

The Interaction of Glycoprotein C of Herpes Simplex Virus Types 1 and 2 with the Alternative Complement Pathway

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Glycoprotein C (gC) of herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) binds the human complement fragment C3b, but the two proteins differ in their ability to bind C3b on infected cell surfaces. In addition, gC-1, but not gC-2, accelerates the decay of the alternative pathway C3 convertase, thereby affecting later steps of the complement cascade. Previously, we constructed linker insertion and deletion mutants of gC-1 and gC-2 and used transient transfection to express mutant proteins in uninfected cells. In spite of the differences between gC-1 and gC-2, C3b binding was localized to residues within the central portion of both proteins, encompassing the first four cysteines. For gC-1, deletion mutants lacking amino acids 33 to 123 or 367 to 469 or lacking both regions still bound C3b. We recombined these deleted forms of gC-1 into gC-39, an HSV-1 strain lacking the gC gene. The altered forms of gC-1 were incorporated into virions, expressed on the surface of infected cells, and bound C3b. We used these proteins to investigate the structural basis for the inhibitory action of gC-1 on the complement cascade. We found that gC-1 does not inhibit formation of the alternative pathway C3 convertase. This convertase is stabilized by the serum protein properdin. Purified gC-1, but not gC-2, inhibits the binding of properdin to C3b, suggesting that this destabilizes the convertase. The mutant lacking amino acids 367 to 449 was able to inhibit properdin binding to a limited extent when present at high concentrations, although it bound to C3b more weakly than wild-type gC. In contrast, the protein lacking amino acids 33 to 123 was unable to inhibit properdin binding to C3b. Thus, gC-1 contains two structural domains, one for C3b binding, residues 124 to 366, and another, residues 33 to 133, which interferes with properdin binding to C3b. © 1994 Academic Press, Inc.

INTRODUCTION

Recently it has become clear that viruses have evolved mechanisms favoring their own survival by modifying the effectiveness of the host immune response (Dubin *et al.*, 1992; Gooding, 1992). A good example is glycoprotein C (gC) of herpes simplex virus type 1 (HSV-1) which binds the C3b fragment of complement (Cines *et al.*, 1982; Friedman *et al.*, 1984, 1986; McNearney *et al.*, 1987). This interaction is postulated to down-regulate the innate response of the host to virus infection (Fries *et al.*, 1986; Harris *et al.*, 1986, 1990). Moreover, HSV-1 gC has also been shown to act as a receptor for coagulation protein factor X (Altieri *et al.*, 1991; Etingin *et al.*, 1990).

We found that C3b binding activity is expressed on the surface of HSV-1 but not HSV-2-infected cells (Cines *et al.*, 1982; Friedman *et al.*, 1984, 1986). Although gC-2 expressed on infected cell surfaces does not bind C3b, both gC-1 and gC-2 bind C3b when they are expressed on the surface of transfected cells or after purification

from infected cells (Eisenberg *et al.*, 1987; Ghosh-Choudhury *et al.*, 1990; Huemer *et al.*, 1989; Hung *et al.*, 1992; McNearney *et al.*, 1987; Seidel-Dugan *et al.*, 1988, 1990). Both gC-1 and gC-2 also bind another C3 fragment, iC3b (Kubota *et al.*, 1987; Tal-Singer *et al.*, 1991), suggesting that gC may enable virions or infected cells to evade complement attack by competing with the iC3b receptor, human complement receptor 3 (CR3), which functions in the phagocytosis of iC3b-coated particles (Lambris, 1988).

Using a series of constructed mutants of gC-1 and gC-2, we localized the C3b-binding regions to similar portions of each protein (Hung *et al.*, 1992; Seidel-Dugan *et al.*, 1988, 1990). Four distinct regions (I, II, III, and IV) in gC-1 and three regions (I, II, and III) in gC-2 are important for this interaction (Hung *et al.*, 1992; Seidel-Dugan *et al.*, 1988, 1990). In addition, amino acids residues 33 to 123 and 367 to 449 of gC-1 (Hung *et al.*, 1992) and residues 26 to 73 of gC-2 (Seidel-Dugan *et al.*, 1988) are not required since deletion mutants lacking these amino acids are still able to bind C3b.

There is *in vitro* evidence which indicates that in addition to binding to C3b, gC modulates the effects of com-

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plement activation. First, both gC-1 and gC-2 protect against complement-mediated viral neutralization (Harris *et al.*, 1990; McNearney *et al.*, 1987). gC-1 also plays a protective role at the infected cell surface (Harris *et al.*, 1990; Hidaka *et al.*, 1990). Of importance for this report, purified gC-1 has a regulatory effect on the complement pathway in that it accelerates the decay of the key enzyme C3 convertase and also reduces the efficiency of complement-mediated lysis (Fries *et al.*, 1986). In contrast, purified gC-2 appears to stabilize the C3 convertase and has no effect on complement-mediated lysis (Eisenberg *et al.*, 1987; Fries *et al.*, 1986). Our goal is to determine whether the differing effects of gC-1 and gC-2 on the complement pathway are related to structural differences which are outside of the C3b-binding regions of these herpes proteins. Second, we are interested in the mechanism by which C3b binding by gC leads to down-regulation of the complement cascade and protects infected cells and viruses from complement-mediated destruction. The convertase is a heterodimer consisting of C3b and the Bb fragment of factor B. This complex is unstable and the enzyme activity decays unless stabilized by a third serum protein, properdin (Fearon and Austen, 1975; Lambris, 1988). We previously proposed (Hung *et al.*, 1992) that although the amino terminus is not needed to bind to C3b, these residues might interfere with the binding of properdin. Our overall assumption is that gC-1 protects the virus and infected cells against complement-mediated lysis by interfering with this pathway.

In the study described in this paper we used an enzyme-linked immunosorbent assay (ELISA) to show that purified gC-1, but not gC-2, interferes with the binding of properdin to C3b. To map the region of gC-1 which is involved in interfering in this activity, we recombined mutated forms of the gC-1 gene with deletions of residues 33 to 123, or 367 to 449, or with both sets of amino acids deleted, into the gC null virus, HSV-1 gC⁻39 (Homa *et al.*, 1986), and used cells infected with the resulting recombinant viruses as the source of purified mutant proteins. We found that all of the mutant proteins bound C3b, verifying predictions from previous studies (Hung *et al.*, 1992). However, the protein lacking residues 367 to 449 bound C3b more weakly than wild-type gC-1, suggesting these residues contribute to some extent to C3b binding. The wild-type protein and the protein lacking residues from 367 to 449 were able to block properdin binding to C3b; although in the latter case, the extent of blocking was not as great. However, the protein lacking amino acids 33 to 123, near the N-terminus, was unable to block properdin binding. Our data indicate that: (1) the central portion of gC-1 is needed for binding to C3b; (2) amino acids near the N-terminus of gC-1 are needed to block properdin binding to C3b and; (3) the region from residue 367 to the transmembrane region is critical for

maintaining the overall protein structure necessary for C3b binding. A preliminary report of these findings was presented at the XVII International Herpesvirus Workshop (Hung *et al.*, 1992).

MATERIALS AND METHODS

Cell culture and virus strains

Vero and CV1 cells were propagated in Dulbecco's minimal essential medium (DMEM) supplemented with 5% fetal bovine serum (FBS) at 37°. Mouse L cells were grown in DMEM with 10% FBS. BHK cells were grown in Eagle's minimum essential medium containing 5% FBS. HSV-1 gC⁻39, is a gC-1 null mutant derived from strain KOS (321) (Homa *et al.*, 1986). HSV-1 strains vSH214, vSH216, vSH217, and vSH218 are recombinant viruses derived from HSV-1 gC⁻39 and contain wild-type or deleted forms of the HSV-1 gene of KOS as described below. HSV-2 strain 333 was used to prepare purified gC-2.

Construction of a flanking sequence vector for recombination of gC mutants into the virus genome

Intracellular viral DNA was isolated from Vero cells infected with gC⁻39 virus at 1 PFU per cell (Muggeridge *et al.*, 1990; Walboomers and Ter Schegget, 1976). Plasmid (S/B T1) containing the *SalI*-*Bam*HI fragment T-1 (0.621 to 0.643) of HSV-1 (KOS) cloned in pBR322 was kindly provided by E. Wagner. The *SalI*-*Bam*HI fragment from S/B T1 was recloned into the *SalI* and *Bam*HI sites of pUC18 to form S/B-pUC18. pSH214 was derived from S/B-pUC18 by digestion with *Hind*III, followed by treatment with mung bean nuclease and religation, which destroyed the *Hind*III site in the vector. Digestion of pSH214 with *Nhe*I and *Bsu*36I yielded a 4.3-kb fragment and a 1.6-kb fragment, the latter containing the complete gC-1-coding region with an additional 35 bp upstream of the initiation codon and 100 bp downstream of the termination codon. The 4.3-kb fragment was isolated and treated with the Klenow fragment of *Escherichia coli* DNA polymerase, followed by ligation with *Hind*III linkers to form the flanking sequence vector pSH215.

The mutated gC genes, gC-1(Δ 33-123), gC-1(Δ 367-449), and gC-1(Δ 33-123; Δ 367-449), were isolated from plasmids pSH186, pSH206, and pSH209 (Hung *et al.*, 1992) and recloned into the flanking sequence vector, pSH215. The resulting plasmids were named pSH216, pSH217, and pSH218, respectively. As a control the gC-1 gene from strain KOS was recloned into pSH215 and the resulting plasmid was called pSH214.

Transfection and isolation of recombinant viruses

Subconfluent Vero cells were transfected with virion DNA from gC⁻39 together with *Bam*HI-*SalI*-digested

plasmid DNA (pSH214, pSH216, pSH217, or pSH218) and salmon sperm DNA as carrier using the calcium phosphate coprecipitation method (DeLuca *et al.*, 1985; Muggeridge *et al.*, 1990). Five hours after transfection, cells were glycerol shocked (DeLuca *et al.*, 1985) for 2 min, and then rinsed and overlaid with DMEM–5% FBS. Virus was harvested after 4 days, when CPE was evident. It was then diluted and replated on CV1 monolayers. Recombinant viruses were identified by their reaction with rabbit polyclonal anti-gC-1 serum (R46) in an immunoperoxidase assay (Holland *et al.*, 1983; Hung *et al.*, 1992; Kousoulas *et al.*, 1984; Muggeridge *et al.*, 1990) and designated as vSH214, vSH216, vSH217, and vSH218. The recombinant viruses were plaque purified until the plaques were 100% gC positive (three to five rounds of plaque purification) as determined by the immunoperoxidase assay.

To ascertain that the proper insert was present, viral DNA was isolated from Vero cells infected with vSH216, vSH217, or vSH218 (Pignatti *et al.*, 1979). For vSH216 and vSH217, *Hind*III digestion of virion DNA generated a fragment which was not found when gC⁻³⁹ virion DNA was similarly digested. These *Hind*III fragments were isolated and each recloned into the *Hind*III site of pUC18. The *Hind*III DNA fragments which were recovered from vSH216- and vSH217-infected cells contained the expected *Apa*I and *Bgl*II sites, respectively, at the deletion junctions. *Hind*III digestion of virion DNA from vSH218 gave a correct size *Hind*III band corresponding to the gC mutant gene contained in pSH218. However, the novel band was faint, which we believe indicates that the virion DNA was a mixture of vSH218 and the parental virus gC⁻³⁹ (no gC gene). We were unable to plaque purify vSH218 and the results should be interpreted with this in mind.

Purification of virus

Recombinant viruses were purified from the extracellular fluid of infected L– cells. Briefly, culture fluids were centrifuged at 10,000 *g* for 10 min and the supernatants were then laid on 5% sucrose in PBS and centrifuged at 100,000 *g* for 2 hr at 4°. Virus pellets were resuspended into disrupting buffer (2.5% SDS, 100 mM Tris (pH 6.9), 10% glycerol, and 10% β -mercaptoethanol), boiled for 3 min, and examined by Western blot analysis.

Neutralization assay

Serum neutralization titers were determined by the plaque reduction technique (Cohen *et al.*, 1978). Rabbit anti-gC-1 polyclonal serum (R47) and mouse monoclonal antibody (MAb) 1C8 were heated at 56° for 30 min to inactivate complement. Complement-dependent neutralization was determined by addition of baby rabbit complement (Cedarlane Laboratories) to the virus prepara-

tion. Serial dilutions of antibodies were added to virus and incubated for 1 hr at 37°. The virus–antibody mixture was added to CV1 monolayers and allowed to adsorb for 1 hr at 37°. The inoculum was removed and the cells were overlaid with DMEM–5% FBS and then incubated until plaques developed. The greatest dilution of serum causing a 50% reduction in virus titer was selected as the neutralizing titer.

C3b rosetting assay

C3b-coated sheep erythrocytes were prepared as previously described (Friedman *et al.*, 1984). Infected cells were treated with 0.05 U of type X neuraminidase from *Clostridium perfringens* (Sigma Chemical Co.) at 37° for 45 min to enhance C3b-binding activity (Eisenberg *et al.*, 1987; Smiley and Friedman, 1985). One plate was used to assay C3b binding by rosetting and the second was used to examine cell surface expression of gC by the immunoperoxidase assay.

Polyclonal and monoclonal antibodies

Rabbit anti-gC-1 serum (R46), prepared against immunosorbant-purified native gC-1, was used for Western blotting, the immunoperoxidase assays, and purification of gC-2 proteins (Eisenberg *et al.*, 1987). Rabbit anti-gC-1 serum (R47), also prepared against immunosorbant-purified native gC-1 (Eisenberg *et al.*, 1987), was used for neutralization assays and for ELISA. MAb 1C8 (Friedman *et al.*, 1984) was used for the viral neutralization assay and for purification of gC-1 proteins (Eisenberg *et al.*, 1987). MAb to properdin (Cytotech, San Diego, CA) was used to detect bound properdin (Daoudaki *et al.*, 1988).

Enzyme digestions and Western blot analysis

The digestion of cytoplasmic extracts of infected cells containing gC-1 proteins with type X neuraminidase, O-glycanase, endo- β -*N*-acetylglucosaminidase H (endo H), and endo- β -*N*-acetylglucosaminidase F (endo F) was performed as described (Sodora *et al.*, 1991; Wilcox *et al.*, 1988). Enzyme-treated and -untreated extracts and purified proteins were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under either nondenaturing (“native”) or denaturing conditions followed by Western blot analysis (Cohen *et al.*, 1986).

Purification of wild-type gC-1 and gC-2 and gC-1 mutant proteins

The wild-type and mutant gC-1 proteins were purified from cytoplasmic extracts of infected BHK cells (Eisenberg *et al.*, 1987). Each mutant form of gC-1 was purified by immunoaffinity chromatography on a separate column

of MAb 1C8 IgG linked to Sepharose 4B, using elution conditions as described (Eisenberg *et al.*, 1987). gC-2 was purified from cytoplasmic extracts of HSV-2-infected cells by affinity chromatography using anti-gC-1 IgG (R46) linked to Sepharose 4B and elution conditions as described (Eisenberg *et al.*, 1987). Protein concentration was determined using the BCA* protein assay reagent (Pierce).

Assay of cleavage of complement factor B

Complement factors B and H were purified from human plasma as previously described (Lambris *et al.*, 1980; Lambris and Müller-Eberhard, 1984; Tsokos *et al.*, 1985) and were greater than 95% pure as assessed by SDS-PAGE and ELISA. ¹²⁵I-labeled factor B was prepared using the Iodogen method (Fraker and Speck, 1978) and had a specific activity of 10⁷ cpm/μg. Factor D was kindly provided by Dr. John Volanakis. Zym-C3b was prepared as previously described (Alsenz *et al.*, 1992). Zym-C3b, in PBS containing 0.1% gelatin and 1 mM Ni²⁺, was incubated for 60 min at 37° with 35 ng of iodinated factor B plus 16 ng of factor D, in the presence or absence of 5 μg gC-1 or factor H. The reaction was terminated using SDS-PAGE sample buffer and cleavage of factor B was detected by the presence of the Bb polypeptide on SDS-PAGE.

Enzyme-linked immunosorbent assay

Purified C3 was prepared as described (Esparza *et al.*, 1991). The binding of either properdin or gC to purified C3 (Esparza *et al.*, 1991; Lambris *et al.*, 1980, 1984) was tested by ELISA. Briefly, 50 μl of purified C3 (8 μg/ml) diluted in PBS was allowed to bind to wells of microtiter plates overnight at 4°. Under these conditions of binding, C3 is converted to iC3 with binding properties similar to those of C3b (Alsenz *et al.*, 1992). The wells were then saturated with 1% bovine serum albumin and 1% ovalbumin in PBS for 30 min at 25°. HSV-negative human serum was used as a source of properdin, or purified properdin was used in some experiments (Perkins *et al.*, 1988). Serial dilutions of EDTA-treated human serum (50 μl) were added to each well and incubated for 1 hr at 25°. Wells were incubated with anti-properdin MAb (kindly provided by C. Koch) for 1 hr to detect the bound properdin, and then incubated with goat anti-mouse IgG horseradish peroxidase (GAM-HRP) for 1 hr. Between each step, the plates were washed three times with 0.05% Tween 20 in PBS. The amount of bound HRP was determined by using an enzyme assay. The substrate was 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate]. Absorbance was measured at 405 nm. The binding of purified gC to C3 was measured in essentially the same manner except that antiserum against gC-1 or against gC-2 (R81) and ProA-HRP were used.

Inhibition of properdin binding to C3 by purified gC proteins was tested by ELISA. Various concentrations of wild-type or mutant forms of gC were incubated for 1 hr at 25° with C3 fixed to microtiter wells. Fifty microliters of HSV-negative normal human serum ($\frac{1}{160}$ dilution) was then added to the gC solution in the wells. After a preincubation period with the various proteins, properdin binding to C3 was carried out as described above. The absorbance (A_{405}) obtained when gC was absent was considered to be 100% properdin binding. When gC proteins were present, the percentage of properdin binding was calculated as follows: (A_{405} of properdin binding in the presence of gC)/(A_{405} of properdin binding without gC) × 100.

RESULTS

Construction and isolation of recombinant viruses expressing deleted forms of gC-1

Previously, we showed that amino acids upstream of residue 123 or downstream of residue 367 of gC-1 are not required for C3b binding by transfected cells expressing mutant forms of gC-1 (Hung *et al.*, 1992). Instead, four other domains of gC-1 are involved and these are located at various sites within residues 124 to 366. To examine which portions of gC-1 play a role in the modulation of the complement pathway, we needed to obtain each mutant protein in a purified form. To achieve this, three mutated forms of the gC-1 gene were first cloned into the flanking sequence vector pSH215 and cotransfected into Vero cells with gC⁻39 virion DNA (Fig. 1). As a control, the wild-type gC-1 gene from strain KOS was also recombined into this null virus. Four recombinant viruses were obtained (Fig. 1): (1) vSH214 expressed wild-type gC-1; (2) vSH216 lacks codons for residues 33 to 123; (3) vSH217 lacks codons for residues 367 to 449; and (4) vSH218 lacks codons for residues 33 to 123 and 367 to 449. The expressed proteins are designated as gC-1-(wt), gC-1-(Δ33-123), gC-1-(Δ367-449), and gC-1-(Δ33-123; Δ367-449), respectively.

Presence of deleted forms of gC-1 protein in virus

To determine whether gC-1 was present in the virion, we isolated virus from culture fluids of cells infected with vSH214, vSH216, and vSH217, extracted the glycoproteins, and electrophoresed them on SDS-denaturing gels. The proteins were Western blotted and probed with anti-gC-1 serum. gC-1 was found for each recombinant virus (Fig. 2). As expected from results with transfected cells, gC-1-(Δ33-123) migrated much faster than gC-1-(wt) in the gel because a large number of amino acids modified with N-CHO were deleted (Hung *et al.*, 1992). gC-1-(Δ367-449) migrated faster than gC-1-(wt) as expected. In the case of vSH218, too little gC was present in the

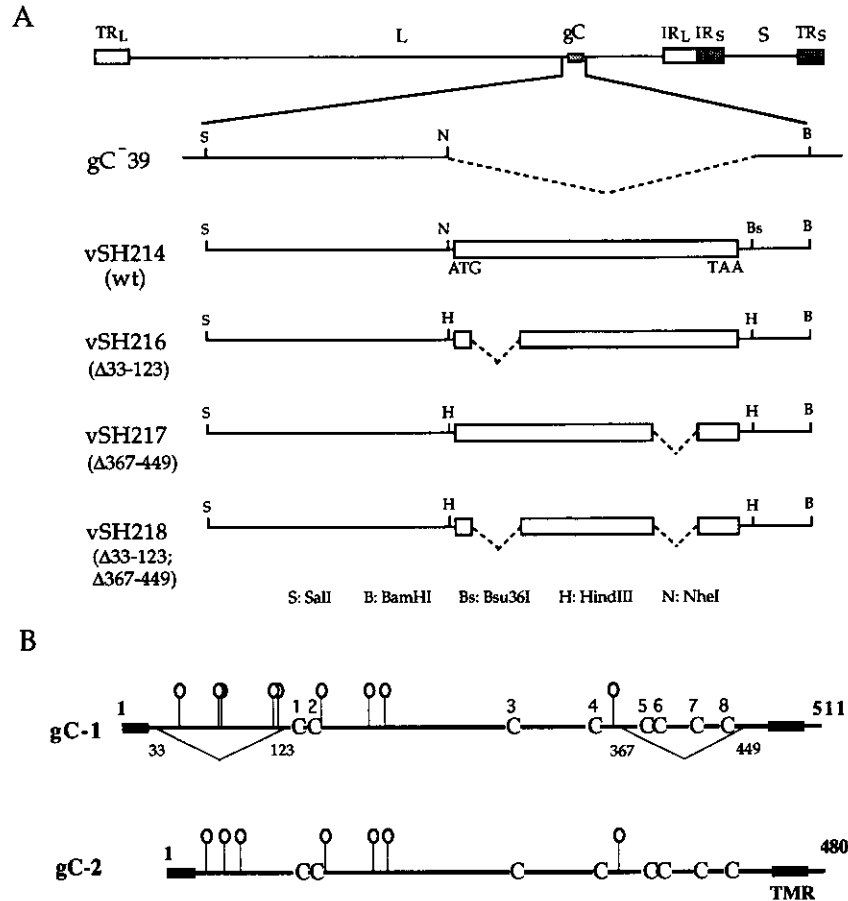


FIG. 1. Diagram of the gC coding regions of the HSV-1 strains used in this study and stick figures of gC-1 and gC-2. (A) Diagram of the HSV-1 genome indicating the long (L) and short (S) unique regions each flanked by inverted terminal repeats (TR_L and TR_S) and internal repeats (IR_L and IR_S). The *Sal*I (S)–*Bam*HI (B) fragment containing the entire gC-1 gene which is deleted from HSV-1 gC⁻³⁹ is shown. For each of the recombinant viruses, the rectangles indicate the protein coding region. Also shown are the translation start and termination sites. The bracketed areas refer to the nucleotides which are deleted from gC⁻³⁹ and each of the recombinant viruses. (B) gC-1 contains 511 amino acids with nine potential N-linked glycosylation (N-CHO) sites (balloons) and eight cysteine (C) residues (C1–C8). The dotted lines refer to the amino acid residues (33 to 123 and 367 to 449) which are deleted in the mutants (Hung *et al.*, 1992). gC-2 contains 480 amino acids with seven potential N-CHO sites and eight C residues which are colinear with those of gC-1.

virus mixture to detect the protein in this fashion. The results suggest that residues 33 to 123 (deleted in vSH216) and 367 to 449 (vSH217) are not required for gC-1 to be incorporated into the virion envelope. However, we cannot exclude the possibility that the virus preparation was contaminated with membrane vesicles.

Virus neutralization

As another means of demonstrating the presence of gC in the mutant viruses, virus neutralization assays (50% plaque reduction method) were carried out with polyclonal anti-gC-1 serum (R47) and with MAb 1C8 (Table 1). As expected, R47 or 1C8 did not neutralize gC⁻³⁹ (no gC present). R47 neutralized both vSH214 and vSH216 in the absence or presence of complement (Cohen *et al.*, 1972). However, R47 neutralized vSH217 at a 1:20 dilution in the presence of complement and had no neu-

tralizing activity in the absence of complement. MAb 1C8 exhibited high neutralization titers against vSH214 and vSH216 in the presence of complement. The neutralization titer against vSH216 was approximately 16-fold higher than that against vSH214. Surprisingly, 1C8 failed to neutralize vSH217, even though MAb 1C8 recognizes this deleted form of gC on the surface of the transfected cells (Hung *et al.*, 1992). In addition, 1C8 reacted with each form of gC-1 when infected cell extracts were analyzed by Western blotting (data not shown) and 1C8 was used to purify gC from vSH217-infected cells.

Assuming that the Western data above are correct, the neutralization data suggest that gC-1-(Δ367–449) present in the virion envelope may be altered so that it cannot function in neutralization. In the case of vSH216, gC-1 (Δ33–123) may bind 1C8 with a higher affinity than gC-1-(wt) so that less antibody is required for neutralization. Alternatively, residues 33 to 123 of gC-1, when present,

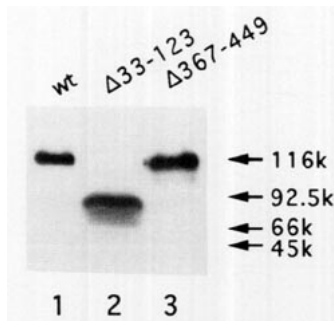


FIG. 2. Presence of gC-1 in purified virions of vSH214, vSH216, and vSH217. Recombinant viruses were purified from the extracellular fluid of infected L cells. Virus pellets were resuspended in disrupting buffer, electrophoresed on an 8% SDS-polyacrylamide gel, and examined by Western blot analysis using polyclonal anti-gC-1 serum. Lane 1, wild-type gC-1 (from vSH214); lane 2, gC-1(Δ 33-123) from vSH216; lane 3, gC-1(Δ 367-449) from vSH217.

may modulate complement-dependent neutralization, and the deletion might cause vSH216 to be more sensitive to complement-dependent neutralization. Such a mechanism was proposed to explain the increased sensitivity to complement-dependent antibody neutralization of gC mutants which do not express gC on the cell surface (Harris *et al.*, 1990).

C3b receptor activity on the surface of recombinant virus-infected cells

In previous studies a rosetting assay was used to determine C3b binding by mutant forms of gC-1 expressed on the surface of transiently transfected cells. Here L cells infected with gC-39 virus, vSH214, vSH216, vSH217, or vSH218 were treated with neuraminidase to enhance C3b binding (Smiley and Friedman, 1985) and incubated with C3b-coated sheep erythrocytes. As expected, cells infected with gC-39 did not form rosettes, whereas cells

TABLE 1

SERUM NEUTRALIZATION OF HSV INFECTIVITY

Virus	gC-1	R47 ^a		1C8	
		+C' ^b	-C' ^c	+C'	-C'
gC-39	No gC	0	0	0	0
vSH214	wt	80	80	10,240	0
vSH216	Δ 33-123	80	20	163,840	0
vSH217	Δ 367-449	20	0	0	0

^a Neutralization was defined as a reduction in virus titer of at least 50% in the presence of polyclonal anti-gC-1 serum (R47) or gC-1 specific MAb 1C8 acites fluid which was depleted of complement by heat inactivation.

^b +C', indicates that the neutralization mixture contained baby rabbit complement (serum from 3- to 4-week-old rabbits) used at a final dilution of 1 to 100.

^c -C', indicates that complement was absent.

TABLE 2

BINDING OF ANTIBODY AND C3b TO THE SURFACE OF CELLS INFECTED WITH RECOMBINANT VIRUSES

Virus	gC-1	Cell surface	C3b rosetting
gC-39	No gC	-	-
vSH214	wt (KOS)	+	+
vSH216	Δ 33-123	+	+
vSH217	Δ 367-449	+	+
vSH218	Δ 33-123; Δ 367-449	+	+

Note. Infected cells were tested for gC expression using an immunoperoxidase assay. A duplicate plate was tested for C3b binding by the rosetting assay. The ability of each mutant to bind antibodies and C3b is indicated as positive (+) or negative (-). Cells were infected with gC-39 (no gC), vSH214 (gC-1-wt), vSH216 (gC-1-(Δ 33-123)), vSH217 (gC-1-(Δ 367-449)), and vSH218 (gC-1-(Δ 33-123; Δ 367-449)).

infected with each of the recombinant viruses were positive for C3b binding (Table 2). However, the extent of rosetting varied. Specifically, we noted that cells infected with vSH216 (gC-1-(Δ 33-123)) bound antibody and formed rosettes as well as cells infected with gC-1(wt). Cells infected with vSH217 (gC-1-(Δ 367-449)) and vSH218 (gC-1-(Δ 33-123; Δ 367-449)) bound antibody but formed fewer rosettes.

Analysis of deleted forms of gC-1 protein by SDS-PAGE and Western blotting

Western blot analysis in combination with carbohydrate-modifying enzymes was used to determine the molecular size and extent of processing of mutant proteins synthesized by recombinant virus-infected cells. Extracts from infected cells were treated with neuraminidase, endo H or endo F, electrophoresed on SDS-denaturing gels, Western blotted, and probed with anti-gC-1 serum (Fig. 3). In the case of cells infected with vSH214 (WT), anti-gC-1 serum reacted with polypeptides of 120, 95, and 65 kDa (Fig. 3A, lane 1). The smallest polypeptide may be a degradation product (Zweig *et al.*, 1983). These polypeptides comigrated with authentic gC-1 purified from either HSV KOS-, KOS 321-, or NS-infected cell extracts (data not shown). As expected, gC-1 proteins with deletions each migrated faster than the wild-type gC-1. In the case of gC-1-(Δ 367-449), expressed by cells infected with vSH217, the gC reactive polypeptides were 115 and 90 kDa (Fig. 3A, lane 5); gC-1-(Δ 33-123) (from vSH216-infected cells) expressed 60- and 70-kDa forms of gC (Fig. 3B, lane 1); and gC-1-(Δ 33-123; Δ 367-449) (from vSH218-infected cells) migrated as two broad polypeptides of 50-55 kDa and 60-65 kDa (Fig. 3B, lane 6).

The pattern of glycosylation of gC-1-(Δ 367-449) was nearly identical to that of gC-1-wt (Fig. 3A, compare lanes 1 and 5). In each case, the higher molecular weight polypeptide was sensitive to digestion with neuraminidase (Fig.

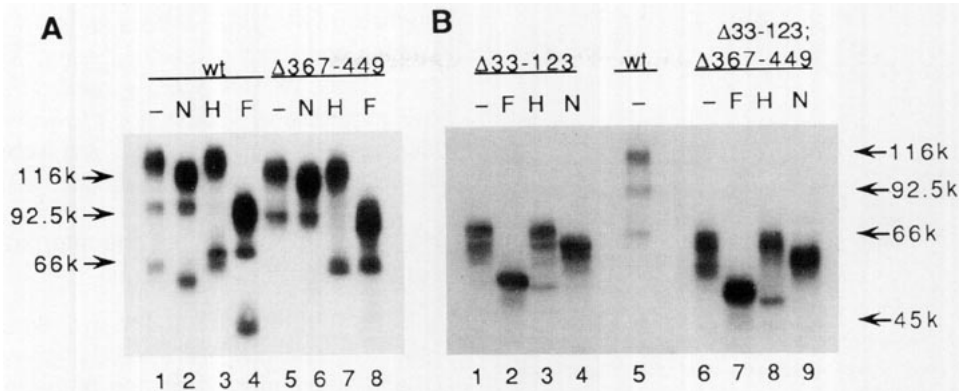


Fig. 3. Effect of carbohydrate-modifying enzymes on the gC-1(wt) and gC-1 deletion mutants synthesized in infected BHK cells. Cytoplasmic extracts from infected cells were treated with enzymes, electrophoresed under denaturing conditions, transferred to nitrocellulose, and reacted with polyclonal anti-gC-1 serum (R46). (A) gC-1(wt) (lanes 1 through 4) and gC-1(Δ 367-449) (lanes 5 through 8); and (B) gC-1(Δ 33-123) (lanes 1 through 4), gC-1(wt) (lane 5), and gC-1(Δ 33-123; Δ 367-449) (lanes 6 through 9). The enzymes used were mock-digested control (-), neuraminidase (N), endo H (H), and endo F (F). The arrows indicate molecular sizes in kilodaltons.

3A, lanes 2 and 6) and endo H (Fig. 3A, lanes 2 and 6), indicating that they contained sialic acid. For both proteins, the lower molecular weight form was sensitive to endo H (Fig. 3A, lanes 3 and 7), indicating that these polypeptides contained N-linked oligosaccharides (N-CHO) of the high-mannose type. The larger molecular weight polypeptides were insensitive to endo H, but sensitive to endo F, indicating that the N-CHO on these polypeptides were of the complex type. The deletion of five potential N-linked glycosylation sites in gC-1(Δ 33-123) or gC-1(Δ 33-123; Δ 367-449) dramatically affected the apparent size of the polypeptides (Fig. 3B, lanes 1 and 6). Nevertheless, the remaining oligosaccharide on these proteins was processed in a similar fashion to the wild-type gC-1 (Fig. 3B, lanes 2 to 4 and 7 to 9), in that the lower molecular weight polypeptide was endo H-sensitive and contained no sialic acid (indicative of a precursor form) and the higher molecular weight form contained N-CHO of the complex type and also contained sialic acid.

In a second experiment, the higher molecular weight form of gC-1-wt was found to be O-glycanase sensitive (Fig. 4A, lanes 4 and 5), indicating the presence of

linked oligosaccharides. The other lanes on this 10% gel repeat the experiment seen in Fig. 3A and serve as controls for that experiment which was done on an 8% gel. This sensitivity to O-glycanase was not seen with gC-1(Δ 33-123) (Fig. 4B, lanes 4 and 5), indicating that most of the O-CHO on gC-1 are located within residues 33-123. This confirms other reports coming to this conclusion (Dall'Olio *et al.*, 1985; Olofsson *et al.*, 1983, 1990).

Immunosorbent chromatography of wild-type and mutant forms of gC

To obtain each mutant protein in a purified form for studies of their effect on the complement cascade, we used immunoaffinity chromatography, employing a separate column for each protein. Very little protein was obtained from vSH218-infected cells, therefore it was not used in further experiments. Immunosorbent-purified proteins obtained from cells infected with vSH214, vSH216, and vSH217 were analyzed by denaturing (data not shown) and nondenaturing SDS-PAGE, followed by Western blotting (Fig. 5). For gC-1(wt), polyclonal anti-

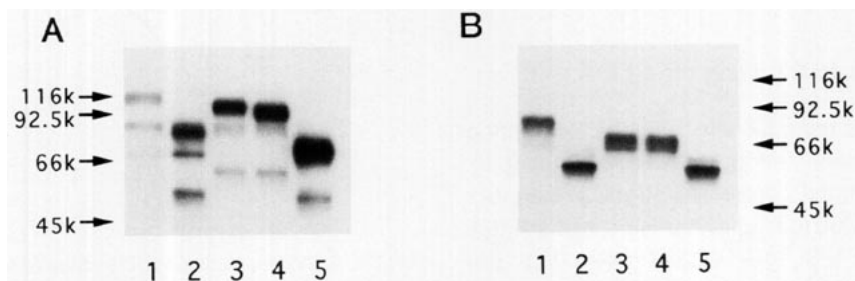


Fig. 4. Effect of carbohydrate-modifying enzymes including O-glycanase on gC-1(wt) and gC-1(Δ 33-123) synthesized in infected BHK cells. Cytoplasmic extracts of infected cells were treated with enzymes, electrophoresed under denaturing conditions, transferred to nitrocellulose, and reacted with polyclonal anti-gC-1 serum (R46). (A) gC-1(wt). (B) gC-1(Δ 33-123). Lane 1, mock-treated extract; lane 2, extracts were treated with endo F; lane 3, treated with neuraminidase; lane 4, treated with neuraminidase + O-glycanase; lane 5, treated with endo F + neuraminidase + O-glycanase.

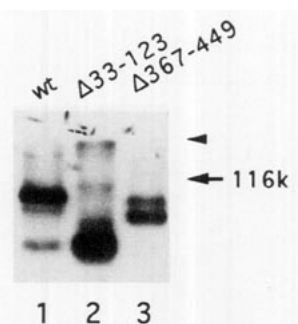


Fig. 5. Western blot analysis of gC-1 purified from vSH214-, vSH216-, and vSH217-infected cells. Cytoplasmic extracts of BHK cells infected with vSH214, vSH216, or vSH217 were passed over separate 1C8 immunosorbent columns and gC-1 was eluted with 0.1 M ethanolamine, pH 10, then concentrated, and dialyzed against Tris-buffered saline, pH 7.3, containing 0.1% NP-40. Samples of the purified proteins were electrophoresed on a 10% native SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with polyclonal anti-gC-1 serum, followed by iodinated protein A. The arrowhead indicates the interface of the stacking and resolving gels. Lane 1, gC-1-(wt) purified from vSH214-infected cells; lane 2, gC-1-(Δ 33-123) purified from vSH216; lane 3, gC-1-(Δ 367-449), purified from vSH217.

gC-1 serum reacted with both product and precursor forms (Fig. 5, lane 1) as well as the smaller polypeptide seen on denaturing gels (Fig. 3). gC-1-(Δ 33-123) migrated more slowly than gC-1-(wt) (compare lanes 2 and 1 in Fig. 5) and a small fraction of the protein appeared to be aggregated (seen as protein at the stacking gel-running gel interface (Fig. 5, lane 2). gC-1-(Δ 367-449) migrated as two polypeptides (Fig. 5, lane 3), the smaller of which migrated more slowly than the precursor form of wild-type gC-1. We recovered significantly less gC-1-(Δ 367-449) from the immunosorbent than gC-1-wt or gC-1-(Δ 33-123). Pursuing this further we found that a portion of gC-1-(Δ 367-449) was bound irreversibly to the MAb 1C8 immunosorbent. A variety of other eluants was also tested without success and only boiling the resin in SDS dislodged the remaining protein. Thus, we believe that deletion of the stretch of amino acids containing the last four cysteine residues destabilizes the gC-1 structure and the protein is more readily denatured, thereby remaining trapped on the column.

Binding of purified gC to C3 using the ELISA

An ELISA assay was used to detect binding of purified gC to C3 which had been fixed to microtiter wells (Fig. 6). Under these conditions the conformation of C3 is altered and resembles that of C3b (called iC3) (Alsenz *et al.*, 1992). gC-1-(wt), gC-1-(Δ 33-123), gC-1-(Δ 367-449) (Fig. 6A) and gC-2 (Fig. 6B) each bound iC3 in a dose-dependent manner. gC-1-(Δ 33-123) bound to iC3 as well as or better than gC-1-(wt). gC-1-(Δ 367-449) also bound to iC3, but not as well. Thus residues 33 to 123 are not required for gC-1 to bind to iC3. The reduced iC3 binding ability of

purified gC-1-(Δ 367-449) is similar to the reduced C3b binding seen in the rosetting assay for vSH217-infected cells (Table 2). This again suggests that residues 367 to 449 contribute to C3b binding. However, since the mutant lacking these residues retains some capacity to bind C3b, it is likely that they are not contact residues.

How does gC destabilize the alternative pathway C3 convertase?

Binding of factor B to C3b deposited on cell surfaces results in cleavage of B into Bb (in the presence of factor D) and subsequent formation of the alternative pathway C3 convertase C3bBb (Farries *et al.*, 1988a,b). In addition to binding to C3b, gC-1 acts as an accelerating factor in the decay of the preformed convertase (Fries *et al.*, 1986). gC-2, although it binds to C3b does not have this activity. Instead, it appears to stabilize the convertase (Eisenberg *et al.*, 1987). Here we investigated two other ways in

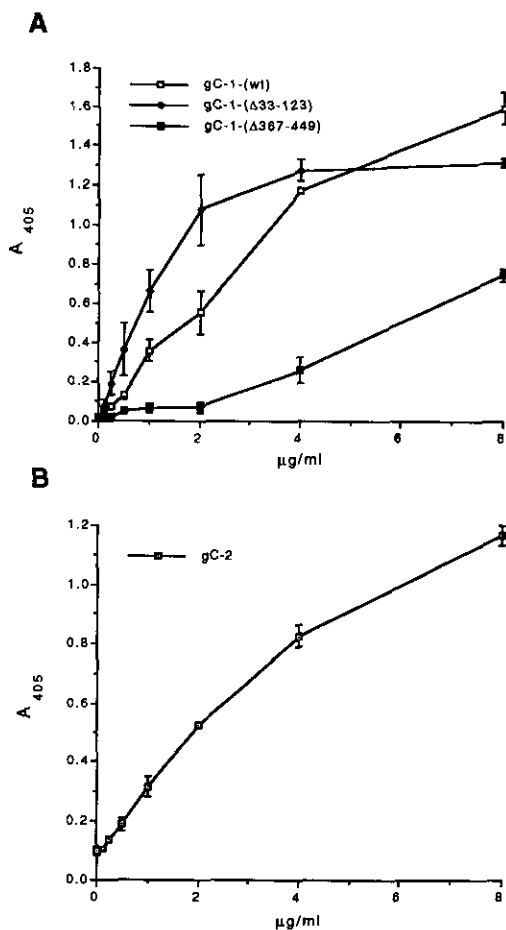


Fig. 6. Binding of purified gC and the mutant proteins to purified C3. The following gC proteins were used: (A) gC-1-(wt), gC-1-(Δ 33-123), gC-1-(Δ 367-449), and (B) gC-2. Fifty microliters of different dilutions of gC (0-8 μ g/ml) was applied to ELISA plates precoated with purified C3. The bound gC was detected with polyclonal antibody against gC-1, R47 (or against gC-2, R81), followed by protein A conjugated with horseradish peroxidase (PrA-HRP) and substrate.

which gC-1 might affect the activity of C3 convertase: (i) gC-1 might inhibit formation of the convertase so that its activity over time would decline in the presence of gC-1 due to turnover; (ii) gC-1 might interfere with binding of properdin which stabilizes the convertase, thereby accelerating its decay.

To assess the potential of gC-1 to inhibit formation of the alternative C3 convertase, we examined its ability to inhibit the cleavage of factor B. We used C3b on zymosan (Zym-C3b) as the source of C3b and measured the effect of gC-1 on cleavage of iodinated factor B (Fig. 7). As a control the reaction was carried out in the presence of EDTA to inhibit the cleavage reaction. A further control was to carry out the cleavage reaction in the presence of factor H whose function it is to inhibit this reaction in serum. As expected, the cleavage took place in the absence but not the presence of EDTA. Factor H completely inhibited the cleavage of factor B, but a similar amount of gC-1 had no effect on the cleavage reaction. We conclude that gC-1 does not inhibit formation of the alternative C3 convertase.

Properdin is a regulatory glycoprotein of the alternative pathway of the complement cascade. Properdin stabilizes the alternative C3 convertase by binding to it and this stabilization is necessary for efficient amplification of the enzyme cascade during complement activation (Fearon and Austen, 1975; Lambris, 1988). It is possible that gC-1 interferes with properdin binding to C3b, thereby accelerating its decay (Fries *et al.*, 1986). gC-2 does not accelerate the decay of the convertase and therefore might not interfere with the binding of properdin to C3b. We used ELISA assays to investigate these possibilities. First, we developed an assay for detecting properdin binding to iC3 (Alsenz *et al.*, 1992). EDTA-treated HSV-negative human serum was used as a source of

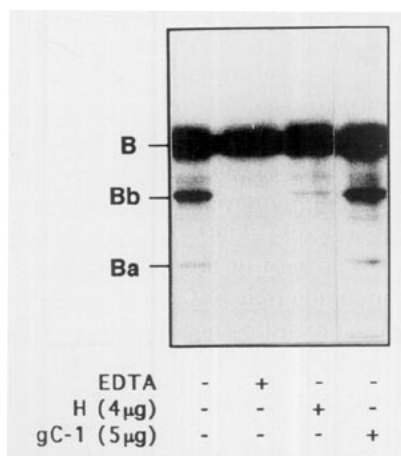


Fig. 7. gC has no effect on the formation of the alternative pathway C3 convertase. Iodinated factor B was incubated with C3b plus factor D for 60 min at 37°, in the presence or absence of EDTA, gC, or factor H. EDTA inhibits the reaction by chelating Ni^{2+} . Factor H inhibits the reaction after binding to C3b.

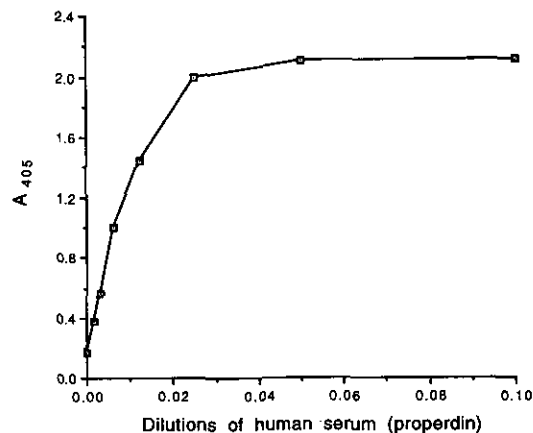


Fig. 8. Binding of properdin to purified C3. HSV-negative human serum was used as a source of properdin. Varying dilutions of serum were added to ELISA plates precoated with purified C3 (which becomes iC3). The bound properdin was detected with a monoclonal antibody to properdin followed by goat anti-mouse IgG peroxidase (GAM-HRP) and substrate. Dilutions ranged from 1:10 (0.10) to 1:640 (0.00156).

human properdin (Daoudaki *et al.*, 1988). The binding of properdin to iC3 was detected by MAb to properdin, followed by GAM-HRP and substrate. Figure 8 shows that the binding of properdin to iC3 was dose dependent. The same effect was observed using purified properdin (Perkins *et al.*, 1988) in this assay. A dilution of 1:160 (0.00625 in Fig. 8) of human serum was chosen for the following studies.

To examine the effect of gC on properdin binding, ELISA plates were coated with C3 and then incubated with purified gC-1(wt), gC-2, gC-1(Δ 33-123), or gC-1(Δ 367-449) before the addition of human serum as a source of properdin (Fig. 9). gC-1(wt) blocked the binding of properdin to iC3 by approximately 40% (Fig. 9A). However, purified gC-2 did not show any such effect (Fig. 9B). gC-1(Δ 33-123) at 20 $\mu\text{g}/\text{ml}$ also had no effect on properdin binding to iC3 (Fig. 9C) even though this form of gC-1 binds to C3b as well as or better than wild-type gC-1 (see Table 2 and Fig. 6). At high concentrations, gC-1(Δ 367-449) blocked properdin binding to a limited extent (Fig. 9D), even though this protein bound poorly to iC3 (Fig. 6). Taken together, the data suggest that residues 33 to 123 of gC-1 are associated with blocking of properdin binding to C3b. Similar results were obtained using purified properdin (data not shown). A puzzling observation was that gC-1(Δ 367-449) enhanced properdin binding when present at low concentrations (Fig. 9D). We have no explanation for this result, but it might be related to the altered conformation of this mutant form of gC-1 as described above.

Characterization of the properdin blocking domain of gC-1

What is it about the N-terminus of gC-1 that interferes with the binding of properdin to C3b? One possibility is

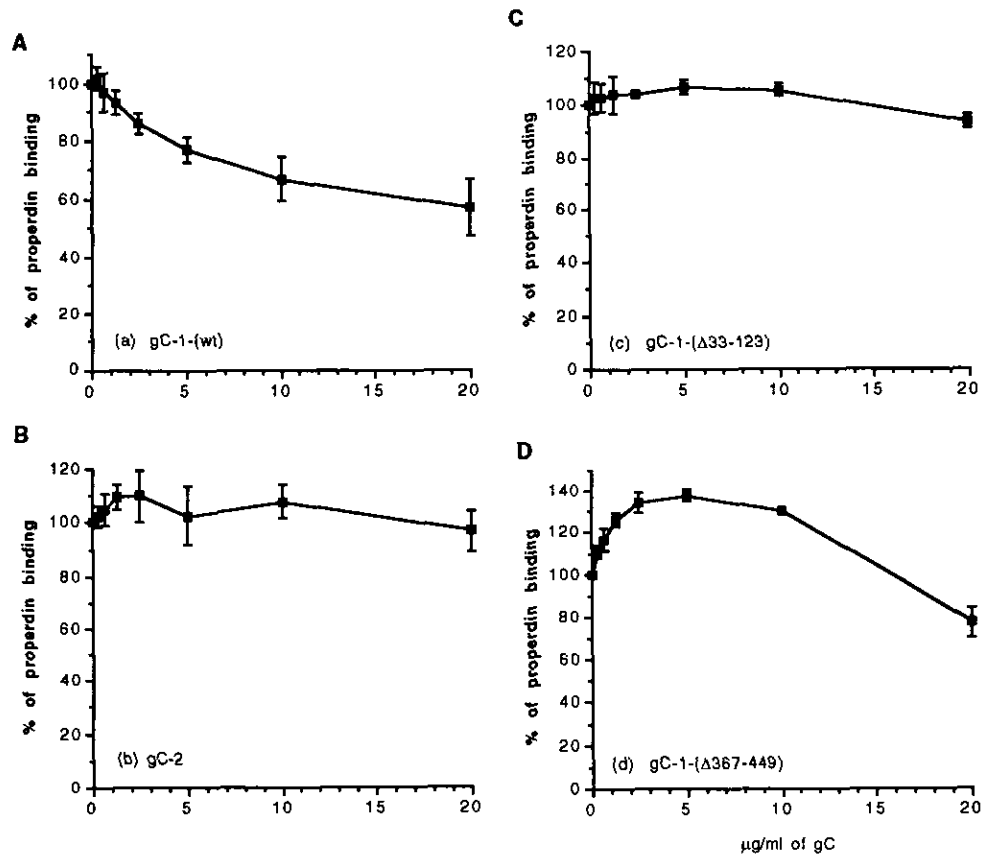


Fig. 9. Inhibition of properdin binding to C3 by gC-1 and gC-2 and mutant forms of gC-1. (A) gC-1-(wt); (B) gC-2; (C) gC-1-(Δ 33-123); and (D) gC-1-(Δ 367-449). Varying dilutions of gC (0-20 μ g/ml) were preincubated with C3-coated wells before addition of normal human serum at a $\frac{1}{160}$ dilution. The bound properdin was detected as described in the legend to Fig. 8. The percentage of properdin binding was calculated as follows: $((A_{405}$ of properdin binding in the presence of gC)/(A_{405} of properdin binding without gC)) \times 100. The results from a series of experiments are shown for each protein. Each experiment was repeated three to five times.

that gC-1 binds to properdin and thereby interferes with its binding to C3b. Using the ELISA assay, we found that purified properdin did not bind to gC-1 fixed to the plate, nor did gC-1 bind to properdin when the assay was reversed (data not shown).

Was the blocking effect due to the polypeptide backbone or to the carbohydrate modifications in the amino terminal portion of gC-1? To address these issues, we treated gC-1-(wt) and gC-1-(Δ 33-123) with glycosidases or neuraminidase and then tested the ability of the treated protein to block properdin binding (Fig. 10). Treatment of gC-1-(wt) with neuraminidase and *O*-glycanase enhanced its binding to C3b (Fig. 10A). Similar treatment of gC-1-(Δ 33-123) had no enhancing effect (Fig. 10B). In contrast, removal of N-CHO with endo F had little effect on binding of gC-1-(wt) or gC-1-(Δ 33-123) (compare Figs. 10A and 10B). A combination of all of the enzymes had no more effect than treatment of gC-1-(wt) with neuraminidase and *O*-glycanase alone. In separate experiments, treatment of gC-1-(wt) with neuraminidase alone enhanced its binding to C3b, an observation that has been noted previously (Eisenberg *et al.*, 1987; Friedman

et al., 1984; Hung *et al.*, 1992; Smiley *et al.*, 1985). These results suggest that sialic acid residues attached to *O*-linked oligosaccharides within residues 33-123 interfere with C3b binding. Second, none of the treatments ablated the properdin blocking capacity of gC-1-(wt) and in fact removal of *O*-linked oligosaccharides or all of the carbohydrate marginally enhanced the blocking effect of gC-1-(wt) (Fig. 10C). However, this enhancement is probably accounted for by the enhanced binding of gC-1-(wt) to C3. In the case of gC-1-(Δ 33-123) none of the carbohydrate modifying enzymes (including neuraminidase) enabled this protein to block the binding of properdin to C3 (Fig. 10D). Thus the data suggest that blocking of properdin binding is associated with the polypeptide backbone of gC-1 within residues 33-123, rather than the carbohydrates or sialic acid residues that modify these amino acids.

DISCUSSION

In previous studies, it was shown that gC-1 accelerates the decay of the alternative pathway C3 convertase

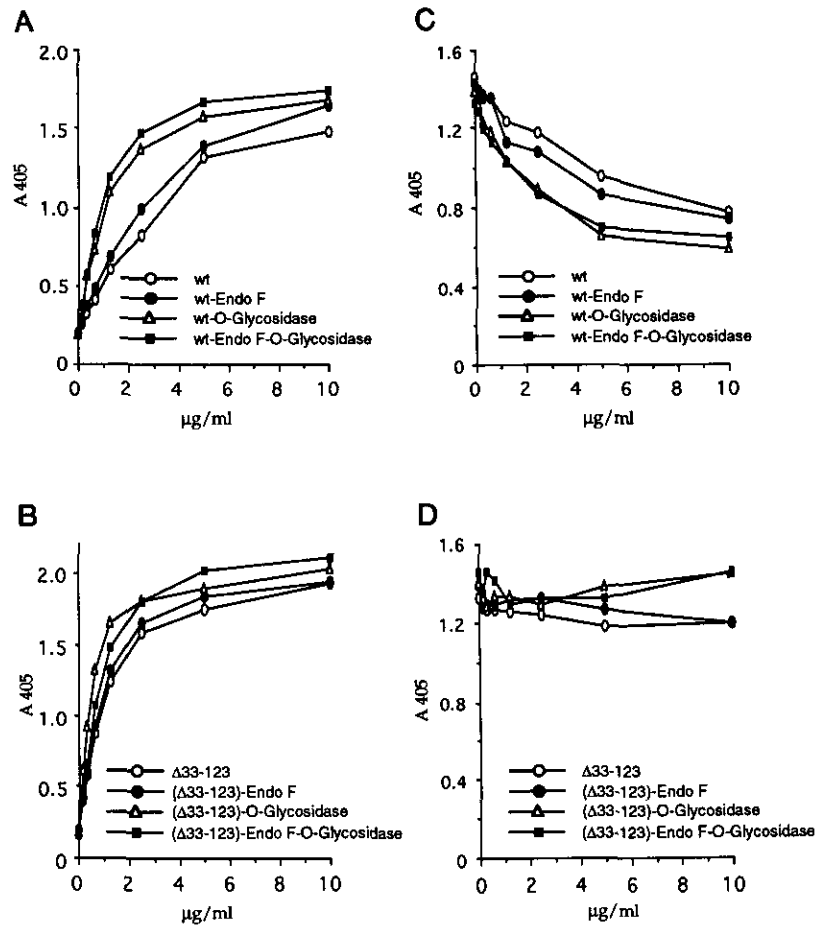


Fig. 10. Effect of glycosidase and neuraminidase treatment on binding of gC to C3 and blocking of properdin binding to C3. gC was treated with one or a combination of the endoglycosidases, and varying dilutions (0–10 µg/ml) were incubated with C3-coated wells of ELISA plates. (A) and (C), wells were incubated with polyclonal antibody against gC-1 (R47) followed by protein A conjugated with horseradish peroxidase (PrA-HRP) and ABTS substrate. (B) and (D), varying dilutions of endoglycosidase-treated or untreated gC (0–10 µg/ml) were preincubated with C3-coated wells before addition of normal human serum at a $\frac{1}{160}$ dilution. The bound properdin was detected as described in the legend to Fig. 8.

and reduces the efficiency of complement-mediated lysis, whereas purified gC-2 appears to stabilize the C3 convertase and has no effect on complement-mediated lysis (Eisenberg *et al.*, 1987; Fries *et al.*, 1986). gC-1 and gC-2 are both able to bind C3b and the regions which are involved in this binding are colinear and highly conserved in the two proteins. Amino acids 33 to 123 of gC-1 and amino acids 26 to 73 of gC-2 (Seidel-Dugan *et al.*, 1988) are not involved in C3b binding. It is noteworthy that these residues are the least conserved in the two proteins (Frink *et al.*, 1983; Swain *et al.*, 1985). It was of interest to determine whether the differing effects of gC-1 and gC-2 on the complement pathway are related to structural differences which are outside of the C3b binding regions of these herpes proteins. Properdin is a regulatory glycoprotein of the alternative pathway of the complement cascade of immune defense. Unlike most of the regulatory control proteins, properdin has an enhancing effect on activation of the complement cascade. Proper-

din is able to bind C3b and stabilize the alternative C3 convertase, C3bBb (Fearon and Austen, 1975; Lambris, 1988). Thus, we examined the effect of gC-1 and gC-2 and mutant forms of gC-1 on the ability of properdin to bind to C3 in an ELISA assay. We also examined whether gC-1 was able to interfere with the formation of the convertase.

To obtain sufficient quantities of each form of gC-1, we recombined the genes for several forms of gC-1 into the HSV-1 gC-null virus, gC⁻³⁹ (derived from KOS 321) (Homa *et al.*, 1986). We were unable to plaque purify the double deletion mutant of gC-1 (vSH218) and did not obtain sufficient quantities of protein for further study. However, the virus recombinant vSH216, which lacked amino acids 33 to 123 and vSH217, which lacked amino acids 367 to 449, were plaque purified. Each of the proteins was shown to be incorporated into virions, to be expressed on the surface of infected cells, and to bind C3b, both on the infected cell surface and in ELISA

assays. These results confirmed that amino acids 33 to 123 and 367 to 449 are not required for C3b binding by gC-1 (Hung *et al.*, 1992). However, using more quantitative means in this study, we found that residues 367 to 449 contribute to some extent to C3b binding. Interestingly, MAb 1C8 in the presence of complement was able to neutralize the infectivity of vSH216 to a much greater extent (16-fold) than vSH214, suggesting that amino acids 33 to 123 may protect the virus from complement-dependent antibody neutralization.

We found that gC-1 does not inhibit formation of the alternative C3 convertase. The convertase consists of C3b plus a cleavage product of factor B, called Bb. We showed that gC-1 was unable to inhibit cleavage of iodinated factor B in the presence of factor D and therefore should have no effect on convertase assembly. In contrast, gC-1, but not gC-2, partially blocked the binding of properdin to C3 fixed to an ELISA plate, and it is possible that a similar blocking effect of properdin binding to C3b on cell surfaces would interfere with stabilization of the alternative pathway C3 convertase (Fearon and Austen, 1975; Lambris, 1988). The results correlate with previous observations about the effect of gC-1 and gC-2 on the alternative C3 convertase (Eisenberg *et al.*, 1987; Fries *et al.*, 1986). However, it should be noted that properdin was absent in those *in vitro* assays (Eisenberg *et al.*, 1987; Fries *et al.*, 1986). It will be of interest to determine the stability of the cell surface bound alternative C3 convertase activity in the presence of both properdin and gC.

To determine whether the differing effects of gC-1 and gC-2 on the complement pathway are related to structural differences which are separate from the C3b binding regions, we tested the ability of proteins deleted for different parts of gC-1 to block the interaction of properdin with C3. The results indicate that although the amino terminus is not needed for gC-1 to bind C3b, these residues, when present, interfere with the binding of properdin to C3b. Thus gC-1(Δ 33–123) was unable to block properdin binding to C3b. In contrast, gC-1(Δ 367–449), though it did not bind to C3b as well as wild-type gC-1, was able to block properdin binding when used at high concentrations. There are at least three ways to explain these observations. First, the amino terminus of gC-1 may interfere by direct protein–protein interaction with the properdin molecule. Second, this region of gC could bind to the same site on C3 as properdin and thereby block its binding. A third possibility is that the binding of wild-type gC-1 to C3b alters the conformational structure of the properdin binding site on C3b, affecting the affinity of binding of properdin. In each case, the action requires the amino terminus of gC-1.

Although the N- and O-CHO of gC-1 may contribute to its structure, they do not appear to contribute to blocking of properdin binding, since their removal with glycosi-

dases or neuraminidase did not alter the properdin blocking effect. Thus, it appears that properdin blocking is associated with the polypeptide portion of the amino terminus of gC-1. Using ELISA assays, we tried to bind gC directly to properdin and found no evidence for a high affinity interaction between these two proteins. It was shown that properdin binds to residues 1402 to 1435 of C3b and a synthetic peptide corresponding to this sequence completely inhibited properdin binding to C3b (Daoudaki *et al.*, 1988). Since the binding site for gC on C3b is not known, it will be of interest to test whether this peptide blocks the binding of gC to C3b. However, the blocking effect of gC-1 on properdin binding was not complete, suggesting that it is unlikely that gC-1 binds to precisely the same site on C3b as does properdin. This is supported by the observation that gC-1 binds to cobra venom C3, while properdin does not (Kostavasili *et al.*, 1993). Likewise, properdin binds to bovine C3, but gC-1 does not. Thus, it is more likely that binding of gC-1 to C3b induces a conformational change that reduces the ability of properdin to bind. Since human serum was used as a source of properdin, it is also possible that the amino terminus of gC-1 interacts with some other factors in serum and this complex in turn blocks properdin binding. However, we did see a similar blocking effect when purified properdin was used (data not shown).

What is it about the structure of the N-terminus that enables gC-1 to block properdin binding to C3b? It has been suggested that this region of gC has characteristics which resemble those of mucins (Strous and Dekker, 1992), such as clustered N- and O-linked oligosaccharides and sialic acid, as well as a high percentage of serine, threonine, proline, glycine, and alanine (Dall'Olio *et al.*, 1985; Olofsson, 1992). In mucins these amino acids are often in tandem repeats and all of these characteristics lead to an extended hydrophilic rod-like structure (Jentoft, 1990). It is of interest that electron microscopy studies of HSV virions suggested that gC-1 assumes an extended conformation in the virion envelope (Stannard *et al.*, 1987). All of the gC homologs that have been sequenced have an N-terminus with structural features similar to HSV gC, though the actual sequences within this region are widely divergent (Spear, 1993).

Although gC-1 and gC-2 may bind to a similar site on C3b (Seidel-Dugan *et al.*, 1990), the binding can still have very different effects on the activity of alternative C3 convertase (Fries *et al.*, 1986) and on properdin binding as shown in this study. A similar situation exists with three of the complement regulatory proteins which bind C3b (Fishelson, 1991). Factor H is able to block the binding of factor B and CR1 to C3b, suggesting that these molecules interact with the same site on C3b (Pangburn, 1986). However, they have different effects after binding to C3b. For example, binding of factor B to C3b results in amplification of the complement cascade, whereas binding of

factor H or CR1 results in the inactivation of C3b (Reid and Porter, 1981). A common binding site for factor H and CR1 has been localized to the amino terminal forty amino acids of the alpha chain of C3b (Becherer *et al.*, 1989, 1992; Becherer and Lambris, 1988). This site may also bind factor B (Ganu and Müller-Eberhard, 1985; Taniguchi-Sidle and Isenman, 1993). However, these three molecules also bind to additional distinct sites on C3b. It is therefore of some interest to map the binding sites on C3b for gC-1 and gC-2 and to compare these to the molecules of the complement cascade.

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