutant of strain US

* Numbered strains are clinical isolates from different unrelated patients.

§ Intensity of fluorescence was noted as negative (-), weak (+), medium (++), and strong (+++).

strains for reactivity with MAb VE8 in an indirect immunofluorescence test. Vero cell monolayers on cover slips were infected with the HSV-2 strains and fixed after appearence of focal CPE. All strains reacted with MAb VE8, although reactivity was quantitatively different (Tab. 1). No reaction was seen using MAb IIIE8. These results indicate that the antigenic determinant necessary for reactivity with MAb VE8 is present in all HSV-2 strains tested.

Considering the reported rarity of cross-reacting gC-specific antibodies, we were surprised that one out of only two gC-specific clones derived from one fusion was cross-reactive. We argued, therefore, whether the strain used for immunization of the mice differed from other HSV-1 strains in respect to the cross-reacting epitope. Therefore, in another series of immunofluorescence tests the reactivity of MAbs IIIE8 and VE8 with 27 HSV-1 strains was assayed (Tab. 1). All gC-expressing HSV-1 strains fluoresced with MAb IIIE8 and with the cross-reacting MAb VE8. Thus, similar to HSV-2, no HSV-1 strain lacks the antigenic determinant site necessary for reactivity with MAb VE8.

Our results clearly show that the type-common gC-specific MAb VE8 reacts with all HSV-1 and HSV-2 strains tested. This reaction strongly exceeded the cross-reactivity of the gC-1 specific MAb HC1 recognizing only 2 of 67 HSV-2 strains (Pereira *et al.*, 1982). Although a cross-reaction of this extent is shown for the first time, it may not be unlikely that antibodies of this specificity are occasionally present in human sera. This will lead to false results, when the type-specificity of human sera is determined using gC-1 and/or gC-2.

It remains unclear why type-common gC-specific antibodies are only rarely detected. One explanation might be that a cross-reacting epitope in native gC-1 and gC-2 is not presented. But this hypothesis appears not to be probably since the acetone-treated glycoproteins of HSV-1 and HSV-2 infected cells, in contrast to SDS-PAGE-separated antigens, are not essentially altered but clearly reacted with MAb VE8 in immunofluorescence tests. Another explanation could be that cross-reactivity is caused by an unusual inaccuracy of the antibody. An originally type-specific epitope may not be very different from an epitope of the heterologous virus type, and an antibody which is not well fitted to its epitope, will recognize them both.

References

Balachandran, N., Harnish, D., Rawls, W. E., and Bacchetti, S. (1982): Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. *J. Virol.* **44**, 344-355.

Balachandran, N., Oba, D. E., and Hutt-Fletcher, L. M. (1987): Antigenic crossreactions among herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus. *J. Virol.* **61**, 1125-1135.

Ching, C. Y., and Lopez, C. (1980): A type-specific antiserum induced by a major herpesvirus type 1 glycoprotein. *J. Immunol. Meth.* **32**, 383-392.

Eberle, R., and Courtney, R. J. (1981): Assay of type-specific and type-common antibodies to herpes simplex virus types 1 and 2 in human sera. *Infect. Immun.* **31**, 1062-1070.

Ejercito, P. M., Kieff, E. D., and Roizman, B. (1968): Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. *J. gen. Virol.* **2**, 357-364.

Friedman, H. M., Yee, A., Diggelmann, H., Hastings, J. C., Tal-Singer, R., Seidel-Dugan, C. A., Eisenberg, R. J., and Cohen, G. H. (1989): Use of a glycocorticoid-inducible promotor for expression of herpes simplex virus type 1 glycoprotein gCl, a cytotoxic protein in mammalian cells. *Mol. Cell. Biol.* **9**, 2303-2314.

Fuller, A. O., Santos, R. E., and Spear, P. G. (1989): Neutralizing antibodies specific for glycoprotein H of herpes simplex virus permit viral attachment to cells but prevent penetration. *J. Virol.* **63**, 3435-3443.

Graham, F. L., and van der Eb, A. J. (1973): A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456-467.

Goldstein, L. C., Corey, L., McDougall, J. K. Tolentino, E., and Nowinski, R. C. (1983): Monoclonal antibodies to herpes simplex viruses: use in antigenic typing and rapid diagnosis. *J. infect. Dis.* **147**, 829-837.

Holland, T. C., Marlin, S. D., Levine, M., and Glorioso, J. (1983): Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. *J. Virol.* **45**, 672-682.

Kleim, J.-P., Eis-Hübinger, A. M., Mohr, K., and Schneweis, K. E. (1990): Inducible expression of herpes simplex virus type 1 glycoprotein C in NIH 3T3 cells. *Zbl. Bakt. Hyg. A.* **274**, 426-432.

Lehtinen, M., Koivisto, V., Lehtinen, T., Paavonen, J., and Leinikki, P. (1985): Immunoblotting and enzyme-linked immunosorbent assay analysis of serological responses in patients infected with herpes simplex virus types 1 and 2. *Intervirology* **24,** 18-25.

Longnecker, R., Chatterjee, S., Whitley, R. J., and Roizman, B. (1987): Identification of a herpes simplex virus 1 glycoprotein gene within a gene cluster dispensable for growth in cell culture. *Proc. natn. Acad. Sci. U.S.A.* **84,** 4303-4307.

Marlin, S. D., Holland, T. C., Levine, M., and Glorioso, J. C. (1985): Epitopes of herpes simplex virus type 1 glycoprotein gC are clustered in two distinct antigenic sites. *J. Virol.* **53,** 128-136.

McGeoch, D. J., Moss, H.W.M., McNab, D., and Frame, M. C. (1987): DNA sequence and genetic content of the *Hind*III 1 region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. *J. gen. Virol.* **68,** 19-38.

Neidhardt, H., Schröder, C. H., and Kaerner, H. C. (1987): Herpes simplex virus type 1 glycoprotein E is not indispensable for viral infectivity. *J. Virol.* **61,** 600-603.

Para, M. F., Zezulak, K. M., Conley, A. J., Weinberger, M., Snitzer, K., and Spear, P. G. (1983): Use of monoclonal antibodies against two 75,000-molecular-weight glycoproteins specified by herpes simplex virus type 2 in glycoprotein identification and gene mapping. *J. Virol.* **45,** 1223-1227.

Para, M. F., Parish, M. L., Noble, A. G., and Spear, P. G. (1985): Potent neutralizing activity associated with anti-glycoprotein D specificity among monoclonal antibodies selected for binding to herpes simplex virions. *J. Virol.* **55,** 483-488.

Pereira, L., Dondero, D. V., Gallo, D., Devlin, V., and Woodie, J. D. (1982): Serological analysis of herpes simplex virus types 1 and 2 with monoclonal antibodies. *Infect. Immun.* **35,** 363-367.

Rector, J. T., Lausch, R. N., and Oakes, J. E. (1982): Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. *Infect. Immun.* **38,** 168-174.

Schneweis, K. E. (1962): Serologische Untersuchungen zur Typendifferenzierung des Herpesvirus hominis. *Z. Immun.-forsch.* **124,** 24-48.

Schneweis, K. E., and Nahmias, A. J. (1971): Antigens of herpes simplex virus type 1 and 2-immunodiffusion and inhibition passive hemagglutination studies. *Z. Immunitätsforsch.* **141,** 471-487.

Schneweis, K. E. (1972): Herpes simplex virus, pp 66-84. In Majer, M., and Plotkins, S. A. (Eds): *Strains of Human Viruses,* S. Karger, Basel.

Schneweis, K. E. Forstbauer, H., Olbrich, M., and Tag, M. (1984): Pathogenesis of genital herpes simplex virus infection in mice. III. Comparison of the virulence of wild and mutant strains. *Med. Microbiol. Immunol.* **173,** 187-196.

Schranz, P., Neidhardt, H., Schröder, C. H., and Kaerner, H. C. (1989): A viable HSV-1 mutant deleted in two nonessential major glycoproteins. *Virology* **170,** 273-276.

Schröder, C. H., Engler, H., and Kirchner, H. (1981): Protection of mice by an apathogenic strain of HSV-1 against lethal infection by a pathogenic strain of HSV-1 *J. gen. Virol.* **52,** 159-161.

Seidel-Dugan, C., De Leon, M. P., Friedman, H. M., Fries, L. F., Frank, M. M., Cohen, G. H., and Eisenberg, R. J. (1988): C3b receptor activity on transfected cells expressing glycoprotein C of herpes simplex virus types 1 and 2. *J. Virol.* **62,** 4027-4036.

Showalter, S. D., Zweig, M., and Hampar, B. (1981): Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. *Infect. Immun.* **34,** 684-692.

Spear, P. G. (1985): Glycoproteins specified by herpes simplex viruses, pp. 315-356. In B. Roizman (Ed.): *The Herpesviruses,* Vol 3, Plenum Press, New York.

Vestergaard, B. F., and Norrild, B. (1978): Crossed immunoelectrophoresis of a herpes simplex virus type 1-specific antigen: immunological and biochemical characterization. *J. infect. Dis.* **138,** 639-643.

Zezulak, K. M., and Spear, P. G. (1983): Characterization of a herpes simplex virus type 2 75,000--molecular-weight glycoprotein antigenically related to herpes simplex virus type 1 glycoprotein C. *J. Virol.* **47,** 553-562.

Zweig, M., Showalter, S. D., Bladen, S. V., Heilman, C. J., and Hampar, B. (1983): Herpes simplex virus type 2 glycoprotein gF and type 1 glycoprotein gC have related antigenic determinants. *J. Virol.* **47,** 185-192.