Cloning and expression of the complement receptor glycoprotein C from *Herpesvirus simiae* (herpes B virus): protection from complement-mediated cell lysis

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Simian herpes B virus (SHBV) is the herpes simplex virus (HSV) homologue for the species *Macaca*. Unlike in its natural host, and unlike other animal herpesviruses, SHBV causes high mortality in accidentally infected humans. SHBV-infected cells, like those infected with HSV-1 and equine herpesvirus types 1 and 4, express complement C3 receptor activity. To study immunoregulatory functions involved in susceptibility/resistance against interspecies transmission, the SHBV glycoprotein C (gC_{SHBV}) gene (encoding 467 aa) was isolated. Sequence analysis revealed amino acid identity with gC proteins from HSV-2 (46–9 %), HSV-1 (44–5 %) and pseudorabies virus (21–2 %). Highly conserved cysteine residues were also noted. Similar to gC_{HSV-2}, gC_{SHBV} is less glycosylated than gC_{HSV-1}, resulting in a molecular mass of 65 kDa if expressed in replication-deficient vaccinia virus Ankara. Stable transfectants expressing full-length gC_{SHBV} on the cell surface induced C3 receptor activity and were substantially protected from complement-mediated lysis; no protection was observed with control constructs. This suggests that expression of the gC homologues on infected cell surfaces might also contribute to the survival of infected cells in addition to decreased virion inactivation. Interestingly, soluble gC_{SHBV} isolated from protein-free culture supernatants did not interfere with the binding of the alternative complement pathway activator properdin to C3b, which is similar to our findings with gC_{HSV-2} and could be attributed to major differences in the amino-terminal portion of the protein with extended deletions in both gC_{SHBV} and gC_{HSV-2}. Binding of recombinant gC_{SHBV} to polysulphates was observed. This, together with the heparin-sensitivity of the gC_{SHBV}–C3 interaction on the infected cell surface, suggests a role in adherence to heparan sulphate, similar to the gC proteins of other herpesviruses.

**INTRODUCTION**

As a rare exception among animal members of the family *Herpesviridae*, which do not usually infect humans, severe disease leading to high mortality in humans is caused by the primate simian herpes B virus (SHBV, *Cercopithecine herpesvirus type 1*). Accidental infection of humans has been described on several occasions with SHBV, originating from old world monkeys of the genus *Macaca*.

In contrast to the situation in its natural host, where disease symptoms resemble those of human herpes simplex virus (HSV), severe encephalitis associated with high mortality has been observed in humans (Davenport *et al.*, 1994). Interestingly, human HSV can also act as a ‘killer virus’ if transferred to certain new world monkey species (Huemer *et al.*, 2002), indicating that the pathogenicity and virulence of herpesviruses are difficult to predict in different host environments.

The DNA sequence of the gC gene of *Cercopithecine herpesvirus type 1* reported in this paper has been deposited (30 October 1998) in EMBL under nucleotide accession no. SHE012474 and in GenBank under no. AJ012474.
Immunocompetent humans are not usually endangered by alphaherpesviruses from other species, a phenomenon that has been also observed in animals, where, in most cases, interspecies transmission seems to be rather restricted (Engels et al., 1992).

In contrast, a broad spectrum of cells from different species is susceptible to infection with alphaherpesviruses in vitro, which is most likely due to attachment to the heparan sulphate 'receptors' found on many cell types. Thus, it seems unlikely that a restriction at the cellular level is solely responsible for the observed host specificity of herpesviruses in vivo and a key topic is to investigate possible mechanisms for this widely observed 'natural immunity' (reviewed by Skinner et al., 2001). Identification of virus and host factors that cause susceptibility or resistance to herpesviruses from other species may represent a possible way forward for future prophylactic or therapeutic measures.

In the early phase of virus infection, neither a specific humoral nor a cellular immune response is available. The fate of an infectious agent depends primarily on its ability to avoid the mechanisms of the so-called 'nonspecific' immune response. One important antibody-independent system, acting without precedent priming with antigen, is the alternative complement pathway, a phylogenetically old system that represents a first barrier against invading microorganisms (Cooper, 1991). Its importance for complex viruses arises from the finding that several members of the Herpesviridae and also the Poxviridae express proteins on the virion and infected cell surface; these proteins are able to interact with proteins of the complement cascade at different levels (Ohmann et al., 1988; Isaacs et al., 1992; Huemer et al., 1992b, 1993a; Rother et al., 1994).

Thus, HSV glycoprotein C (gC HSV-1/2) of both serotypes has been shown to bind to the central complement component C3 and thereby interfere with the antibody-independent alternative complement pathway (Fries et al., 1986; McNearney et al., 1987). gC HSV-1 was found to be very similar to known human complement regulatory proteins, thus competing for binding with the alternative pathway activator properdin or regulator protein factor H, which was not observed using purified gC HSV-2 (Huemer et al., 1993b). Absence of gC HSV-1 or pseudorabies virus (PRV) gIII (gCPRV) on virions has been shown to cause increased virus lysis via the alternative complement pathway and interestingly, species variations in the efficiency of lysis using different complement sources have been observed (Hidaka et al., 1991; Ikeda et al., 2000; Maeda et al., 2002). This is in accordance with the finding of differences in the binding to complement C3 of herpesvirus gC proteins of different species (Huemer et al., 1993a).

Expression of gC homologues is highly conserved and the gC proteins of different HSV serotypes are found in clinical isolates, with the exception of isolates originating from immunologically 'privileged' locations (Hidaka et al., 1990). Moreover, the finding of an attenuated phenotype of other species isolates with deletions or diminished expression of the gC homologues (Mettenleiter et al., 1988; Liang et al., 1992; Moffat et al., 1998) also suggests that these proteins perform vital functions in vivo.

Furthermore, the gC proteins have been shown to contribute to binding to cell surface heparan sulphate, which is also true for other herpesvirus glycoproteins such as gB (reviewed by Sawitzky et al., 1990; Liang et al., 1992). However, it should be noted that binding to charged surface molecules, like heparan or chondroitin sulphate, appears to be a general mechanism of virus adsorption in nature, which is also found in many other viruses from different families.

gC binding to heparan sulphate is not essential for HSV-2 attachment (Gerber et al., 1995) and studies using gC-deficient HSV-1 mutants suggest that gC plays little role in the adsorption process (Griffiths et al., 1998). Although this is still a controversial topic (Laquerre et al., 1998), there is growing evidence that binding to heparan sulphate may not represent the predominant biological function of the gC homologues in vivo, at least in some of the strains tested. Additionally, the C3-binding sites on gC HSV-1 and the binding sites for heparin, dextran sulphate and other polysulphates seem to be related, as these substances block the C3 receptor function of gC HSV-1 (Huemer et al., 1992a).

In this study, we have investigated the complement receptor of SHBV, and cloned and sequenced gC SHBV. Expression of the gene in eukaryotic expression systems facilitates characterization of the antigenic structure and furthers studies of possible immune regulatory properties. The constructs have also been used for diagnostic serology testing. Serodiagnosis of SHBV is difficult due to extensive cross-reactivity with HSV specimens (Falke, 1964; Hilliard et al., 1989; Norcott & Brown, 1993). In addition, vaccinia virus constructs using the established modified vaccine virus strain Ankara (MVA) have the potential for use in immunization studies.

### METHODS

#### Cells and viruses.

The macaque isolate from SHBV used in this study has been described (Falke, 1961). HSV-1 strain Ang and equine herpesvirus type 1 (EHV-1) strain Piber were used for comparison. Virus stocks of SHBV were produced in Vero cells (ATCC, #CCL-81). For expression experiments, the rabbit kidney RK13 cell line (ATCC, #CCL-37) was used. Chicken-adapted MVA was kindly provided by A. Mayr, LMU-Munich, Germany. Wild-type virus and recombinants were raised in primary chicken fibroblasts (CHF).

#### DNA purification, identification of fragments and cloning procedures.

As isolation of the full-length gC SHBV gene by PCR amplification using HSV-specific primers was not successful, and because amplification of long SHBV fragments proved to be difficult due to the high G/C content of the SHBV genome, a conventional cloning strategy was chosen. Viral DNA was isolated from SHBV-infected roller bottles of Vero cells, which were lysed in 1 % lauryl sarcosine and digested with pronase E. Viral DNA was separated from genomic DNA by density centrifugation in CsCl gradients. Fractions containing viral DNA were precipitated with isopropanol.
and digested with rare-cutting restriction enzymes. Restriction digests were separated on 1% agarose, blotted onto nylon membranes and hybridized with \( g_C_{HSV-1} \) and \( g_C_{EHV-1} \)-specific probes under conditions of low stringency (50°C, 0-5% SSC wash). As probes, restriction fragments were excised from plasmids containing the coding sequences of HSV-1 strain KOS (Huemer et al., 1989) and EHV-1 strain Piber (Huemer et al., 1995) and labelled by random priming using Klenow polymerase and digoxigenin-labelled UTP. Blots were developed using the anti-digoxigenin system from Boehringer Mannheim. Cross-hybridizing SHBV fragments were isolated, subcloned into cloning plasmids and subjected to sequence analysis. For comparison, the \( g_C \) gene of HSV-1 strain Ang was isolated in a similar manner.

Sequence analysis. Subcloning of several subfragments was necessary as the high G/C content of SHBV prevented long sequencing runs due to the formation of secondary structures. Constructed plasmids were analysed by automated sequencing in an ABI Prism DNA sequencer and a MWG LiCor using both dye labelling and primer sequencing techniques. Homology alignments with \( g_C \) sequences obtained from EMBL were performed using the DNAStar program.

Construction of expression plasmids and expression of recombinant proteins. After sequence analysis, a 1.4 kb BamHI-Sacl fragment was excised and religated to a 570 bp Ncol–BamHI fragment producing the complete \( g_C_{EHV-1} \)-encoding sequence. The whole coding sequence following a Ncol site containing the start codon was inserted into the expression vector pCR3.1 (Invitrogen) under the control of the cytomegalovirus immediate-early promoter.

A construct lacking the putative transmembrane sequence was generated subsequently by PCR amplification of the 5'–terminal sequence, including the \( PstI \) site at position 1420 using kinase-treated forward (5'–GATGGAGATCCGGGACGGGCA-3') and reverse (5'–GACCCCGGGGACGAATATACCATCATCATCATCATGAG-3') primers. The PCR fragment was cloned unidirectionally into pCR3.1 using a single-sided dephosphorylated vector (Invitrogen). The fragment was excised with \( PstI/EcoRI \) and inserted into the corresponding sites of pCR3.1_SHBV, thus replacing the hydrophobic putative transmembrane sequences following aa 418 with six histidine residues and a stop codon.

For comparison, similar expression plasmids were constructed, inserting the full-length \( g_C \) genes from EHV-1 (Huemer et al., 1995) and Marek's disease virus (MDV, kindly provided by K. Osterrieder, IMB/BFAV, Insel Riems, Germany) into pCR3.1.

Plasmids were transfected into Vero and RK13 cells using Lipofection (Qiagen) and the expression of \( g_C_{EHV-1} \) was monitored by immunofluorescence. Additionally, stable cell lines were generated by electroporation of RK13 cells using a Biorad GenePulser (0.4 cm cuvettes, 350 V, 500 μF). Recombinant cell lines were selected by outgrowth in medium containing 500 μg genetin ml⁻¹ (G418). These stable cell clones were finally moved to protein-free culture conditions, which enabled isolation of the secreted recombinant protein by filtration. Among the protein-free media tested, Cyoeffect CHO medium (PAA Laboratories) supported growth of our transfected RK13 cells. Supernatants were concentrated to 100-fold by Amicon filtration (Millipore) using membranes with a 10 kDa cut-off pore. Filtrates were used as a source of recombinant protein.

Construction of vaccinia virus recombinants. As insertion into the thymidine kinase gene leads to loss of replicative activity of MVA (Schefflinger et al., 1996), insertion into the haemagglutinin (HA) gene of vaccinia virus was performed using recombination plasmid pHA11k-gpt, described recently by Huemer et al. (2000a). Full-length and truncated \( g_C_{EHV-1} \) and the homologous sequences from HSV-1 and EHV-1 were ligated into the recombination vector pHA11k-gpt and transfected into CHF cells infected earlier with MVA. Recombinant viruses expressing gpt-resistance were selected in medium containing xanthine, hypoxanthine and mycophenolic acid and isolated by several rounds of plaque purification using semi-solid agar overlays.

Antisera and immunoassays. A polyclonal anti-SHBV antisera has been raised in rabbits (Falke, 1964). Sera against cytomolgus- and rhesus-derived isolates of SHBV have been kindly provided by M. Slomka (CPhL, Colindale, UK). Macaque sera were obtained from C. Coulibaly (Paul-Ehrlich-Institute, Germany). Production of antisera and monoclonal antibodies against \( g_C_{HSV-1} \) has been described previously (Huemer et al., 1989). Monoclonal and polyclonal antibodies against \( g_C \) homologues from PRV, bovine herpesvirus type 1 (BHV-1) and EHV-1 have been used as described earlier (Huemer et al., 1992b, 1995). Antisera were tested by immunofluorescence using 12-well microscopic slides coated with acetone/methanol-fixed SHBV-infected Vero or RK13 cells stably transfected with \( g_C_{EHV-1} \) or control constructs. Infected cell lysates, transfected cells and recombinant \( g_C_{EHV-1} \) protein were reacted with polyclonal anti-SHBV rabbit sera for ELISA and for immunofluorescence assays, according to the methods described for \( g_C_{HSV-1} \) (Huemer et al., 1989, 1992a).

Radioimmunoprecipitation of vaccinia virus lysates was performed by injecting \( \text{¹³}S \)-labelled RK13 cells with recombinant \( g_C_{EHV-1} \)-MVA constructs for 2 days. Cells were lysed with 0.5% NP-40 and 0.5% deoxycholate and supernatants were obtained by low-speed centrifugation. Lysates were then reacted with the indicated mono- or polyclonal antibodies and precipitated by formalin-fixed Staphylococcus aureus protein A (Sigma). Samples were washed in lysis buffer and analysed by SDS-PAGE. MVA constructs containing \( g_C_{HSV-1} \) and the parental MVA were used for comparison.

Complement factors, C3-binding assays and polyanion binding. The purification of complement component C3 from human serum and other species has been described elsewhere (Huemer et al., 1992b, 1993a, b). Macaque C3 was purified accordingly from cyto- mollus monkey serum by ion exchange chromatography over Mono-Q and subsequent molecular size fractionation on CL4b–Sepharose (Pharmacia). Haemolytic activity of the purified C3 protein was tested by lysis of rabbit erythrocytes using C3-deficient human serum (Quidel).

Binding of C3 to surface-expressed \( g_C_{EHV-1} \) was tested by FACS analysis of recombinant RK13 cells expressing full-length \( g_C_{EHV-1} \). Stable transformants producing the truncated \( g_C_{EHV-1} \) lacking the transmembrane domain and the parental RK13 cell line were used as controls. Cells were incubated with complement component IC3 (100 μg ml⁻¹ in PBS) on ice; IC3 represents haemolytically inactive C3 with the internal thioester hydrolysed, leading to C3b/IC3b-like properties of the molecule, including binding to different human complement receptors (Cole et al., 1985). This was confirmed by binding of the samples used to different complement receptor-carrying human cell lines, such as Raji and U937. Cells were washed with PBS and reacted with FITC-labelled antisera directed against C3d or C3e (Dako). After a final washing step, cells were fixed with formaldehyde and the intensity of fluorescence was determined using a FACScanII (Beckton-Dickinson).

Binding of \( g_C_{EHV-1} \) from virus-infected cell lysates was performed according to the immunoprecipitation described for \( g_C_{PRV} \) (Huemer et al., 1992b). In brief, purified C3 was coupled to CNBr–Sepharose and incubated with membrane extracts of SHBV- or HSV-1-infected Vero cells, which were labelled previously with \( \text{¹⁴}C \)glucosamine.
Sepharose coupled with ovalbumin or purified mouse immunoglobulin served as negative controls. Washed precipitates were run on 10% SDS-polyacrylamide gels and exposed to radiography.

Initial testing for binding of C3 to SHBV-infected Vero cells was performed by rosetting using sheep red blood cells (SRBC) coated with human complement component C3b. This has been found previously to be useful for detection of complement receptors expressed by HSV-1, EHV-1, EHV-4 and a guinea pig herpesvirus, but not for HSV-2, BHV-1 or PRV (Huemer et al., 1992a, 1993a, 1995). Inhibition of SRBC binding of SHBV-infected cells was performed using heparin sulphate, dextran sulphate and other polysulphates under conditions identical to those described for HSV-1 (Huemer et al., 1992a).

Binding of recombinant gC SHBV from protein-free cell culture supernatants to the polysulphates dextran sulphate and chondroitin sulphate was tested in ELISA. Thus, different concentrations of the substance were used to examine the following: (1) plates coated with BSA only and wells without viral antibodies. Plates coated with BSA only served as background controls. Washed precipitates were run on 10% SDS-polyacrylamide gels and exposed to radiography.

Comparable to gCHSV-1 (Huemer et al., 1992a, 1993a, 1994), SHBV-infected Vero cells showed binding of complement receptors expressed by HSV-1, BHV-1 or PRV (Huemer et al., 1992a, 1993a, 1995). Inhibition of SRBC binding of SHBV-infected cells was performed using heparin sulphate, dextran sulphate and other polysulphates under conditions identical to those described for HSV-1 (Huemer et al., 1992a).

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RESULTS

Expression of complement receptor activity on SHBV-infected cells sensitive to polyanions

SHBV-infected Vero cells showed binding of complement component C3 coupled to SRBC and CNBr–Sepharose. Comparable to gC HSV-1 (Huemer et al., 1992a), this binding was inhibited by heparin or dextran sulphate (Fig. 1). SRBC, lacking C3 only, showed no binding to SHBV-infected cells; binding was also inhibited by anti-C3 antibodies (data not shown).

Precipitations of SHBV-infected Vero cells labelled with [14C]glucosamine revealed a C3-binding protein of about 65 kDa precipitated by human and macaque C3–Sepharose, whereas the fully glycosylated form of gC HSV-1 appeared at 120 kDa. Furthermore, no binding was observed of gC HSV-1 and gC SHBV to the porcine complement component as well as to the control–Sepharose (Fig. 1).

Isolation and sequencing of the gC SHBV gene

SHBV DNA hybridized to gC HSV-1 but not to gC EHV-1-specific DNA probes under conditions of low stringency (data not shown).

The gC SHBV-encoding sequence was contained within two BamHI fragments of 960 bp and 3 kb. An open reading frame of 1401 bp encoding 467 aa was found and in contrast to the HSV proteins, two additional cysteine residues were identified in the leader sequence, in addition to the eight cysteines in the coding sequences at highly conserved positions (Fig. 2).

There are six potential N-glycosylation sites in gC SHBV and comparison of the protein sequence with the HSV homologues revealed major structural differences in the amino-terminal domain. Both gC HSV-2 and gC SHBV show deletions in the amino-terminal domain when compared with gC HSV-1, with the extended SHBV deletions preceding...
the deletion of the HSV-2 protein. Despite these differences, the overall amino acid identity was about 44-5% for gCHSV-1 and 46-9% for gC HSV-2. The highly conserved central region of the molecule suggests a comparable structure, which is also reflected by C3 binding.

Comparison with the homologous proteins of human varicella-zoster virus (VZV), PRV, BHV-1, EHV-1 and MDV revealed an overall amino acid identity of 21-2% for PRV, 20-3% for BHV-1, 20-1% for EHV-1, 16-9% for MDV and only 16-3% for VZV.
Detection of C3 receptor activity on transfected cells expressing surface-bound gC_{SHBV}

Construction of stable cell lines expressing full-length and truncated gC_{SHBV}. Soluble gC_{SHBV} was detected by Western blotting in the supernatant of cells lacking the transmembrane anchor sequence as a protein of approximately 65 kDa, whereas no secretion was detected in cells expressing the full-length protein (Fig. 3).

Expression of recombinant proteins was detected on the surface and within transfected cells by immunofluorescence using a polyclonal anti-SHBV antiserum. Whereas the full-length protein is expressed homogeneously on the cell surface, constructs lacking the gC transmembrane domain show a protein staining mainly in the perinuclear (presumably Golgi) region (Fig. 4).

Stable RK13 cell lines were obtained expressing full-length and truncated gC_{SHBV} as well as clones expressing gC_{MDV}. Interestingly, no clones showing sufficient expression of gCHSV-1 were obtained, presumably due to toxicity of the HSV-1 protein in eukaryotic cells; this presents a further difference between the simian and the human herpesvirus gC protein.

Cells expressing gC_{SHBV} on the cell surface were compared with gC_{SHBV} constructs lacking the membrane anchor sequence in addition to the parental RK13 cell line for binding of complement C3 by FACS analysis. As depicted in Fig. 5, binding of human C3 was observed only to surface-bound gC_{SHBV} but not to parental RK13 cells or gC_{SHBV} constructs lacking the membrane anchor.

Inhibition of complement-mediated cell lysis by surface-expressed gC_{SHBV}

Protection from alternative complement pathway lysis of the stable RK13 cell lines expressing surface-bound gC constructs was studied in chromium-release assays. As shown in Fig. 6, complement lysis of untransfected RK13 cells occurred in a concentration-dependent manner up to serum dilutions of 1:80.

Of interest in RK13 cells expressing gC_{SHBV} on the cell membrane was that complement lysis was diminished substantially at different serum concentrations, with something similar to a plateau or saturation point being reached around dilutions of 1:20–1:40. Higher serum concentrations exceeded the complement regulatory capacity of the membrane protein, although a significant reduction was also observed at lower dilutions.

As controls, the untransfected RK13 ancestor cell line and stable transformants expressing gC_{MDV} on the cell membrane were used, with the latter also showing no protective effect against complement-mediated cell lysis. The
percentage of lysis of the gC SHBV RK13 transfectants seemed to be largely dependent on the amount of gC SHBV protein expressed on the cell surface, as variations in lysis among different stable cell clones correlated with the rate of membrane expression of gC SHBV, as detected by immuno-fluorescence, and no inhibition was found with the truncated gC SHBV RK13 clone produced subsequently (data not shown).

**Binding of recombinant gC SHBV to polysulphates and failure to block C3b binding of alternative pathway complement activator properdin**

As depicted in Fig. 7, secreted gC SHBV bound to dextran sulphate coupled covalently to polystyrol surfaces in a dose-dependent manner. No binding was observed to microtitre plates coated with ovalbumin or BSA. Weak binding was also observed to sodiumhyaluronate, which is a charged substance found in connective tissue.

Soluble gC SHBV did not inhibit the binding of purified alternative complement pathway activator properdin to surface-bound C3b, which is similar to gCHSV-2. The different concentrations of the viral protein represented by the data-points (Fig. 7, lanes 1–3) did not lead to a reduction in properdin binding to C3b (Fig. 7, ○), but, interestingly, even led to slightly enhanced levels of properdin binding as compared to the data-point without viral protein added (Fig. 7, lane 4). BSA-coated wells were used as a comparison; no binding of properdin was observed, as detected by monoclonal anti-properdin antibody (Fig. 7, △).

Binding of factor B from human serum to our C3b-coated plates was rather weak compared to the strong binding of the purified properdin and also the use of a polyvalent antiserum led to considerable background. Furthermore, gC SHBV did not have any visible influence on this interaction (Fig. 7, ▼).

**DISCUSSION**

Infection of humans with SHBV occurs after animal bites among animal care workers but infected organ or cell culture material is also hazardous for laboratory staff and the disease can be transferred to close contacts (Davenport et al., 1994). Serological testing for SHBV is rather difficult due to extensive cross reactivity with human HSV (Falke, 1964; Hilliard et al., 1989; Norcott & Brown, 1993). The recombinant constructs and proteins produced in this study may consequently provide a useful tool for serological testing of infected animals and human populations that may be at risk.
The sequencing of the gC<sub>SHBV</sub> gene revealed a close relationship with the HSV homologues, which was expected from their comparable genome organization (Harrington et al., 1992). Regions with high amino acid identities and conserved cysteine residues indicate a similar structure, which is also reflected in the functional properties of the protein. Regarding the differences in the amino-terminal domain of gC<sub>SHBV</sub>, it should be noted that there are also major differences within the two human HSV subtypes in this region, with the deletion of 27 aa in the HSV-2 protein.

These differences in the amino terminus of the gC proteins possibly explain the different functional behaviour of gC<sub>HSV-1</sub> and gC<sub>HSV-2</sub> upon competition with complement regulatory proteins, including properdin, factor H or complement receptors CR1 and CR2, with the failure of gC<sub>HSV-2</sub> to inhibit factor H or alternative pathway activator properdin (Huemer et al., 1993b). Therefore, it was not surprising that the recombinant gC<sub>SHBV</sub> was also unable to block binding of properdin but may even partially stabilize the properdin–C3b interaction. Whether this observation has any significance for the function of gC<sub>SHBV</sub> and gC<sub>HSV-2</sub> remains questionable, as these proteins might possibly exert their function only in their physiological (i.e. membrane-associated) conformation, which is also true for other membrane receptors.

Regarding the binding to surface-bound C3, SHBV, despite the HSV-2-like amino-terminal deletions, seems to be functionally similar to HSV-1, as gC<sub>HSV-2</sub> does not mediate binding of C3-coated SRBC to HSV-2-infected cells. Whether this reflects influences such as differing densities or clustering of receptors, differences in lateral mobility and/or other membrane/receptor peculiarities of the behaviour of gC<sub>HSV-2</sub> remain unclear. A suggested inhibitory influence of other HSV-2 glycoproteins in the cell membrane seems rather unlikely, as there were no detectable interferences of HSV-2 glycoproteins using intertypic recombinant strains expressing gC<sub>HSV-1</sub> (Huemer et al., 1992a).

The sensitivity of the gC<sub>SHBV</sub>–C3 interaction to heparin and dextran sulphate and the binding of gC<sub>SHBV</sub> to surface-bound polysulphates is also analogous to the situation found with gC<sub>HSV-1</sub> (Huemer et al., 1992a), suggesting a comparable heparan sulphate ‘receptor’ activity in SHBV. However, studies investigating adherence of gC-null HSV strains from both subtypes suggested that mediating adherence to heparan sulphate may not be the predominant function of gC (Gerber et al., 1995; Griffiths et al., 1998). Of interest, heparin also interacts with a variety of proteins in the clotting system, the immune system, growth factors, adherence molecules and receptors, including different endogenous complement regulatory proteins (Edens et al., 1993).

From the sequence data, it appears that SHBV seems to be more closely related to HSV-2. Furthermore, PRV was found as the most closely related animal herpesvirus, which is in accordance with the neurotropic characteristics of both viruses. One of the characteristics of PRV is its broad host-range, which is unusual for the Alphaherpesvirinae, and it would be interesting to know more about the tropism of SHBV in this respect.

The interference of gC<sub>SHBV</sub> with cell lysis by porcine complement was somewhat surprising, as there was no binding to purified porcine C3, resisting the vigorous washing procedure in immunoprecipitation. Whether this indicates differences in affinity or the numbers of binding sites, etc., has to be clarified. Failure of direct binding on the surface does not necessarily correspond to a lack of function, like the situation with known human complement regulators such as decay-accelerating factor (DAF). DAF is a cell surface glycoprotein that regulates complement activity by accelerating the decay of C3/C5 convertases. Interacting directly with membrane-bound C3b/C4b, it prevents uptake of C2 and factor B but is not a C3b receptor detectable by the rosetting technique (Medof et al., 1984).

Furthermore, gC<sub>HSV-2</sub> has been shown to interfere with the alternative complement pathway, although no direct binding of C3 was observed on the cell surface (McNearney et al., 1987). Similar observations have been made most recently with PRV, which has been shown to be protected against porcine serum by gC<sub>PRV</sub> (Maeda et al., 2002), although binding to porcine C3 was only found with the purified protein and not by binding of complement-coated SRBC to PRV-infected cells (Huemer et al., 1992b, 1993a). This difficult scenario is complicated further by the fact that herpesviruses, as well as other enveloped viruses, are able to incorporate host cell-derived complement regulatory proteins into their membrane (Spear et al., 1995). This has been shown to play an important role in variations of complement-mediated lysis of PRV grown in different host cells (Maeda et al., 2002) and highlights further the complex differences among human and animal complement regulatory proteins, as well as possible influences of conformational requirements or varying affinities for different C3 species of the virally encoded complement receptor proteins.

Purified gC<sub>HSV-1</sub> also has a reduced binding capacity for C3 derived from other species and binds very poorly to porcine C3 (Huemer et al., 1993a). This observation has been confirmed most recently by plasmon surface resonance analysis (Biacore), whereas human alternative complement pathway regulator factor H was able to bind to the porcine complement C3 in this set-up (M. Holmberg, Helsinki, personal communication).

Certain species-specific selectivity is observed among human complement regulators (Horstmann et al., 1985; Yu et al., 1997) and marked differences in the efficiency of complement-mediated herpesvirus lysis using serum of different species as a complement source have been described (Hidaka et al., 1991; Maeda et al., 2002). The findings that HSV was lysed far more efficiently by serum of
rodents as compared to human serum than earlier reports have suggested. It appears to play a crucial role in animal studies using herpesvirus vectors for anti-tumour therapy (Ikeda et al., 2000). This suggests a close evolutionary relationship of microbial-encoded complement escape mechanisms and the ‘unspecific’ host defence and highlights once more the importance of using appropriate animal hosts as models of herpesvirus infections.

Nevertheless, this class of molecules, acting as virus pathogenicity factors due to their conservation among herpesviruses, represents a good candidate for further vaccination studies, which are under way also with gC antigens from other species (Huemer et al., 2000b).

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