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Involvement of glycoprotein C (gC) in adsorption of herpes simplex virus type 1 (HSV-1) to the cell

Brief Report

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Summary. Results demonstrating involvement of glycoprotein C (gC) of herpes simplex type 1 virus (HSV-1) in attachment of the virus to the cell are presented. Monoclonal antibodies against gC-1 inhibited adsorption of gC^+-strains. The gC^--mutant, MP, attached to cells but at a reduced rate. Attachment of the MP-mutant was unaffected by presence of anti-gC-1 antibody. Purified truncated gC-1 adsorbed to cells at a rate essentially the same as that of gC^+-virus. Glycoprotein C-1 pretreated with heparin did not adsorb to cells. The results are compatible with a suggested role for gC in HSV attachment.

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Of the HSV envelope glycoproteins, currently known as glycoprotein B (gB), gC, gD, gE, gG, gH and gI, three (gB, gD and gH) are required for a productive infection [1, 3, 10], whereas the other four including gC are dispensable for infectivity [11, 14, 17]. Nevertheless recent reports have suggested that gC plays a role in attachment of the virion to the cellular receptor. Thus, binding to the cell surface was reported to be dependent upon gC, gB as well as gD, and the glycoproteins reacted in a noncooperative way [7]. Monoclonal antibodies against gC of HSV type 1 (gC-1) interfered with the adsorption [4, 7] and virus lacking gC demonstrated a reduced rate of adsorption [9]. A region on the HSV-1 genome encoding proteins associated with HSV-1 attachment has been located [9] and found to include the gene for gC. Furthermore, by means of intertypic HSV-1 × HSV-2 recombinants two pathways for HSV adsorption have been observed, one which is sensitive to the polycationic aminoglycosides, neomycin and polylysine [8, 9] and gC-1 dependent, and another which was gC-1 independent and insensitive to the polycations and co-mapped with gC-

2 [2]. In line with these observations the gC counterpart of pseudorabies, glycoprotein III (gIII), interacts with a heparin-like cellular receptor [13, 15] during the process of adsorption, and as for HSV there exists a way of attachment which was independent of gIII activity [15].

In the present study we report results which support the assumption that gC-1 has a function during HSV-1 adsorption. Green monkey kidney cells (GMK AH-1) were cultured as monolayers with Eagle's MEM supplemented with 10% calf serum, 100 μg streptomycin/ml, and 100 IU of penicillin/ml. The same medium supplemented with 2% calf serum was used as maintenance medium. Six HSV-1 strains (F, McIntyre, KJ502, 276290, 90237, and E5) all expressing gC-1 and one gC-mutant (MP) were studied in attachment and plaque reduction experiments. The particle/PFU ratios of the McIntyre and MP strains were 3.5 and 28.8, respectively. The particle/PFU ratios of the other 5 strains studied ranged from 2.7 to 192. Techniques for preparation of virus stocks and for plaquing of virus in GMK cells have been described in detail elsewhere [16].

Hybridoma cell lines secreting MAb were produced by immunizing mice using a deoxycholate (DOC) solubilized membrane fraction [5] of HSV-1 infected Balb/c embryonic mouse cells as immunizing antigen. The monoclonal antibodies (MAb) obtained were screened with HSV-1 specific and HSV type-common antigens. MAb isotypes were determined with peroxidase-conjugated subclass specific antimouse IgG (Serotec Ltd, Bicester, England) and the concentration of immunoglobulins in hybridoma supernatants was estimated by Mancini technique. Moreover 6 mouse ascites fluid preparations of MAb kindly provided by Dr. Patricia Spear, and a rabbit anti-HSV-1 hyperimmune serum (K642) were used.

For attachment studies of HSV we have developed an ELISA method, which is described in detail elsewhere [12]. Confluent monolayer cultures of GMK cells in microtiter plates were allowed to adsorb HSV at 4 °C to prevent internalization of virus and at a high multiplicity of infection (100 PFU/cell). After varying intervals, the cells were washed 5 times with 0.1 M phosphate-buffered saline (PBS pH 7.2) and fixed with 0.2% glutaraldehyde in PBS for 1 h. The cells were then washed three times with PBS containing 0.05% Tween 10 and anti-HSV antibody and after 1 h at 37 °C the plates were washed three times with PBS-Tween. Peroxidase-conjugated antimouse of antirabbit IgG (Dako Immunoglobulins Ltd, Copenhagen, Denmark) diluted 1/400 in PBS-Tween containing 1% BSA was supplemented, and the plates were incubated for 2 h at 37 °C, followed by another three washes in PBS-Tween. After addition of the substrate (p-phenylenediamine, 1 mg/ml) and 0.01% hydrogen peroxide in PBS, and 10 min of incubation at 20 °C, the reaction was stopped by means of 3 M H_2SO_4. The absorbance was read at 492 nm (Multiscan; Flow Laboratories, McLean, VA). For evaluation of antibody influence on virus adsorption, virus was mixed with antibody diluted in PBS, incubated at 37 °C for 15 min and added to cells in microtiter plates, which subsequently were treated as outlined

above. According to the attachment curves, expressed as increase in absorbance against time, there was a rapid attachment rate at 4 °C with a maximum within 1 h. The binding kinetics was thus in close agreement with kinetics based on measurements of residual non-cell bound infectivity or cell-associated radiolabelled HSV, but considerably more sensitive. As indicated by the standard errors, the assay is highly reproducible and the specificity clearly demonstrable. Background absorbance values (PBS controls without virus) of approximately 0.25 might be related to non-specific adhesion of antibodies to the fixed cells or a low content of endogenous peroxidase in the cells.

In addition to the ELISA procedure for detection of cell-associated HSV antigens, attachment of radio-labelled purified HSV to GMK cells was assayed. 10^6 PFU of [^{3}H]-thymidine-labelled McIntyre (80,000 cmp/ml) were mixed with MAb diluted to 0.01 mg/ml in PBS containing 1% of BSA and incubated for 15 min at 37 °C. Subsequently, monolayer cultures of GMK cells in petri dishes (5 cm) were infected by adding 1 ml of the virus-antibody mixtures. The first set of cultures were assayed immediately after inoculation of virus and the second set after 120 min of gentle agitation at 4 °C. Adsorption of virus was terminated by washing the cultures 5 times with PBS followed by addition of 1 ml 5% SDS in Tris HCl. The dissolved cells were scraped off the petri dishes and Rotiscint was used as scintillator. Five out of 20 anti-HSV-1 MAb preparations studied (14 hybridoma cell culture supernatants with MAb against gB, gC, gD, and gE and 6 ascites fluid preparations containing MAb against different gC-1 epitopes) demonstrated neutralizing capacity. Two of the 11 anti-gC-1 MAb preparations interfered with binding of HSV-1 and one of these (B1C1) blocked the binding also at the lowest protein concentration tested (10 μg/ml). This MAb was of the G1 IgG-subclass and neutralized HSV-1 infectivity, but was markedly less potent in neutralization than as inhibitor of attachment. It is known that inhibition of virus adsorption and neutralization not always have parallelled [4]. The B1C1 MAb was selected for further studies.

The attachment of gC-1 expressing HSV-1 strains is illustrated in Fig. 1. It is seen that a rapid attachment occurred during the initial 60 min at 4 °C, and that pretreatment of virus with the B1C1 MAb or anti-HSV-1 rabbit hyperimmune serum effectively abrogated virus adsorption. The inter-strain variation between the 6 HSV-1 strains tested (Fig. 2) was moderate and the blocking effect of B1C1 was demonstrable with all the strains. The results were confirmed by assaying [^{3}H]-thymidine-labelled HSV-1 (McIntyre) and mixtures of virus and monoclonal antibodies (data not shown).

We next compared the attachment of the gC^- HSV-1 mutant, MP, with that of gC^+ strains (Fig. 3). MP demonstrated a markedly lower relative attachment rate with a binding extending over a prolonged period of time. First after a 3 h of incubation the amounts of cell-attached MP reached levels comparable to those of the gC^+-strains. The B1C1 MAb did not affect attachment of MP. The binding to cells of lectin- and immunosorbent-purified truncated gC-1 is illustrated in Fig. 4, and yielded adsorption profiles identical with those

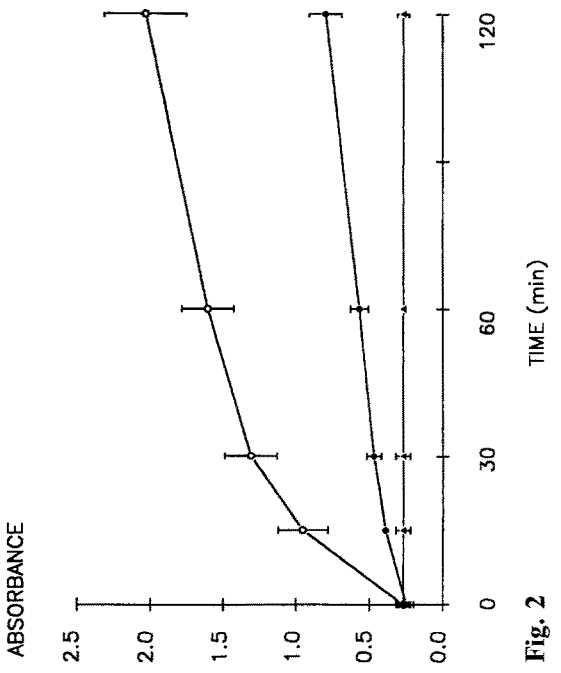

Fig. 2

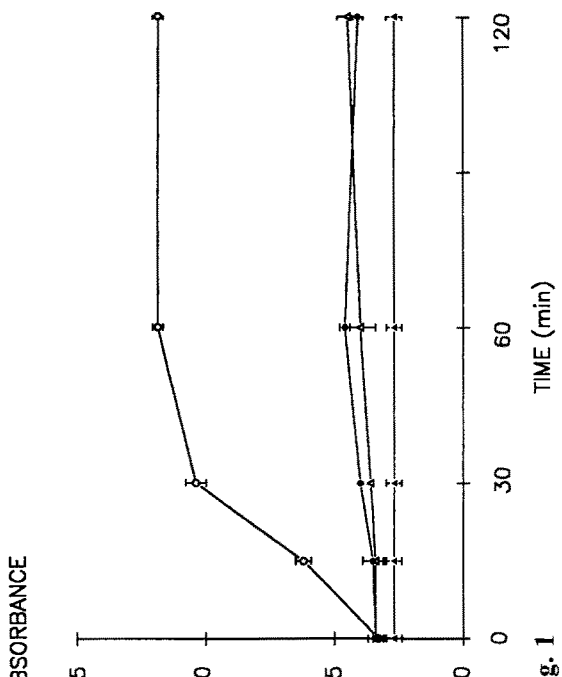

Fig. 1

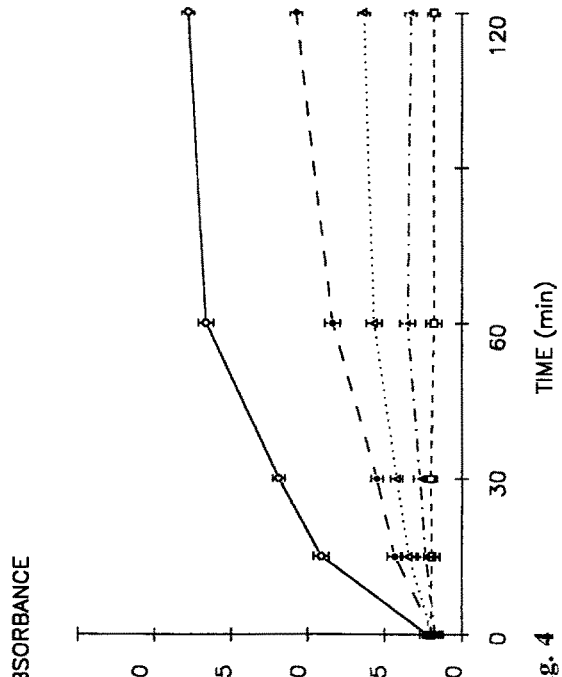

Fig. 4

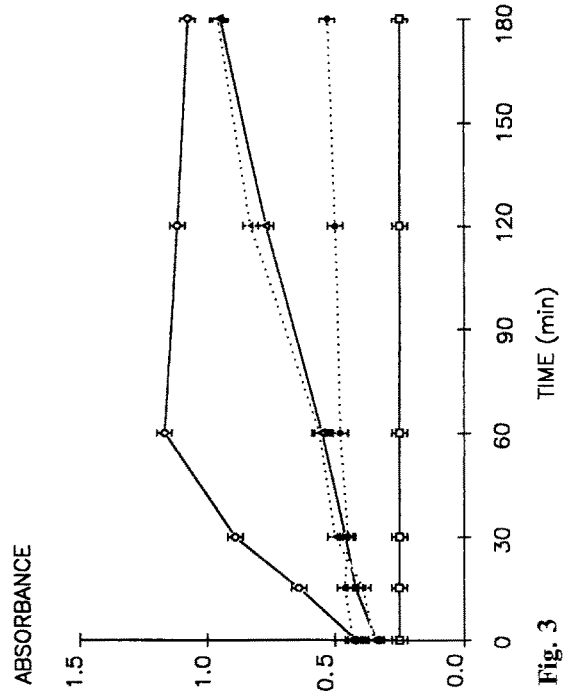

Fig. 3

Table 1. Adsorption to cells of glycoprotein C of HSV-1 (15 μg/ml), pretreated with heparin (20 μg/ml)

gC-1	Heparin	Absorbance (492 nm)
+	−	1.333 ± 0.023
+	+	0.189 ± 0.006
−	−	0.155 ± 0.007

of gC^+ HSV-1. Pretreatment of gC-1 with heparin (20 μg/ml) for 10 min at 37 °C eliminated the binding to cells (Table 1).

At least three of the HSV glycoproteins (gB, gC, gD) have been suggested playing roles in the attachment of the virion to the surface of the target cells. Of these gB and gD are indispensable for infection while the function of the dispensable gC is less evident. Recently the role of gC in the process of HSV attachment has been strengthened, however, and gC-1 is believed to mediate attachment of HSV-1 virions by a neomycin/polylysine sensitive pathway [2]. The results of the present report confirm the involvement of gC-1 insomuch

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Fig. 1. Attachment profiles of the gC^+- strain McIntyre untreated (○) or pretreated with antibody K 642, a rabbit anti-HSV-1 hyperimmune serum (●), or B1C1, an anti-gC-1 MAb (△). Relative amounts of virus adsorbed at 4 °C and at times indicated. Assays were performed by an ELISA-based technique by which virus attached to GMK AH-1 cells was assayed. Absorbed virus was demonstrated by mouse or rabbit anti-HSV-1 antibodies and peroxidase labelled anti-mouse or anti-rabbit IgG. Absorbance at 492 nm plotted against adsorption time in minutes after inoculation of virus. ▲ PBS control represents background values. Means and SEM of 4 assays

Fig. 2. Mean attachment profile constructed on means and SEM of assays with 6 gC^+-strains untreated (○) or treated with anti-gC-1 MAb, B1C1 (●). Absorbance at 492 nm plotted against time in minutes after inoculation of virus. ▲ PBS control

Fig. 3. Attachment profiles of gC^+-strain F (●, ○) and gC^--mutant MP (▲, △), untreated (○, △) or pretreated with anti-gC-1 MAb, B1C1 (●, ▲). Means and SEM of 4 assays. Absorbance at 492 nm plotted against time in minutes for virus adsorption. □ Results with virus-free controls

Fig. 4. Attachment profiles of purified, truncated gC-1. Four concentrations of gC-1 (○ 15, ● 7.5, △ 3.8, and ▲ 1.9 μg/ml, respectively). Background values with sham-treated cells (□). Means and SEM of 4 assays. Absorbance at 492 nm plotted against time in minutes

that a MAb against a gC-1 epitope effectively blocked adsorption, the gC^- mutant, MP, demonstrated reduced rate of attachment, and the adsorption of gC-1 was inhibited after treatment of gC with heparin. It has been suggested that HSV-1 utilizes the fibroblast growth factor (FGF) receptor for attachment [6]. Both the low-affinity and the high-affinity FGF receptor can bind HSV [6]. The less specific binding to the low-affinity receptor is dependent upon interactions of the virus with the heparin-like, heparan sulphate moiety of the receptor [12, 18]. We suggest that this binding is mediated by reactions with glycoprotein C projections on the viral envelope.

Acknowledgements

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