Mechanism of Complement Inactivation by Glycoprotein C of Herpes Simplex Virus[1]

Ioanna Kostavasili,[2*] Arvind Sahu,[2*] Harvey M. Friedman,[†] Roselyn J. Eisenberg,[‡] Gary H. Cohen,[§] and John D. Lambris[3*]

Glycoprotein C (gC) of both herpes simplex virus type 1 (HSV-1) and HSV-2 interacts with complement C3b and protects the virus from complement-mediated neutralization. To study the mechanism by which gC modulates complement activation, we expressed both gC-1 and gC-2 in a baculovirus expression system. Baculovirus recombinants containing gC genes spanning the entire gC-1 sequence (gC-1-TMR) or only the extracellular domain(s) of gC-1, gC-2, or a deletion mutant of gC-1 lacking residues 33 through 123 were expressed in sf9 insect cells. Binding of the expressed proteins to human C3 and C3 fragments was assessed by direct and competition ELISA. All four expressed proteins bound to C3, C3b, and C3c but not to C3d, suggesting 1) that the binding sites for these proteins are located in the C3c region of C3; and 2) that gC, in contrast to other C3-binding proteins, interacts with native C3. We have also examined the interaction of native C3 with gC-1 expressed on the HSV-1-infected cells. Analogous to recombinant proteins, gC-1 expressed on the infected cells also bound to native C3. The ability of baculovirus-expressed gCs to inhibit the interaction of C3b with its ligands was also analyzed. We found that gC-1, but not gC-2, inhibited the binding of C5 and properdin to C3b and also inhibited the alternative pathway-mediated lysis of rabbit erythrocytes. Inhibition of alternative pathway-mediated lysis and properdin binding to C3b, but not of C5 binding to C3b, required the transmembrane segment of the gC-1. The specificity of gC interactions was examined by studying the interaction of gC with C3 from various species. In contrast to properdin, both gCs bound to cobra C3; this finding suggests that gC-1 and properdin bind to different sites on C3b. Further analyses suggested that gC-1 sterically hindered access of C5 and properdin to C3b. ***The Journal of Immunology,*** **1997, 158: 1763–1771.**

The complement system serves as both an innate and an acquired defense against viral infection (1). Activation of complement can lead to virus neutralization (2) and phagocytosis of C3b-coated viral particles (3). To circumvent these defenses, viruses not only have developed mechanisms to control complement, but have also turned these interactions to their own advantage (4). Important examples of viral defense proteins are glycoprotein C (gC)[4] of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), which are expressed on the virion envelope and on the surface of infected cells (5). Studies have clearly indicated that gCs regulate complement and provide protection against complement-mediated viral neutralization (6, 7).

The gC of HSV-1 (gC-1) and HSV-2 (gC-2) are highly glycosylated proteins with numerous *O*-linked oligosaccharides and nine and seven potential sites for *N*-linked glycosylation, respectively (8–10). Both proteins bind to C3b when they are expressed on the surface of transfected cells (11, 12) or in the form of purified proteins (13, 14). As purified proteins, they also bind to the proteolytically inactivated fragment of C3, iC3b (15). Previous reports have suggested that gC-1, like other regulators of complement activation (factor H, CR1, and DAF), accelerates the decay of a bimolecular C3 convertase (C3b,Bb) into its subunits; however, in contrast to factor H, and CR1, it does not mediate the proteolytic inactivation of C3b by factor I (16). gC-2, on the other hand, seems to stabilize the C3 convertase (13).

Recently, we have found that gC-1, but not gC-2, can destabilize C3 convertase by inhibiting the binding of properdin to C3b (17). Comparison of gC-1 with human C3 receptors CR1 and CR3 revealed that, unlike CR3, C3b binding to gC does not depend on the presence of divalent cations. Furthermore, a mAb recognizing CR3 failed to inhibit C3b binding to gC-1, whereas an anti-CR1 mAb inhibited the binding significantly. Thus, gC-1 was thought to be an analogue of CR1 (18).

The binding sites for C3b and the regions required to block properdin binding to C3b have been identified on gC-1 (19). By the use of linker insertion and deletion mutants, the areas involved in C3b binding have been localized to four distinct regions in the central portion of gC-1. Three analogous regions have also been identified on gC-2 as C3b binding sites (12). A similar series of constructed mutants has been used to identify the residues of gC-1 that interfere with the binding of properdin to C3b (17). Binding studies have shown that the N-terminal region is necessary for inhibition of properdin binding to C3b; this region is also implicated in virus attachment to cell surface proteoglycans (14, 20).

*Laboratory of Protein Chemistry, Department of Pathology and Laboratory Medicine, †Department of Medicine, School of Medicine, ‡Department of Microbiology, School of Veterinary Medicine, and §Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104

Received for publication July 15, 1996. Accepted for publication November 8, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Science Foundation Grant MCB93-19111, National Institutes of Health Grants AI30040 and HL28220, and Cancer and Diabetes Centers Core Support Grants CA16520 and DK19525.

[2] I.K. and A.S. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. John D. Lambris, Laboratory of Protein Chemistry, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104-6079.

[4] Abbreviations used in this paper: gC, glycoprotein C; HSV, herpes simplex virus type 1; C3, the third component of complement; C3b, the proteolytically activated form of C3; C3(H_2O), hydrolyzed C3; iC3, immobilized C3; C3c, the 137,300 M_r fragment of C3 generated by trypsin digestion; C3d, the 35,000 M_r fragment of C3 generated by trypsin digestion; CR1, complement receptor type 1; ABTS, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); DAF, decay-accelerating factor; MCP, membrane cofactor protein.

Copyright © 1997 by The American Association of Immunologists

0022-1767/97/$02.00

From these studies it is clear that both gC-1 and gC-2 modulate the complement system and protect the two viruses from complement attack. In the present study we asked whether both proteins inhibit complement through a common mechanism. Our results suggest that unlike other regulators of complement activation that interact with C3b (factor H, MCP, DAF, and CR1), both gC-1 and gC-2 bind to native C3. Furthermore, we present data indicating that the binding sites for gC-1 and gC-2 are located in the C3c region of C3 and that the C3 region spanning residues 727 through 768, which are implicated in the binding of CR1 to C3b, are not important for the gC-C3 interaction. Thus, gC-1 does not act as an analogue of CR1. We propose that gC-1 inhibits complement activation by inhibiting the interaction of properdin and C5 with C3.

Materials and Methods

Cloning, expression, and purification of recombinant gCs

Recombinant proteins gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), and gC-2(426t) (see Fig. 1) were synthesized using the baculovirus expression system. The construction, expression, and purification of the recombinant proteins were described in detail previously (14). Plasmids pCP250, pCP247, pCP248, and pCP249, containing gC genes encoding gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), and gC-2(426t), respectively, were each recombined in baculovirus (*Autographa californica* nuclear polyhedrosis virus) by using Baculogold-linearized wild-type viral DNA (PharMingen, San Diego, CA). Recombinant viruses were purified and used to infect Sf9 cells. As a potential aid in purification, all the recombinant proteins except gC-1(TMR) contained a histidine tag at the C-terminal. gC-1(TMR), gC-1(457t), and gC-1(Δ33–123t) were purified from culture supernatants of baculovirus-infected cells by immunoaffinity chromatography (13); gC-2(426t) was purified on a heparin column (Pharmacia, Piscataway, NJ) (14). The purified proteins were analyzed by SDS-PAGE and Western blotting. With the exception of gC-1(TMR), all the other expressed proteins yielded 10 to 15 mg of purified protein/l of infected cell medium. The m.w. of the recombinant proteins were determined by matrix-assisted laser desorption spectrophotometry (VG Tofspec, Fisons Pharmaceuticals Ltd., Manchester, U.K.) (21, 22).

Complement proteins and reagents

Human C3 (23, 24) and factor H (25) were purified from plasma as previously described. C5 was kindly provided by Dr. T. Kinoshita (Department of Immunoregulation, University of Osaka, Osaka, Japan) and was iodinated using ^{125}I and Iodogen (Pierce Chemical Co., Rockford, IL) to a sp. act. of 1.8×10^6 cpm/μg. C3b was generated from C3 by digestion with 1% trypsin (*N*-α-*p*-tosyl-L-lysine chloromethyl ketone-treated, Sigma Chemical Co., St. Louis, MO) and purified on a Mono Q column (Pharmacia, Piscataway, NJ) (26). C3c and C3d were generated from C3 by digestion with 5% trypsin and purified on a Mono Q column. To remove the small amount of C3c contamination detected, C3d was further purified by passing through a Superose 12 column (Pharmacia). To separate native C3 from C3(H_2O) and the stable conformational intermediate form of C3, the C3 sample was loaded onto a Mono S column and eluted with a linear salt gradient (27). Bovine C3 ($C3_{Bo}$) and cobra C3 ($C3_{Cv}$) were purified as previously described (24). Construction and purification of wild-type human C3 ($wtC3_{Hu}$) and truncated human C3 with residues 727 to 768 deleted ($C3\Delta727–768_{Hu}$) were described previously (28).

Rabbit blood was purchased from Cocalico Biologicals (Reamstown, PA), and $Na^{125}I$ was obtained from DuPont-New England Nuclear Research Products (Boston, MA). Zymosan-C3b/iC3b was prepared by incubating zymosan with normal human serum in the presence of 5 mM MgEGTA as previously described (24). Control (zymosan-EDTA) samples were incubated with normal human serum in the presence of 10 mM EDTA.

Buffers used were GVB (5 mM barbital, 145 mM NaCl, and 0.1% gelatin, pH 7.4), DGVB (2.5 mM barbital, 73 mM NaCl, and 5% dextrose, pH 7.4), GVBE (GVB with 10 mM EDTA), MgEGTA (0.1 M $MgCl_2$ and 0.1 M EGTA), PBS (10 mM phosphate buffer and 145 mM NaCl, pH 7.4), and PBS-gelatin (PBS containing 0.1% gelatin).

ELISA for measurement of gC binding to C3 and C3 fragments

Binding of different gC molecules to C3 and C3 fragments (C3b, C3c, and C3d) was assessed by ELISA; C3 or C3 fragments (50 μl at 2 μg/ml) were adsorbed to Maxisorp plates (Nunc, Inc., Naperville, IL) for 1 h at 37°C, and plate was saturated with PBS, pH 7.4, containing 2% milk for 30 min at 22°C. The amount of C3 or C3 fragments bound to the plate was determined using mAbs recognizing the C3c and C3d regions of C3 (133-H11 and anti-C3–15, respectively) (29, 30). For ELISA assays, gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), or gC-2(426t) was serially diluted and added to the plate, then incubated for 1 h at 22°C. Bound gC was detected with a 1/200 dilution of rabbit anti-gC Ab (R47) (13), followed by a 1/1000 dilution of peroxidase-conjugated goat anti-rabbit-IgG Ab (Bio-Rad, Hercules, CA). 2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was used as substrate, and the OD was measured at 405 nm. All Abs and gCs were diluted in PBS, pH 7.4, containing 2% milk. The volume of each incubation was 50 μl/well, except for the saturation step (100 μl/well). After each incubation the plates were washed three times with PBS, pH 7.4, containing 0.05% Tween-20.

Competition ELISA for measurement of gC binding to C3 and C3 fragments

The ability of fluid phase C3 and C3 fragments to inhibit the binding of gC-1(457t) or gC-2(426t) to C3 was also assessed by ELISA. Microtiter plates were coated with C3 as described above (C3 at 2 μg/ml). Serial dilutions of C3 or C3 fragments (25 μl/well) and a constant amount of gC-1(457t) or gC-2(426t) (25 μl/well at 5 μg/ml) were then added to the plate and incubated for 1 h at 22°C. The amount of gC bound was determined using a polyclonal rabbit anti-gC Ab and peroxidase-conjugated second Ab as described above.

Flow cytometry for measurement of C3 binding to gC-1 expressed on infected cells

Binding of native C3 and C3b to virally infected cells was determined by flow cytometry. Vero cells were infected with HSV-1 (wild-type) or NS-gC_{null} virus (gC-1 DNA was replaced with ICP6::*lacZ*) (7) at a multiplicity of infection of 3 for 18 to 24 h as previously described (31). Infected cells (1×10^6) were mixed with 3 μg of native C3 or C3b in a total volume of 20 μl and incubated for 1 h on ice. The cells were centrifuged, mixed with 40 μl of 1/50 diluted FITC-conjugated $F(ab')_2$ anti-C3 goat IgG (Cappel Laboratories, Warrington, PA) and further incubated on ice for 1 h. After incubation, cells were washed with 2 ml of PBS-gelatin, resuspended in 1 ml of the same buffer, and analyzed for C3/C3b binding on a FACS Caliber (Becton Dickinson, Mountain View, CA). Results are expressed as mean channel fluorescence of 10,000 cells.

Measurement of inhibition of properdin binding to C3 by gC

Binding of properdin to C3 was determined by ELISA as previously described (32). Microtiter wells were coated with 50 μl of C3 (at 8 μg/ml) as outlined above. After coating, the wells were saturated with PBS containing 2% milk and incubated for 1 h at 22°C with 25 μl of serially diluted gC and 25 μl of a 1/5 dilution of normal human serum with 20 mM EDTA (as a source of properdin). The amount of properdin bound to C3 was quantitated by adding 50 μl of monoclonal anti-properdin Ab (2 μg/ml) followed by 50 μl of peroxidase-conjugated anti-mouse Ab. Color was developed and was measured as described above.

Measurement of inhibition of ^{125}I-labeled C5 binding to C3b by gC

The ability of gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), and gC-2(426t) to inhibit the binding of ^{125}I-labeled C5 to zymosan-C3b/iC3b was assessed as follows: 50 μl of zymosan-C3b/iC3b (at 3 mg/ml in DGVB) was allowed to react with serial dilutions of gC at 22°C for 30 min. Twenty microliters of ^{125}I-labeled C5 (0.25×10^6 cpm) was then added, the volume was adjusted to 120 μl, and the mixture was allowed to incubate at 22°C for 60 min. Thereafter, 50 μl of each sample was centrifuged through 20% sucrose in DGVB to separate bound from free ^{125}I-labeled C5, and binding of the labeled C5 was measured. The data obtained were normalized by considering 100% ^{125}I-labeled C5 bound to be equal to the amount bound in the absence of gC. Nonspecific binding was measured by incubating ^{125}I-labeled C5 with zymosan-EDTA (zymosan lacking C3b).

Hemolytic assay

To determine the effect of gCs on the alternative pathway of complement, 10 μl of rabbit erythrocytes (1×10^9/ml in GVB) were mixed with 5 μl of normal human serum and 5 μl of MgEGTA (0.1 M) in the presence of various concentrations of gC-1(457t), gC-2(426t), gC-1(TMR), or factor H. The volume was adjusted to 100 μl with GVB, and the reaction mixture was incubated at 37°C for 20 min. The reaction was then stopped by adding 200 μl of GVBE. After centrifugation, the absorbance of 200 μl of the

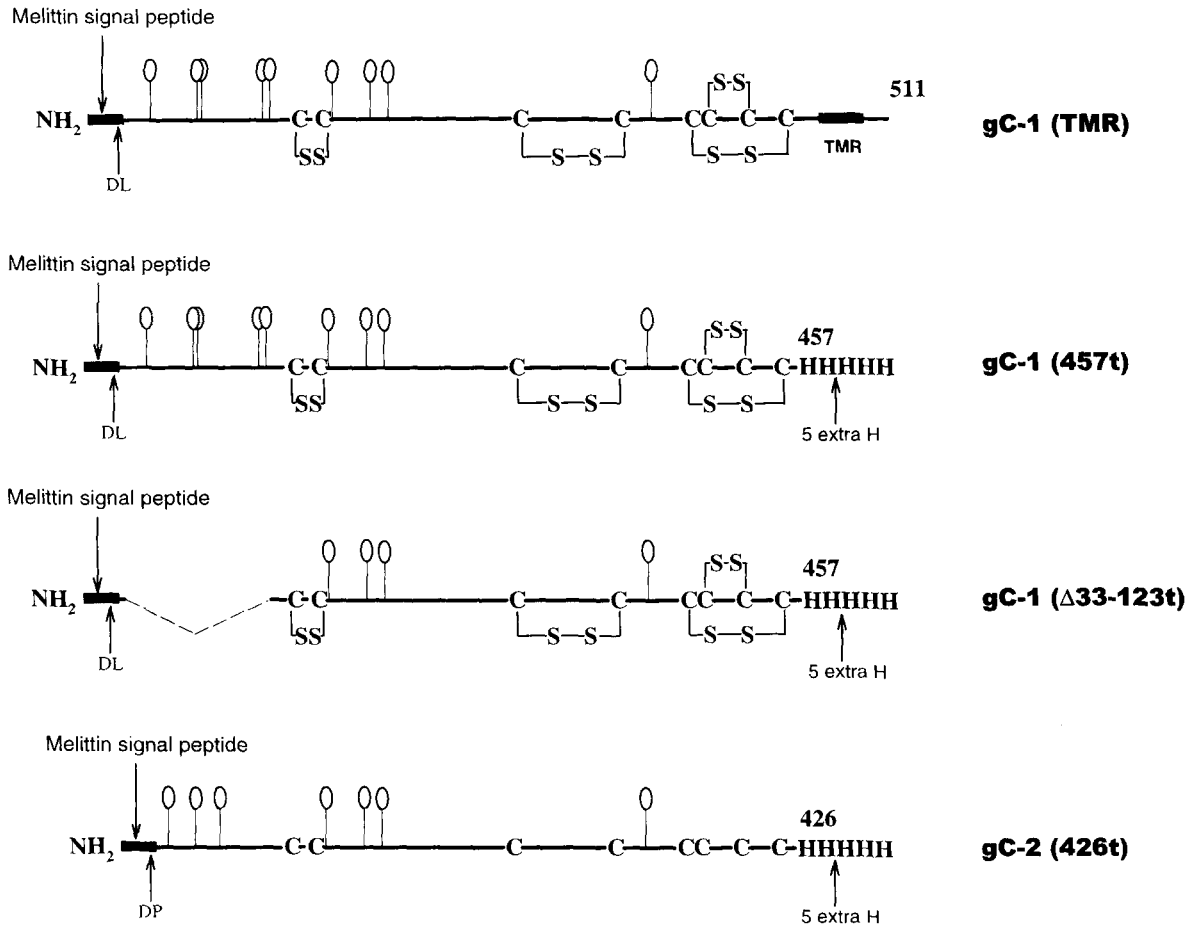

FIGURE 1. Schematic models of recombinant gC molecules used in this study. Various constructs were subcloned into the baculovirus vector pVT-Bac and recombined into baculovirus as described (see *Materials and Methods*). In all constructs the N-terminal signal peptide was replaced with the melittin signal peptide, cleavage of which leaves two additional amino acids at the N-terminus: aspartic acid (D) and leucine (L) in gC-1, and aspartic acid and proline (P) in gC-2. Five extra histidine (H) residues were added at the C-terminus of truncated proteins to aid purification. Both gC-1 and gC-2 contain eight conserved cysteine (C) residues that form four disulfide bonds (22). The predicted N-linked carbohydrate sites are indicated with balloons. TMR, transmembrane region.

supernatant of each sample was determined at 405 nm. The percentage of lysis was normalized by considering 100% lysis equal to the lysis occurring in the absence of the gC.

Measurement of C3 convertase-mediated cleavage of C3

The effect of gCs on C3 cleavage by C3 convertase (C3b,Bb) was examined by incubating 2 μg of C3 with 5 μg of gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), gC-2(426t), or factor H in GVB at 37°C for 15 min. Thereafter, 2 μg of factor B and 0.04 μg of factor D were added in the presence of 5 mM MgEGTA, in a total volume of 20 μl to activate the pathway. After 2 h at 37°C, the reaction mixtures were mixed with sample buffer containing 2-ME, boiled for 3 min, and electrophoresed on a 7.5% SDS-PAGE gel. Proteins were visualized by staining gel with Coomassie blue. The gel was scanned for densitometric analysis, and the percentage of C3 cleaved was calculated. Controls were incubated in GVBE (GVB with 10 mM EDTA) to inhibit cleavage.

Results

Localization of the gC binding site on C3

In this study we have used direct and competitive ELISAs to sublocalize the binding sites of gC-1 and gC-2 on C3. These experiments involved four baculovirus recombinant forms of gC, including gC-1 containing its transmembrane domain (gC-1-TMR) and three truncated proteins lacking the TMR, gC-1(457t), gC-1(Δ33–123t), and gC-2(426t) (Fig. 1). GC-1(Δ33–123t) was examined because it had previously been shown that residues 33 through 123 of gC-1 are not involved in C3b binding (19). For our assays, C3 and C3 fragments (C3b, C3c, and C3d) were individually bound to ELISA plates. The binding of each fragment was confirmed by use of specific mAb (data not shown). Our results showed that all four gCs bound to immobilized C3, C3b, and C3c, but not to C3d or BSA (control; Fig. 2).

To demonstrate that the binding was specific, a competition ELISA was performed (Fig. 3). Binding of gC-1(457t) and gC-2(426t) to immobilized C3 was inhibited by the addition of increasing concentrations of C3 and C3 fragments. As expected, C3b and C3c, but not C3d, inhibited the binding in a concentration-dependent manner. It was surprising, however, that the soluble C3 also inhibited the binding of gC-1(457t) and gC-2(426t) to immobilize C3 (Fig. 3), indicating that gC can bind to native C3. This inhibition could have been due to the presence of $C3(H_2O)$, since this C3 preparation was a mixture of C3 (72%) and $C3(H_2O)$ (28%). However, additional experiments (described below) verified that this inhibition was indeed due to the binding of gC-1 and gC-2 to native C3.

gC-1 was previously suggested to be an analogue of CR1 (18). Since residues 727 through 768 of C3 are implicated in the binding of CR1 to C3, we sought to determine whether these residues are important for gC-C3 interactions. For this purpose recombinant wild-type C3 ($wtC3_{Hu}$) or truncated C3 ($C3\Delta727–768_{Hu}$) was

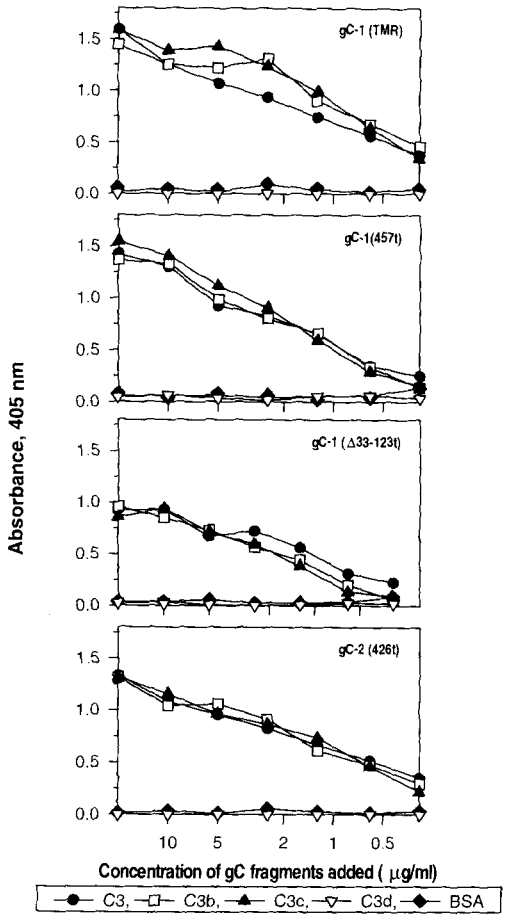

FIGURE 2. Binding of gC proteins to C3 and C3 fragments. An ELISA plate was coated with purified C3 and C3 fragments, saturated with 2% milk, washed with PBS, pH 7.4, containing 0.05% Tween-20 and incubated with twofold dilutions of one of the purified recombinant gC proteins. The amount of each gC bound was determined using a polyclonal anti-gC Ab (1/200 dilution), peroxidase-coupled anti-rabbit Ab (1/1000 dilution), and ABTS peroxidase substrate.

fixed to an ELISA plate, and serial dilutions of gC-1(457t) or gC-2(426t) were added. The results showed that gC-1 and gC-2 bound to both wild-type and truncated C3, suggesting that the deleted residues (727–768) are not involved in the binding of gCs to C3 (Table I).

Do gCs bind to native C3?

To determine whether gC-1 and gC-2 do indeed bind to native C3 we used freshly purified C3 that had been chromatographed on a Mono S column (Pharmacia) to remove any small amount of C3 that might have been hydrolyzed during the purification procedure; this method allowed us to separate native C3 from C3(H_2O) (27). C3(H_2O) was purified from a C3 sample that was subjected to repeated cycles of freezing and thawing. When we compared the affinity of gC-1(457t) for native C3 and C3(H_2O) in an ELISA, we observed that both inhibited the binding of gC-1(457t) to immobilized C3 (Fig. 4*A*). Although the inhibition by native C3 was eightfold less efficient than that of C3(H_2O). Similar results were obtained for gC-2(426t) (data not shown). These results indicate that gCs bind to native C3. However, it is possible that C3 could have been hydrolyzed during its incubation in the wells of the ELISA plate. To rule out this possibility, we tested the ability of native C3 and C3(H_2O) to inhibit the binding of properdin to immobilized C3 (iC3) under similar conditions. As expected, C3(H_2O) inhibited the binding of properdin to iC3, but native C3 did not (Fig. 4*B*). Furthermore, when C3 incubated in an ELISA plate was rerun on a Mono S column, no detectable amount of C3(H_2O) was observed. These findings suggest that purified gC-1 and gC-2 do indeed bind to native C3.

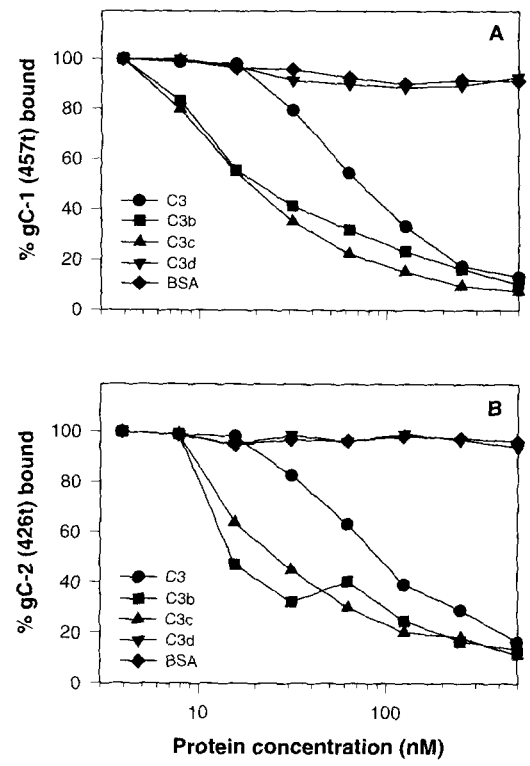

FIGURE 3. *Inhibition of gC-1(457t) and gC-2(426t) binding to immobilized C3 by various concentrations of C3 and C3 fragments. C3-coated wells were incubated with either purified gC-1(457t) or gC-2(426t) and increasing concentrations of C3, C3 fragments (C3b, C3c, or C3d), or BSA (control). Bound gC proteins were detected by a polyclonal anti-gC Ab, peroxidase-coupled anti-rabbit Ab, and ABTS peroxidase substrate. The data were normalized by setting the value for 100% bound equal to that obtained for the binding of gC in the absence of a competitor.*

Table I. *Binding of gC-1 and gC-2 to various C3 molecules*

	C3 Molecule[a]				
	$C3_{Hu}$	$C3_{Bo}$	$C3_{Co}$	$wtC3_{Hu}$	$C3\Delta727–768_{Hu}$
gC-1 (457t)	+	−	+	+	+
gC-2 (426t)	+	−	+	+	+
Properdin	+	+	−	+	+

[a] $C3_{Hu}$ indicates C3 from human serum; $C3_{Bo}$, C3 from bovine serum; $C3_{Co}$, C3 from cobra serum; $wtC3_{Hu}$, wild-type baculovirus-expressed human C3; $C3\Delta727–768_{Hu}$, baculovirus-expressed human C3 with residues 727–768 deleted.

The above experiments suggest that gC-1 and gC-2, when present in solution, bind to native C3. gC are intrinsically acting membrane proteins; therefore, it was important to determine whether gC present on virally infected cells bind to native C3. There is a possibility that gC, when expressed as a membrane protein, may not bind to native C3 due to intrinsic protection. To exclude this contingency, we assessed the direct binding of

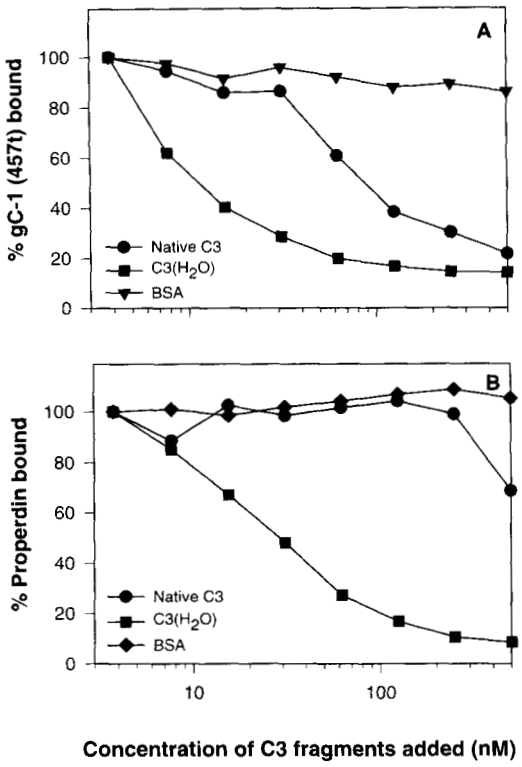

FIGURE 4. Inhibition of gC-1(457t) (*A*) and properdin (*B*) binding to immobilized C3 by various concentrations of native C3 and C3(H_2O). A microtiter plate was coated with C3 (2 μg/ml), saturated with 2% milk, washed, and incubated with gC-1(457t) or with normal human serum containing 10 mM EDTA (as a source of properdin). Inhibition of binding was assessed by adding various concentrations of purified native C3 or C3(H_2O).

native C3 and C3b to infected cells by flow cytometry. As depicted in Figure 5, both native C3 and C3b bound to HSV-1-infected cells. Control cells infected with NS-gC_{null} virus showed no binding to either protein. These findings clearly indicate that unlike any other C3 binding protein, gC bind to native C3. It is apparent that the binding of native C3 is 6.5-fold less efficient than that of C3b; these results are similar to those found for fluid phase binding (Fig. 4*A*).

Inhibition of properdin binding to C3 by gCs

Using protein purified from cytoplasmic extracts of infected BHK cells, we have previously shown that gC-1, but not gC-2, inhibits the binding of properdin to iC3 (17). Furthermore, we have found that residues 33 through 123 of gC-1 are associated with blocking of properdin binding to C3b. To confirm our previous findings we used the recombinant gCs to inhibit properdin binding to iC3. gC-1(TMR) inhibited properdin binding with an IC_{50} of 0.5 μM. However, the three truncated proteins had no effect on properdin binding to C3b (data not shown).

Does gC compete with properdin for the same binding site on C3? It is known that properdin interacts differently with C3 from different species. We took advantage of this variability to compare the interaction of gC and properdin with C3 from various species. Human C3 (2 mg/ml) and C3 from cobra and cattle (4 mg/ml) were allowed to bind to microtiter plates, then were exposed to serial dilutions of gC-1(457t), gC-2(426t), or normal human serum (a source of properdin). Binding of gCs and properdin was determined by using polyclonal Abs recognizing the respective proteins. Both gC-1(457t) and gC-2(426t) bound to cobra C3; however, no binding was detected to bovine C3 (Table I). In contrast, properdin bound to bovine C3 but failed to bind to cobra C3, suggesting that gC-1 and properdin bind to different sites on C3.

Inhibition of ^{125}I-labeled C5 binding to C3 by gC

The interaction of gC with C3b may interfere with the binding of C3b to other known ligands. To examine this possibility we used a particle-based binding assay. This assay measures the competition between a fluid phase inhibitor and ^{125}I-labeled C5 for binding to C3b-coated zymosan particles (zym-C3b/iC3b). Zym-C3b/iC3b were prepared by incubating zymosan particles with normal human serum, which results in efficient activation of complement. As a control, particles were incubated with serum in the presence of EDTA (zym-EDTA). gC-1(TMR) and gC-1(457t) inhibited the binding of ^{125}I-labeled C5 to zym-C3b/iC3b with IC_{50} of 40 and 80 nM, respectively (Fig. 6). In contrast, neither gC lacking residues 33 through 123 nor gC-2(426t) inhibited the binding of [^{125}I]C5. These results suggest that residues 33 through 123 of gC-1 or carbohydrates associated with this region of the molecule are able to interfere with the binding of C5 to C3b.

Inhibition of alternative pathway of complement activation by gCs

We also examined the effects of gC-1(TMR), gC-1(457t), and gC-2(426t) on complement activation. Inhibition of lysis of rabbit erythrocytes in the presence of MgEGTA was used as an indicator of inhibition of the alternative pathway (Fig. 7). For comparison purposes, the inhibition capacity of factor H was also tested. gC-1(TMR) inhibited erythrocyte lysis with an IC_{50} of 2 μM; factor H had an IC_{50} of 0.3 μM. The truncated form of gC-1 (gC-1–457t), however, did not inhibit the alternative pathway, suggesting that a transmembrane domain could be involved in generating proper structural changes (e.g., formation of the gC-1 dimers) needed to inhibit complement activity. It is important to add here that gC-1 exists as a dimer on the virion (33) and on the plasma membranes of infected cells (34).

Effect of gC on C3 convertase-mediated cleavage of C3

Binding of gC to native C3 could result in inhibition of C3 cleavage and thus of complement activation (Fig. 8). We therefore examined the interaction of gCs with C3 in a fluid phase assay in which purified gCs (*lanes 3–6*) were incubated together with C3, factor B, and factor D in the presence of MgEGTA so as to allow C3 convertase-mediated cleavage of C3. None of the gC proteins showed inhibition of C3 or factor B cleavage, suggesting that gC have no effect on C3 activation or on the formation of C3b,Bb (Fig. 8). Factor H (*lane 7*), which was used as a control, completely inhibited C3 cleavage at a similar concentration.

Discussion

Previous studies have demonstrated that gC-1 and gC-2 bind to C3b and provide protection against complement-mediated neutralization (6, 7). gC-1 has been shown to accelerate the decay of alternative pathway C3 convertase and inhibit properdin binding to C3, whereas gC-2 stabilizes C3 convertase and does not inhibit properdin binding (Table II) (16, 17). Therefore, the mechanism of complement inhibition by gC-2 has not yet been identified. In our current study using recombinant proteins (Fig. 1), we demonstrate that unlike all other proteins that are known to regulate complement, gC-1 and gC-2 bind to native C3. The proteins used in this study were expressed in the baculovirus system, which allowed us to obtain large quantities of protein for conducting multiple assays. Four distinct regions of gC-1 and three distinct regions of gC-2 are

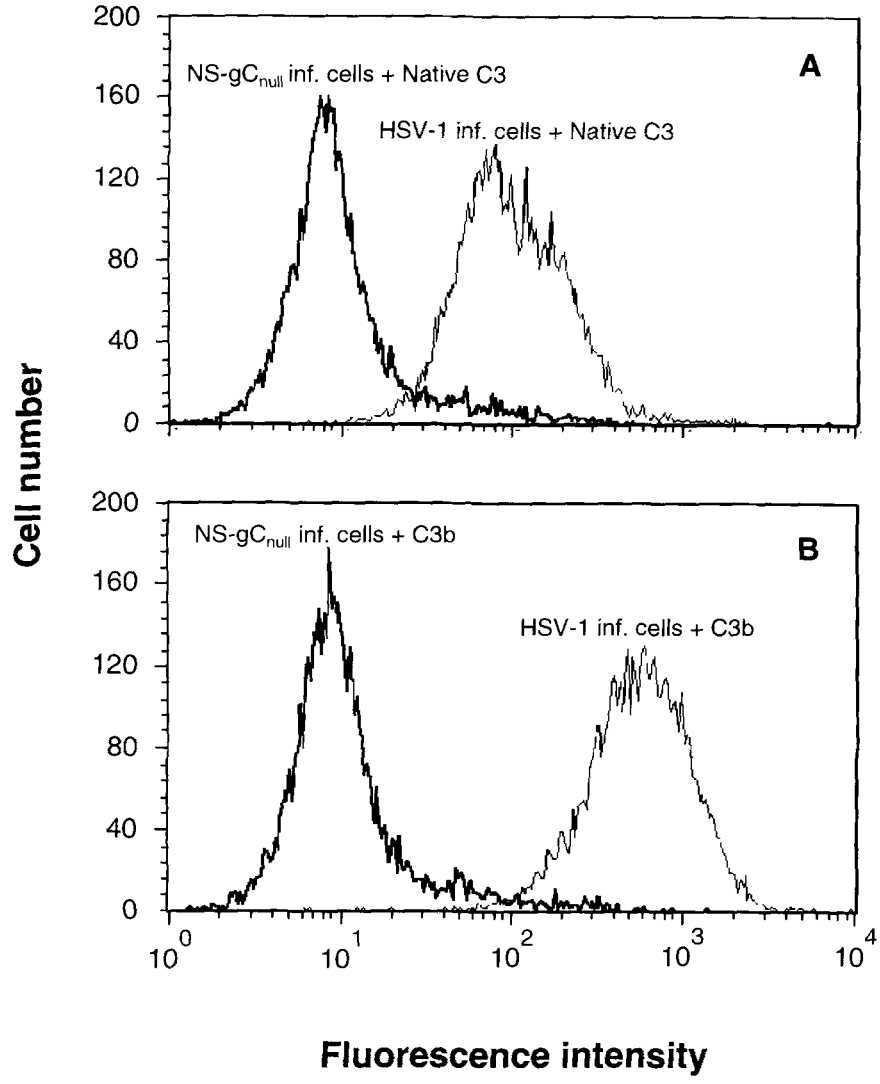

FIGURE 5. Binding of native C3 (*A*) and C3b (*B*) to HSV-1-infected cells. Vero cells were infected with either HSV-1 or NS-gC$_{null}$ (control) virus at a multiplicity of infection of 3 for 18 to 20 h. Infected cells were incubated with native C3 or C3b, and bound proteins were detected by FITC-conjugated (Fab')$_2$ anti-C3 Ab using a flow cytometer.

known to be important for binding to C3b (12, 19). Therefore, preservation of this C3b-binding function in the baculovirus-expressed forms of gC is a good indication that they have maintained their proper conformation. All four of these gC proteins also reacted with conformation-dependent mAbs, again suggesting that they retained a proper conformation (14, 22).

Binding of gC-1 and gC-2 to native C3

Earlier studies from our group and others (11–13, 15) have shown that gC-1 and gC-2 bind to C3b and iC3b and that the regions of gC involved in this binding are highly conserved in both proteins. However, none of these studies has characterized the binding to native C3. In the present study we have found, as has previously been reported, that both gCs bind to C3 (iC3) immobilized on an ELISA plate (Fig. 2). We presumed that the binding of gC to adsorbed C3 indicated that the conformation of C3 had been altered during the process of coating the ELISA wells, since iC3 behaves like C3b (e.g., it binds to properdin). However, to test the possibility that gCs bind to native C3, we analyzed the ability of purified native C3 and C3(H_2O) to inhibit the binding of gC-1 and gC-2 to iC3 (Fig. 4). In this competition assay we observed a concentration-dependent inhibition of gC-1(457t) binding to immobilized C3 by native C3 and by C3(H_2O); although the inhibition by native C3 was eightfold less efficient than that by C3(H_2O). Similar results were obtained for gC-2(426t). These findings suggest that the gCs bind to native C3. However, they do not rule out the possibility that the native C3 might have been hydrolyzed during incubation with the gC. Hydrolysis of the thioester of C3 initiates a series conformational transition. Previous studies demonstrated that one of the intermediates is sufficiently stable to be detected and isolated by cation exchange chromatography (35, 36). Spontaneous hydrolysis of C3 is very slow under physiologic conditions and has been estimated to be 0.005%/min (37). Nevertheless, to identify any possible induced/spontaneous hydrolysis on the surface of the wells we 1) examined the inhibition of properdin binding to iC3 by native C3 and C3(H_2O) under same conditions, and 2) analyzed the gC-C3 mixture incubated on an ELISA plate for C3(H_2O) and the stable intermediate conformational form of C3 (35). Since the native C3 failed to inhibit properdin binding to iC3 (Fig. 4) and no C3(H_2O) or the intermediate form of C3 could be detected in the samples incubated on the ELISA plate, we conclude that hydrolysis did not occur and that both gC-1 and gC-2 were binding to native C3. These experiments show that gC, when present in a solution, bind to native C3. To determine whether gCs present on virally infected cells bind to native C3, we analyzed the direct binding of native C3 and C3b to HSV-1-infected cells by flow cytometry. The results showed that native C3 bound to gC-1 expressed on infected cells, although the binding of native C3 was

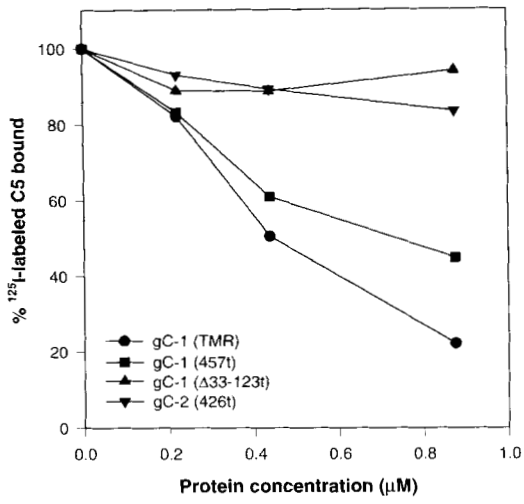

FIGURE 6. Inhibition of ^{125}I-labeled C5 binding to C3b-coated zymosan particles by gC-1 and gC-2. Zymosan particles coated with C3b (from human serum) were incubated with increasing concentrations of gC-1(TMR), gC-1(457t), gC-1(Δ33–123t), or gC-2(426t) for 30 min at 22°C. The reaction mixture was then incubated with ^{125}I-labeled C5 at 22°C for 1 h. Bound and free C5 were separated by centrifugation through 20% sucrose, and samples were counted in a gamma counter. Data are expressed as a percentage of the ^{125}I-labeled C5 bound relative to control values of zymosan particles prepared by incubation with human serum in the presence of EDTA.

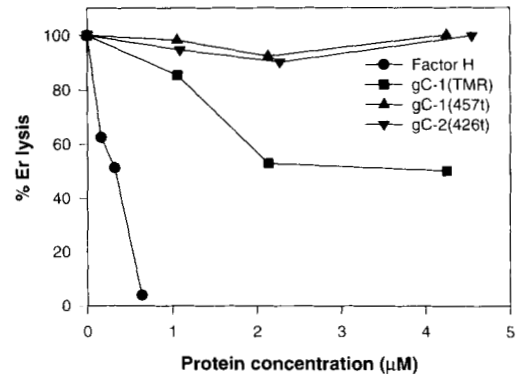

FIGURE 7. Effect of gC-1 and gC-2 on the alternative pathway activity. The alternative pathway activity was measured by lysis of rabbit erythrocytes (Er) in NHS. Er were incubated for 20 min with NHS containing MgEGTA and various concentrations of gC-1(TMR), gC-1(457t), or gC-2(426t). The amount of lysis was measured at 405 nm, and values were normalized. Factor H was used for comparison purposes.

6.5-fold lower than that of C3b (Fig. 5). The interaction of gC-1 present on infected cells or the viral surface with native C3 could play a role in inhibiting complement activation. It is conceivable that C3 molecules bound to gC-1, even if cleaved to C3b on the surface, would be unable to form a stable C3 convertase or C5b-9 complex as gC-1 inhibits the binding of properdin and C5 to C3b (17). In contrast to the host C3 binding proteins that coevolved with C3 and interact specifically with its activation fragments to the benefit of the host, there is no specific requirement for gCs to selectively interact with either native C3 or its fragments. In fact, it is more probable that the virus interact with native C3, as it is most abundant species of C3 in serum. To our knowledge these gCs are the only complement-related proteins that bind native C3.

gC binding site on C3

The sites on the C3 molecule to which various complement proteins bind have been a subject of intensive research. These findings have been identified by use of synthetic peptides representing various regions of C3, by using mAbs and site-specific anti-peptide Abs, and recently by constructing chimeric C3 molecules (28, 38). We now report that both purified gC-1 and gC-2 bind to the C3c region of C3 and that the C3d region is not involved in the gC-C3 interaction (Fig. 2). These observations support a previous study (12), which suggested that both proteins bind to a similar site on C3b. This suggestion was based on a competition experiment in which both gC-1 and gC-2 inhibited the binding of iodinated gC-1 to C3b.

Kubota and coworkers (18) have reported that a mAb directed against CR1 (5C11) can inhibit the rosetting of EAC3b cells with HSV-1-infected cells. This finding led to the hypothesis that gC-1 is an analogue of CR1 and that gC-1 shares a binding site with CR1 on C3. Recently, using chimeric and truncated C3 molecules, we have shown that a major binding site of CR1 on C3 is located within residues 727 through 768 (28). This region is also involved in binding to factor B and factor H (24, 39, 40). To determine whether these residues are involved in the gC-C3 interaction, we measured direct binding of purified gC-1 and gC-2 to a truncated C3 molecule (C3Δ727–768; Table I). Both gCs bound to the truncated C3, suggesting that residues 727 through 768 do not contribute to the interaction. This conclusion is also supported by the observation that both gCs bind to native C3. The binding sites for factor B, factor H, and CR1 are buried in native C3 and become available only after the conformational change that occurs upon the cleavage of C3 to C3b.

Our previous study on the interaction of gC with C3 has shown that gC-1, but not gC-2, inhibits the binding of properdin to iC3 (17). However, the blocking effect of gC-1 on properdin was not complete, suggesting that it is unlikely that gC-1 competes with properdin for the same site. The properdin binding site has been localized to residues 1402 to 1435 of C3 (32). In the present study, comparison of the interactions of gC and properdin with C3 from various species provided further indication that the gCs and properdin have distinct binding sites on C3; our data clearly indicate that gC-1 and gC-2, but not properdin, bind to cobra C3, and that properdin, but not gC-1 and gC-2, interacts with bovine C3; thus, gC-1 and properdin appear to bind to different sites (Table I). The ability of gC-1 to inhibit properdin binding to iC3, therefore, may be the result of a steric or an allosteric effect and not of direct competition for the same binding site.

Effect of gC on complement activation

gC-1 has been shown to inhibit C5b6-initiated reactive lysis of C3b-coated erythrocytes (16). Although the proposal was made that gC-1 inhibits the binding of C5 to C3b, direct binding assays were not performed to confirm these findings. In the present study we have found that gC-1(TMR) and gC-1(457t), but not gC-2(426t), inhibit the binding of ^{125}I-labeled C5 to surface-bound C3b. We also observed that gC-1(457t) lacking amino acids 33 to 123 (gC-1-Δ33–123t), was unable to inhibit the binding of C5 to C3b (Fig. 6 and Table II). It is noteworthy that in our previous study, we demonstrated that residues 33 through 123 of gC-1 are also involved in blocking the binding of properdin to C3b. It should be pointed out here that these residues are among the least conserved in gC-1 and gC-2 and are not involved in C3b binding. Thus, it appears that the inhibition by gC-1 of C5 and properdin binding to C3 is not caused by competition for the same binding

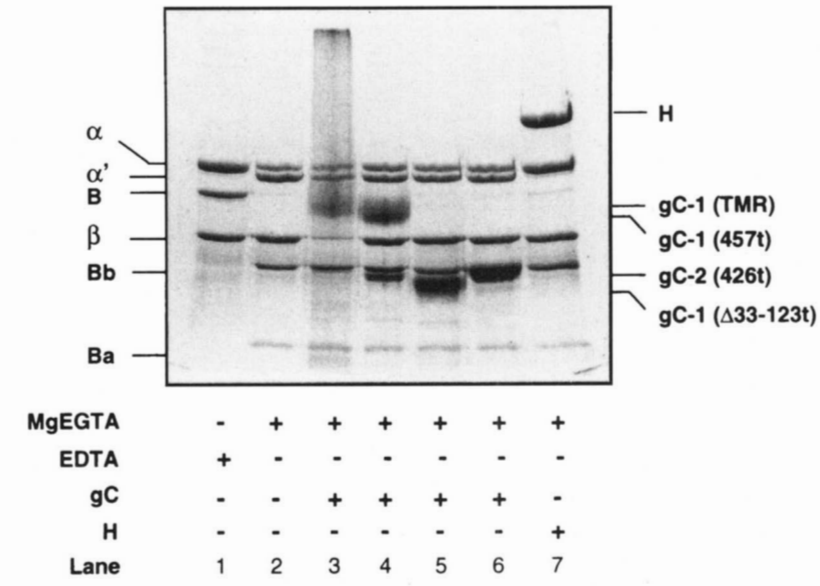

FIGURE 8. Effects of gC-1 and gC-2 on C3 convertase-mediated cleavage of C3. The alternative pathway was reconstituted by adding C3 and factors B and D together with gCs. The recombinant gC proteins used were: *lane 3*, gC-1(TMR); *lane 4*, gC-1(457t); *lane 5*, gC-1(Δ33–123t); *lane 6*, gC-2(426t). Samples were analyzed by 7.5% SDS-PAGE under reducing conditions. The gel was visualized by staining with Coomassie blue. Cleavage of C3 or factor B is indicated by the generation of α' or Bb and Ba, respectively. Controls contained EDTA. Factor H (*lane 7*) was included as a negative control.

Table II. *Biologic properties of gC-1 and gC-2*

Properties	gC-1	gC-2	Reference
Protection against C'-mediated neutralization	+	+	Present study and Refs. 6 and 7
Ligand binding	Native C3, C3b, iC3b, and C3c	Native C3, C3b, iC3b, and C3c	Present study and Refs. 11, 12, 13–15
Decay of C3b,Bb	+	–	Ref. 16
Cofactor activity for factor I	–	–	Ref. 16
Inhibition of properdin binding to C3b	+	–	Present study and Ref. 17
Inhibition of C5 binding to C3b	+	–	Present study
Inhibition of the alternative pathway C' activity	+	ND	Present study
Inhibition of factor B binding to C3b	–	–	Present study
Inhibition of C3 cleavage by C3b,Bb	–	–	Present study

ND, not determined for gC-2 (TMR).

site or by a conformational change in the C3 molecule, but is, rather, a result of sterically hindered access to these sites. To gain a full appreciation of the role of residues 33 through 123 in blocking complement activation we must await the demonstration that gC-1 mutant viruses lacking these residues are markedly susceptible to complement-mediated neutralization.

To our knowledge, no efforts have been made to date to evaluate the effects of gC-1 on the entire complement cascade. Experimental verification of the negative effect of gCs on the complement pathway was obtained in the present study by inhibiting complement-mediated lysis of erythrocytes (Fig. 7 and Table II). In comparison to factor H, a sevenfold higher concentration of gC-1 was required to inhibit complement activation. Based on previous findings and on the data presented here, the observed inhibition of lysis by gC-1(TMR) could be attributed to an accelerated decay of the C3b,Bb complex or to inhibition of the binding of properdin or C5 to C3b. In addition, binding of gC to native C3 could also cause an inhibition of C3 activation that would lead to inhibition of cell lysis. It is unlikely that the inhibition was due to an accelerated decay of C3b,Bb, since no effect was observed on factor B cleavage when gC-1 was incubated with C3b, factor B, and factor D and allowed to form C3 convertase (17). In the current study, reconstitution of the alternative pathway with C3 and factors B and D in the presence of the gC showed no inhibition of the conversion of C3 to C3b (Fig. 8 and Table II), suggesting that the expressed gCs, at least in the fluid phase, do not inhibit C3 activation. However, we cannot rule out this possibility when gC is present on viral particles.

A central question in this system is the mechanism by which gC-1 and gC-2 protect HSV from complement-mediated neutralization. On the basis of the data obtained to date, we propose that activation of complement is not necessary for gC-C3 interaction and that gC-1 can inhibit complement activity by blocking properdin and C5 binding to C3b. The mechanism by which gC-2 modulates complement-mediated neutralization remains unclear and requires further investigation.

Acknowledgments

The authors thank Yvonne H. Shahan and Liyang Wang for their excellent technical assistance, Dr. William T. Moore for helpful discussions, and Dr. D. McClellan for editorial assistance.

References

1. Gooding, L. R. 1992. Virus proteins that counteract host immune defenses. *Cell 71:5.*
2. Welsh, R. M. J., N. R. Cooper, F. C. Jensen, and M. B. Oldstone. 1975. Human serum lyses RNA tumour viruses. *Nature 257:612.*
3. Solder, B. M., T. F. Schulz, P. Hengster, J. Lower, C. Larcher, G. Bitterlich, R. Kurth, H. Wachter, and M. P. Dierich. 1989. HIV and HIV-infected cells differentially activate the human complement system independent of antibody. *Immunol. Today 22:135.*
4. Dierich, M. P., C. F. Ebenbichler, P. Marschang, G. Fust, N. M. Thielens, and G. J. Arlaud. 1993. HIV and human complement: mechanisms of interaction and biological implication. *Immunol. Today 14:435.*
5. Spear, P. G. 1985. Antigenic structure of herpes simplex viruses. In *Immunochemistry of Viruses: The Basis for Serodiagnosis and Vaccines.* M. V. H. van Regenmortel and A. R. Neurath, eds. Elsevier Science Publishers, Amsterdam, pp. 425–446.
6. McNearney, T. A., C. Odell, V. M. Holers, P. G. Spear, and J. P. Atkinson. 1987. Herpes simplex virus glycoproteins gC-1 and gC-2 bind to the third component

of complement and provide protection against complement mediated neutralization of viral infectivity. *J. Exp. Med. 166:1525.*
7. Friedman, H. M., L. Wang, N. O. Fishman, J. D. Lambris, R. J. Eisenberg, G. H. Cohen, and J. Lubinsky. 1996. Immune evasion properties of herpes simplex virus type 1 glycoprotein gC. *J. Virol. 70:4253.*
8. Olofsson, S. I., I. Sjoblom, M. Lundstrom, S. Jeansson, and E. Lycke. 1983. Glycoprotein C of herpes simplex virus type 1: characterization of O-linked oligosaccharides. *J. Gen. Virol. 64:2735.*
9. Dall'Olio, F., N. Malagolini, V. Speziali, G. Campadelli-Fiume, and F. Serafini-Cessi. 1985. Sialylated oiligosaccharides O-glycosidically linked to glycoprotein C from herpes simplex virus type 1. *J. Virol. 56:127.*
10. Johnson, D. C., and P. G. Spear. 1983. O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the Golgi apparatus. *Cell 32:987.*
11. Seidel-Dugan, C., M. Ponce de Leon, H. M. Friedman, L. F. Fries, M. M. Frank, G. H. Cohen, and R. J. Eisenberg. 1988. C3b receptor activity on transfected cells expressing glycoprotein C of herpes simplex virus types 1 and 2. *J. Virol. 62: 4027.*
12. Seidel-Dugan, C., M. Ponce de Leon, H. M. Friedman, R. J. Eisenberg, and G. H. Cohen. 1990. Identification of C3b-binding regions on herpes simplex virus type 2 glycoprotein C. *J. Virol. 64:1897.*
13. Eisenberg, R. J., P. Ponce de Leon, H. M. Friedman, L. F. Fries, M. M. Frank, J. C. Hastings, and G. H. Cohen. 1987. Complement component C3b binds directly to purified glycoprotein C of herpes simplex virus types 1 and 2. *Microbial Pathol. 3:423.*
14. Tal-Singer, R., C. Peng, M. Ponce de Leon, W. R. Abrams, B. W. Banfield, F. Tufaro, G. H. Cohen, and R. J. Eisenberg. 1995. Interaction of herpes simplex virus glycoprotein gC with mammalian cell surface molecules. *J. Virol. 69:4471.*
15. Talsinger, R., C. Seideldugan, L. Fries, H. P. Huemer, R. J. Eisenberg, G. H. Cohen, and H. M. Friedman. 1991. Herpes simplex virus glycoprotein C is a receptor for complement component iC3b. *J. Infect. Dis. 164:750.*
16. Fries, L. F., H. M. Friedman, G. H. Cohen, R. J. Eisenberg, C. H. Hammer, and M. M. Frank. 1986. Glycoprotein C of herpes simplex virus 1 is an inhibitor of the complement cascade. *J. Immunol. 137:1636.*
17. Hung, S. L., C. Peng, I. Kostavasili, H. M. Friedman, J. D. Lambris, R. J. Eisenberg, and G. H. Cohen. 1994. The interaction of glycoprotein C of herpes simplex virus types 1 and 2 with the alternative complement pathway. *Virology 203:299.*
18. Kubota, Y., T. A. Gaither, J. Cason, J. J. O'Shea, and T. J. Lawley. 1987. Characterization of the C3 receptor induced by herpes simplex virus type 1 infection of human epidermal, endothelial, and A431 cells. *J. Immunol. 138:1137.*
19. Hung, S. L., S. Srinivasan, H. M. Friedman, R. J. Eisenberg, and G. H. Cohen. 1992. Structural basis of C3b binding by glycoprotein-C of herpes simplex virus. *J. Virol. 66:4013.*
20. Herold, B. C., D. Wudunn, N. Soltys, and P. G. Spear. 1991. Glycoprotein-C of herpes simplex virus type-1 plays a principal role in the adsorption of virus to cells and in infectivity. *J. Virol. 65:1090.*
21. Hillenkamp, F., M. Karas, R. C. Beavis, and B. T. Chait. 1991. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. *Anal. Biochem. 63:1193A.*
22. Rux, A. H., W. T. Moore, J. D. Lambris, W. R. Abrams, C. Peng, H. M. Friedman, G. H. Cohen, and R. J. Eisenberg. 1996. Disulfide bond structure determination and biochemical analysis of glycoprotein C from herpes simplex virus. *J. Virol. In press.*
23. Hammer, C. H., G. H. Wirtz, L. Renfer, H. D. Gresham, and B. F. Tack. 1981. Large scale isolation of functionally active components of the human complement system. *J. Biol. Chem. 256:3995.*
24. Alsenz, J., D. Avila, H. P. Huemer, I. Esparza, J. D. Becherer, T. W. Kinoshita, Y. S. Oppermann, and J. D. Lambris. 1992. Phylogeny of the third component of complement, C3: analysis of the conservation of human CR1, CR2, H, and B binding sites, concanavalin A binding sites, and thiolester bond in the C3 from different species. *Dev. Comp. Immunol. 16:63.*
25. Alsenz, J., T. F. Schulz, J. D. Lambris, R. B. Sim, and M. P. Dierich. 1985. Structural and functional analysis of the complement component factor H with the use of different enzymes and monoclonal antibodies to factor H. *Biochem. J. 232:841.*
26. Becherer, J. D., and J. D. Lambris. 1988. Identification of the C3b receptor-binding domain in third component of complement. *J. Biol. Chem. 263:14586.*
27. Pangburn, M. K. 1987. A fluorometric assay for native C3: the hemolytically active form of the third component of human complement. *J. Immunol. Methods 102:7.*
28. Lambris, J. D., Z. Lao, T. J. Oglesby, J. P. Atkinson, E. Hack, and J. D. Becherer. 1996. Dissection of CR1, factor H, MCP, and factor B binding and functional sites in third complement component. *J. Immunol. 156:4821.*
29. Servis, C., and J. D. Lambris. 1989. C3 synthetic peptides support growth of human CR2-positive lymphoblastoid B cells. *J. Immunol. 142:2207.*
30. Hack, C. E., J. Paardekooper, R. J. T. Smeenk, J. Abbink, A. J. M. Eerenberg, and J. H. Nuijens. 1988. Disruption of the internal thioester bond in the third component of complement (C3) results in the exposure of neodeterminants also present on activation products of C3: an analysis with monoclonal antibodies. *J. Immunol. 141:1602.*
31. Friedman, H. M., J. C. Glorioso, G. H. Cohen, J. C. Hasting, S. L. Harris, and R. J. Eisenberg. 1986. Binding of complement component C3b to glycoprotein gC of herpes simplex virus type 1: mapping of gC-binding sites and demonstration of conserved C3b binding in low-passage clinical isolates. *J. Virol. 60:470.*
32. Daoudaki, M. E., J. D. Becherer, and J. D. Lambris. 1988. A 34-amino acid peptide of the third component of complement mediates properdin binding [published erratum appears in *J. Immunol. 141:1788, 1988*]. *J. Immunol. 140:1577.*
33. Zhu, Q., and R. J. Courtney. 1988. Chemical crosslinking of glycoproteins on the envelope of herpes simplex virus. *Virology 167:377.*
34. Kikuchi, G. E., J. C. Glorioso, and R. Nairn. 1990. Cross-linking studies show that herpes simplex virus type 1 glycoprotein C molecules are clustered in the membrane of infected cells. *J. Gen. Virol. 71:455.*
35. Pangburn, M. K. 1992. Spontaneous reformation of the intramolecular thioester in complement protein C3 and low temperature capture of a conformational intermediate capable of reformation. *J. Biol. Chem. 267:8584.*
36. Pangburn, M. K. 1992. Spontaneous thioester bond formation in alpha 2-macroglobulin, C3 and C4. *FEBS Lett. 308:280.*
37. Pangburn, M. K., R. D. Schreiber, and H. J. Müller-Eberhard. 1981. Formation of the initial C3 convertase of the alternative pathway: acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. *J. Exp. Med. 154:856.*
38. Lambris, J. D. 1993. Chemistry, biology and phylogeny of C3. *Complement Profiles 1:16 (Rev.).*
39. Ganu, V. S., and H. J. Müller-Eberhard. 1985. Inhibition of factor B and factor H binding to C3b by synthetic peptide corresponding to residues 749–789 of human C3. *Complement 2:27 (Abstr.).*
40. Fishelson, Z. 1991. Complement-C3: a molecular mosaic of binding sites. *Mol. Immunol. 28:545.*