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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

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The sites on glycoprotein gC of herpes simplex virus type 1 (HSV-1) which bind complement component C3b were evaluated by using (i) anti-gC monoclonal antibodies and (ii) mutants which have alterations at defined regions of the glycoprotein. Monoclonal antibodies were incubated with HSV-1-infected cells in a competitive assay to block C3b binding. Each of 12 different monoclonals, which recognize the four major antigenic sites of gC, completely inhibited C3b binding. With this approach, no one antigenic group on gC could be assigned as the C3b-binding region. Next, 21 gC mutants were evaluated for C3b binding, including 1 which failed to synthesize gC, 4 which synthesized truncated forms of the glycoprotein such that gC did not insert into the cell's membrane, and 16 which expressed gC on the cell's surface but which had mutations in various antigenic groups. Eleven strains did not bind C3b. This included the 1 strain which did not synthesize gC, the 4 strains which secreted gC without inserting the glycoprotein into the cell membrane, and 6 of 16 strains which expressed gC on the cell surface. In these six strains, the mutations were at three different antigenic sites. One hypothesis to explain these findings is that C3b binding is modified by changes in the conformation of gC which develop either after antibodies bind to gC or as a result of mutations in the gC gene. Attachment of C3b to gC was also evaluated in 31 low-passage clinical isolates of HSV-1. Binding was detected with each HSV-1 isolate, but not with nine HSV-2 isolates. Therefore, although mutants that lack C3b binding are readily selected in vitro, the C3b-binding function of gC is maintained in vivo. These results indicate that the sites on gC that bind C3b are different from those that bind monoclonal antibodies, that antibodies directed against all sites on gC block C3b binding, and that C3b binding is a conserved function of gC in vivo.

Herpes simplex virus type 1 (HSV-1) encodes at least six viral glycoproteins, designated gB, gC, gD, gE (40), gH (4), and a newly recognized glycoprotein not yet given an alphabetical designation (35). These glycoproteins are incorporated into the virion envelope and are expressed on the surface of infected cells. Although knowledge remains incomplete, certain functions have been assigned to several of these glycoproteins. gB is involved in the penetration of virus into cells (27, 37), gC serves as a receptor for complement component C3b (16), gD plays a role in inducing cell fusion (30), and gE functions as a receptor for the Fc portion of immunoglobulin G (IgG) (2, 32, 33). HSV glycoproteins serve as targets of the host's cellular and humoral immune attack (3, 5, 17, 26; reviewed in reference 31). It is of interest that two of the viral glycoproteins, gC and gE, have binding functions that are likely to diminish the effectiveness of this attack. The binding of nonimmune IgG to the viral Fc receptor, gE, protects the virus from neutralization by immune serum (12). In experimental models using aggregated IgG, the Fc receptor also protects HSV-infected cells from complement-dependent and cell-mediated immune lysis (1). Current work in our laboratory indicates that the C3b receptor, gC, has similar functions. It diminishes the efficiency of complement-mediated viral neutralization and inhibits complement-mediated lysis of HSV-1-infected cells (S. Harris, G. Cohen, R. Eisenberg, and H. Friedman, manuscript in preparation).

HSV-1 is the first infectious agent shown to have C3b receptor activity. Blood cells, including monocytes, macrophages, neutrophils, eosinophils, mast cells, B lymphocytes, some T lymphocytes, and erythrocytes, have C3b receptors on their plasma membrane (reviewed in reference 15). On these cells, C3b receptors have several well-described functions. First, the receptors inhibit complement activation by accelerating the decay of both the classical and alternative pathway C3 convertase enzymes (14, 22). These convertases are generated during activation of the complement cascade; *C4b2a* serves as the convertase of the classical pathway, while *C3bBb* functions as the alternative pathway C3 convertase. C3b receptors on the membrane of human blood cells enhance the decay of C2a and Bb from the convertases, which renders them enzymatically inactive. By accelerating the decay of the C3 convertases, C3b receptors decrease the amount of C3b generated which has an inhibitory effect on complement activation. A second mechanism by which C3b receptors inhibit complement activation is by interacting with serum factor I to cleave C3b to its degradation product iC3b (14). This decreases the amount of C3b available for

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participation in the complement cascade. A third function for C3b receptors on neutrophils, monocytes, and macrophages is that they promote binding and phagocytosis of C3b-coated particles, although phagocytosis may not occur unless IgG-bearing particles are also bound to the cell's Fc receptors (13).

Our previous work to characterize the binding of C3b to gC showed that C3b receptors are expressed on a variety of different cell types following HSV-1 infection. These include endothelial, epithelial, and fibroblastic cells (39). The extent of C3b binding, however, varied in the different cell types. We noted that the binding of C3b to gC is greatly increased if sialic acid is stripped from the glycoprotein on the surface of HSV-1-infected cells (38). This indicates that glycosylation affects the binding of C3b to gC, which may account for the variation in C3b binding following HSV-1 infection of various cell types. Although HSV-1 and HSV-2 both induce receptors for the Fc portion of IgG, HSV-2-infected cells do not bind C3b (16). Infection with other viruses also fails to induce C3b receptors. Viruses examined include cytomegalovirus, varicella-zoster virus, measles virus, mumps virus, adenovirus type 7 (39), pseudorabies virus, and infectious bovine rhinotracheitis virus (H. Friedman, unpublished observation).

In this report, we examine the effects of mutations in gC on C3b binding. Recent studies have shown that the antibody-binding sites on gC are clustered into two distinct antigenic regions (29). We evaluated whether C3b binding to gC maps to a particular antigenic site. We also examined the role of mutations at various positions in gC in modifying C3b binding to gC. Defining the sites on gC which bind C3b is necessary if we are to distinguish those regions of this glycoprotein which serve as targets of the immune attack from those that help the virus escape from immune surveillance.

MATERIALS AND METHODS

Virus strains. Wild-type HSV-1 (strain KOS 321) was used for selection of gC mutants as previously described (21). All mutants were selected on the basis of resistance to neutralization by anti-gC monoclonal antibody and complement. Mutants with the gC-negative phenotype included strains which failed to synthesize gC and those which secreted truncated forms of the glycoprotein (20, 24). Monoclonal antibody-resistant (*mar*) mutants, which express an antigenically altered form of gC on the cell surface, were characterized as described previously (29). Viruses were grown on African green monkey kidney cells (Vero) at 37°C, and titers were determined by plaque assay.

Cell cultures and infection. Studies to detect binding of C3b to gC were performed on cultures of human umbilical vein endothelial cells which were grown in 24-well plates on a fibronectin matrix as previously described (39). Human umbilical vein endothelial cells were chosen for study because C3b-coated erythrocytes form rosettes which are distinct and easy to evaluate after HSV-1 infection (16, 39). Confluent monolayers were infected with virus at a multiplicity of infection of 3 to 10. At 24 to 48 h postinfection, infected cells were evaluated for the expression of C3b receptors as described below.

Detection of C3b receptors by erythrocyte-binding assays. C3b-coated erythrocytes were prepared as previously described (16, 39). Briefly, sheep erythrocytes were radiolabeled with ^{51}Cr (Na_2CrO_4; New England Nuclear Corp., Cambridge, Mass.) and were sensitized with subagglutinating concentrations of rabbit anti-sheep erythrocyte IgM (Cordis Laboratories Inc., Miami, Fla.). IgM-coated erythrocytes (10^8/ml) were then incubated in a stepwise manner with purified complement components (Cordis Laboratories Inc.) consisting of 1,000 U of human C1, 100 U of human C4, 75 U of human C2, and 200 U of human C3 per ml. The complement-coated erythrocytes were pelleted by centrifugation and suspended in calcium- and magnesium-free gelatin Veronal (Winthrop Laboratories, New York, N.Y.)-buffered saline containing 10 mM EDTA. The final preparation consisted of EIgMC1423 cells which were stored at 4°C for up to 3 weeks. As a control for the requirement of C3b, erythrocytes were prepared as described above except that C3 was omitted, which resulted in the preparation of EIgMC142 cells.

To increase the sensitivity of the C3b-binding assay to KOS-infected cells, the endothelial monolayers were treated with 0.05 U of neuraminidase per well (type x, from *Clostridium perfringens*; Sigma Chemical Co., St. Louis, Mo.) (160 U per mg of protein) for 30 min at 37°C (38). The enzyme was removed by washing, and ^{51}Cr-labeled C3b-coated erythrocytes, or control erythrocytes, were added to the monolayer for 2 h at 37°C (9). Unbound erythrocytes were removed by washing, and the monolayer was viewed for rosettes. At least four erythrocytes adherent to the surface of a cell were required for the identification of a rosette. After viewing, adherent erythrocytes were lysed with distilled water and the lysate was counted for gamma emission (Minnigamma 1275; LKB, Hicksville, N.Y.). The ^{51}Cr binding was calculated as the ratio of bound/total ^{51}Cr added (16).

Blocking of C3b binding. The preparation and characterization of the monoclonal antibodies that recognize gD and defined antigenic sites on gC have previously been described (16, 29). Ascitic fluid was diluted 1:10 or 1:20 and incubated for 30 min with endothelial cells that had been infected with virus 24 to 48 h earlier. Unbound antibody was removed by washing and C3b-coated erythrocytes were added. Rosetting assays were performed as described above. A monoclonal antibody was considered to block C3b attachment if the antibody reduced the binding of C3b-coated erythrocytes to background levels, that is, to those levels obtained when infected monolayers were incubated with EIgMC142 (control) erythrocytes.

Immunofluorescence for detection of gC or gD on the surface of infected cells. Immunofluorescence experiments were performed to determine whether mutants negative for C3b binding expressed either gC or gD on the surface of infected cells. Endothelial cells were grown on 12-mm circular glass cover slips and were inoculated with virus. Twenty-four hours later, anti-gC or anti-gD monoclonal antibodies, diluted 1:100, were added to unfixed cells for 30 min at 37°C. Unbound antibody was removed by washing and bound antibody was detected with fluorescein-conjugated $F(ab')_2$ goat anti-mouse IgG (Cooper Biomedical, Inc., West Chester, Pa.).

Detection of C3b receptors on low-passage clinical isolates of HSV-1 and HSV-2. HSV-1 and HSV-2 were isolated from oral or genital clinical samples. Viruses were grown on human embryonic lung fibroblast cells (MRC-5 strain), and isolates were identified as type 1 or 2 by immunofluorescence, using commercially available monoclonal antibodies (Syva Corp., Palo Alto, Calif.). The isolates were passaged once or twice in MRC-5 cells to build up the titers and then were inoculated onto endothelial cells for detection of C3b receptors by rosetting assays.

RESULTS

Blocking C3b binding to KOS-infected cells, using monoclonal antibodies. Previously we showed that monoclonal antibodies to gC block C3b binding while monoclonal antibodies to gB, gD, and gE have no effect (16). The anti-gC monoclonal antibodies evaluated included antibodies 5S, 17S, 19S, and 27S, which bind to the group IIc site on gC (29), and antibody 1C8 (39), which has not been mapped to a particular gC epitope. A possible interpretation of these results is that C3b binds solely or predominantly to the group IIc site of gC. To evaluate this in more detail, we attempted to block the binding of C3b-coated erythrocytes to gC on KOS-infected cells, using monoclonal antibodies which bind to each of the defined antigenic sites on the glycoprotein.

Table 1 shows that monoclonal antibodies which bind to antigenic group I (antibodies C2, C14), group IIa (antibodies C3, C16), group IIb (antibodies C13, C17), and group IIc (antibodies C10, 17S, 18S, 19S, 26S, 27S) all blocked rosetting of C3b-coated erythrocytes to KOS-infected cells. The percentage of ^{51}Cr-labeled C3b-coated erythrocytes which bound to the monolayer, in the absence of monoclonal antibody blocking, ranged from 7.6 to 24.1% (mean of three experiments, 16.6%). Each of the monoclonal antibodies to gC blocked binding of C3b-coated erythrocytes to <2.0%, which was similar to control erythrocytes (EIgMC142 cells) binding to infected monolayers (mean of three experiments, 1.2%). In contrast, monoclonal antibodies to gB, gD, and gE did not reduce binding of C3b-coated erythrocytes. Therefore, antibodies to each of four sites on gC block C3b binding, while antibodies to other HSV-1 glycoproteins have no effect.

These results have several possible interpretations. First, C3b may bind to sites on gC that include domains from each antigenic group. Second, monoclonal antibody binding to any site on gC may block C3b binding by steric hindrance. Third, antibody binding to any antigenic site on gC may alter the conformation of the glycoprotein which reduces C3b binding.

C3b binding to gC on *mar* mutants. To further evaluate these possibilities, we examined 21 KOS strains which had mutations in the gC gene. Three general categories of gC mutants were examined, including (i) a gC-minus strain which failed to synthesize gC in infected cells, and (ii) truncated gC mutants in which various sized fragments, including the transmembrane insertion piece, were missing from the carboxy end of the glycoprotein. Cells infected with these mutants secreted gC into the culture supernatant and failed to insert gC onto the plasma membrane (20, 24). (iii) Mutants in which gC was present on the plasma membrane but in an altered form (29) were also included.

Each of the 21 *mar* mutants was inoculated onto human umbilical vein endothelial cultures. At 24 to 48 h postinoculation, infected cells were evaluated for expression of gC on the cell surface and for binding of C3b-coated erythrocytes, by rosetting and ^{51}Cr binding. Table 2 shows that C3b receptors were detected on the parent KOS strain. As expected, C3b receptors were not detected on the gC-minus mutant or on the four mutants which secreted gC into the supernatant without inserting the glycoprotein into the cell membrane. Of the remaining 16 *mar* mutants, 10 were positive for C3b binding. This included three mutants in antigenic group I, four in group IIb, and three in group IIc. On cells infected with these 10 mutants, the binding of C3b was to gC because anti-gC monoclonal antibodies totally blocked C3b binding.

TABLE 1. Role of anti-gC monoclonal antibodies in blocking C3b binding to gC on KOS-infected cells

Blocking reagent[a]	Binding of C3b-coated erythrocytes	
	Rosette assay[b]	^{51}Cr-binding assay[c]
Monoclonal antibody (MCA) to gC		
Antigenic group I		
MCA C2	No	1
MCA C14	No	1
Antigenic group IIa		
MCA C3	No	1
MCA C16	No	1
Antigenic group IIb		
MCA C13	No	1
MCA C17	No	1
Antigenic group IIc		
MCA C10	No	1
MCA 17S	No	1
MCA 18S	No	1
MCA 19S	No	1
MCA 26S	No	1
MCA 27S	No	1
Monoclonal antibody to gB, MCA 24S	Yes	6
Monoclonal antibody to gD, MCA 1D3	Yes	3
Monoclonal antibody to gE, MCA 1E3	Yes	5
Saline control	Yes	4

[a] A 200-μl portion of a 1:20 dilution of ascites was used in blocking experiments. For antibodies C2, C3, C17, and C10, ascites was also diluted 1:40, 1:80, and 1:160. Each dilution totally blocked C3b binding. Antibodies were added 24 h postinfection and incubated for 30 min at 37°C. They were then removed by washing before adding C3b-coated erythrocytes. Results are the mean of two separate blocking experiments.

[b] Rosettes were viewed by light microscopy (× 100 magnification). To be counted as a rosette, the binding of four or more erythrocytes to an endothelial cell was required.

[c] ^{51}Cr assay results are expressed as the ratio of binding of C3b-coated erythrocytes (EIgMC1423 cells) to infected cells which are blocked with monoclonal antibody compared with background binding, that is, binding of C2-coated erythrocytes (EIgMC142 cells) to infected cells. A ratio of 1 indicates no binding of C3b-coated erythrocytes; a ratio of 2 or more indicates C3b binding.

The six *mar* mutants which did not express C3b receptors included three of six mutants in antigenic group I, two of two mutants in group IIa, and one of four mutants in group IIc. Immunofluorescence was performed on unfixed cells to document that gC was expressed on the cell surface after infection with these six mutants. Antibodies to group II sites were used to detect gC on cells infected with group I mutants, while antibodies to group I sites were used to detect gC after infection with group IIa or IIc mutants. Bright immunofluorescence was noted with each mutant, indicating that gC was expressed on the cell surface. This indicates that the mutation in C3b had converted these six mutants to C3b receptor-negative strains.

Three possible interpretations were discussed above to explain the results shown in Table 1. The findings shown in Table 2 help to exclude one of these interpretations: that which postulates that C3b binds to each of the four antigenic groups on gC. The support for this exclusion is that mutations at site IIb (C9.6, C17.2, C17.3, C13.1) have no effect on C3b binding, suggesting that this domain is nonessential. Nevertheless, monoclonal antibody to this domain blocked C3b binding. Whether this block occurred because of steric

TABLE 2. Detection of C3b receptors on cells infected with HSV-1 strain KOS and KOS mutants

Virus strain	Binding of C3b-coating erythrocytes[a]	
	Rosettes	^{51}Cr assay
KOS parent	Yes	10
gC negative		
gC-551	No	1
Truncated gC mutants		
gC-3	No	1
gC-49	No	1
gC-8	No	1
syn LD70	No	1
Mutants in antigenic group I		
C2.1	No	1
C4.4	No	1
C15.4	No	1
C11.1	Yes	12
C14.1	Yes	9
C15.1	Yes	6
Mutants in antigenic group IIa		
C3.1	No	1
C16.1	No	1
Mutants in antigenic group IIb		
C9.6	Yes	20
C17.2	Yes	22
C17.3	Yes	21
C13.1	Yes	22
Mutants in antigenic group IIc		
C10.1	No	1
C7.1	Yes	7
C7.2	Yes	13
C13.2	Yes	15

[a] Results are the mean of two to three separate infections for each virus strain except for KOS parent strain, which is the mean of seven experiments. Rosettes are measured as described in footnote *b*, Table 1. ^{51}Cr assay is expressed as the ratio of binding of C3b-coated/C2-coated erythrocytes. Ratios are rounded to the nearest whole number. A ratio of >2 indicates that C3b receptors are present. In all cases, the actual binding of C2-coated erythrocytes was <2%. To prove that C3b-coated erythrocytes are binding to gC, blocking assays were performed on cells infected with *mar* mutants that demonstrate C3b binding. Infected cells were incubated with a monoclonal antibody to either group I or II antigenic determinants prior to adding C3b-coated erythrocytes. In all cases, C3b binding was reduced to background levels (i.e., those obtained with C2-coated erythrocytes). Mutants which do not bind C3b were shown to express gC on the cell surface, using indirect immunofluorescence and antibodies to group II sites to detect gC after infection with group I mutants or antibodies to group I sites to detect gC after infection with group IIa and IIc mutants.

hinderance or because of antibody-induced changes in gC conformation cannot be determined from these results.

Within antigenic site 1, considerable heterogeneity exists since some mutants bind while others fail to bind C3b. It is of interest that antibody C14, which recognizes antigenic group 1, blocks C3b binding (Table 1), yet the mutant virus selected by growth in the presence of this antibody (C14.1) maintains the property of binding C3b. This finding also supports the hypothesis that anti-gC antibody can block C3b binding without occupying a domain on gC essential for this function.

Eleven of the 21 *mar* mutants examined have lost the ability to bind C3b (Table 2). These results indicate that C3b binding is not required for viral survival in vitro. To determine whether this function is conserved in vivo, we examined low-passage clinical isolates for C3b binding. Endothelial cell monolayers were infected with either HSV-1 or HSV-2. Twenty-four hours postinfection, C3b-coated or control erythrocytes (EIgMC142 cells) were added. Specific binding of C3b-coated erythrocytes was confirmed by blocking assays, using anti-gC monoclonal antibodies. Each of 31 HSV-1 clinical isolates bound C3b (Table 3). As expected, each of the HSV-1 isolates expressed both glycoproteins gC and gD on the surface of infected cells as monitored by monoclonal antibodies in an immunofluorescence assay. The HSV-2 isolates expressed gD but not the type-specific epitope on gC recognized by monoclonal antibody 1C8. None of the nine HSV-2 isolates bound C3b (Table 3).

DISCUSSION

We attempted to define the regions of gC that bind C3b. Multiple monoclonal antibodies were evaluated for their ability to block C3b binding to gC. All antibodies to gC blocked C3b binding. KOS strain mutants in the gC gene were then examined for C3b binding. Unexpectedly, strains with mutations at one site (IIb) had no effect on C3b binding and mutations at another site (IIa) eliminated C3b binding, while mutations at the remaining two sites (I and IIc) had a variable effect. These results indicate that antigenic groups I and IIc, which were defined by resistence to neutralization by monoclonal antibodies (29), are not homogeneous, since within each group is a subpopulation which either binds or fails to bind C3b. More important to the purpose of this investigation, the results indicate that the antigenic grouping of gC does not define one particular domain of the glycoprotein that binds C3b.

Two interpretations are offerred to explain the results of blocking experiments with monoclonal antibodies (Table 1). One is that antibodies block by steric hinderance, such that C3b binding is prevented even if antibody binds to a nonessential domain on gC, such as site IIb. An alternate hypothesis is that antibody binding to gC alters the conformation of the glycoprotein, which affects C3b binding.

Several interpretations are also possible to explain the results of *mar* mutant binding studies shown in Table 2. Perhaps C3b binds to several sites on gC, such as those composed, in part, of groups I, IIa, and IIc. Alterations in any one of these sites may then eliminate C3b binding. An alternate explanation is that mutations in gC affect C3b binding by altering the conformation of the glycoprotein. The latter explanation provides a unifying hypothesis with the results obtained by monoclonal antibody blocking assays. In support of this hypothesis is recent DNA sequencing results. The gC genes of four *mar* mutants which express

TABLE 3. C3b binding to low-passage clinical isolates of HSV-1 or HSV-2

HSV type	No. tested	No. of strains positive		
		Expression of type-common gD epitope on infected cells[a]	Expression of HSV-1 gC on infected cells[b]	Binding of C3b[c]
HSV-1	31	31	31	31
HSV-2	9	9	0	0

[a] gD was detected with monoclonal antibody 1D3 (16), which recognizes a type-common epitope on gD.

[b] gC was detected with anti-gC antibody 1C8, C10, C13, or C16.

[c] Binding of C3b was measured as described in the footnote to Table 2. For each HSV-1 isolate, the specificity of the binding of C3b to gC was shown by blocking assays, using anti-gC monoclonal antibody 1C8, C10, C13, or C16.

gC on the cell's surface, but which fail to bind C3b, have been sequenced (J. C. Glorioso and M. Levine, manuscript in preparation). The strains include group I mutant C2.1, group IIa mutants C3.1 and C16.1, and group IIc mutant C10.1. Each strain has a mutation in a single nucleotide which is predicted to produce a single amino acid substitution. The mutations span a length separated by 244 amino acids, from amino acids 129 to 373. Of interest, group I mutant C11.1 also has a single nucleotide substitution, resulting in a predicted single amino acid change, yet this strain maintains C3b-binding activity. The most likely explanation for the results is that certain point mutations alter a critical conformation of gC required for C3b binding. Evidence using protein-denaturing agents, such as Formalin and paraformaldehyde (18), indicate that C3b binding to gC can be eliminated by fixing infected cells with these reagents (Friedman, unpublished observations). Definitive evidence to support the importance of gC conformation in C3b binding, however, awaits results of studies examining C3b binding to the purified glycoprotein which is either in its native state or denatured.

Our previous studies indicated that removal of sialic acid from gC on infected cells increased the binding of C3b to gC (38). In view of the DNA-sequencing results mentioned above, it is apparent that changes in the protein backbone can also modify C3b binding. None of the mutations involving single amino acid substitutions occurred at sites which would be predicted to modify N-linked or O-linked glycosylation. In addition, neuraminadase treatment did not modify C3b binding to these mutants. These results, therefore, indicate that both carbohydrate and amino acid variations of gC affect C3b binding.

The *mar* mutant results reveal that the virus need not retain C3b-binding ability for it to survive in vitro. This is consistent with the observation that gC-negative mutants also replicate efficiently in vitro (19, 28). Complement components are not part of the constituents present in cell culture fluids; therefore, no selective pressure is exerted on the virus to maintain C3b-binding function. However, in vivo, a different situation exists. Each of 31 low-passage clinical isolates of HSV-1 examined in this study expressed gC on the cell's surface and bound C3b (Table 3). Glycoprotein gC was also detected in each of 63 isolates tested in a study performed by Pereira et al. (34). These results suggest an important function for gC and for C3b binding in vivo.

Studies which document an important role in vivo for gC as a C3b-binding protein are lacking. Experiments in mice indicate that gC is not essential for virus-induced disease of the central nervous system (11). However, it is interesting to note that in the murine studies of Dix et al., when the gC-minus strains Miyama and MP were inoculated by the footpad route, they were avirulent (11). When inoculated directly into the brain, these strains were virulent, although the Miyama strain had diminished virulence compared with wild-type virus. Kumel et al. also noted decreased virulence of gC mutants after intracerebral inoculation (25). The intracerebral route of infection is not natural and bypasses certain advantages that gC may confer for viral survival at more proximal sites of entry of virus into the host. In rabbits, the gC-negative MP strain was found to be virulent, but the virus was inoculated directly onto a scarified cornea (6). An important consideration for future evaluations of gC in animal pathogenesis studies is, first, to determine the optimal route of inoculation for these studies and, second, to examine the species specificity of C3b for gC. The binding of C3b to gC may not cross species barriers. A similar situation has recently been described for the Fc receptor of HSV-1, which binds human but not murine IgG (23).

Although the function of gC in vivo remains undefined, recent in vitro studies have advanced our understanding of this glycoprotein. Experiments in our laboratory indicate that gC on the surface of infected cells protects the cell from lysis by antibody and complement or complement alone (Harris et al., in preparation). Cytotoxicity was examined in BHK-21 cells infected with wild-type HSV-1 or with a mutant which failed to bind C3b. Cells infected with the mutant virus were more readily lysed by anti-HSV antibody and complement or complement alone. Studies performed with purified gC indicate the possible mechanism by which this glycoprotein prevents complement-mediated lysis. Glycoprotein gC inhibits alternative pathway complement activation by accelerating the decay of the alternative pathway C3 convertase. Purified gC also reduces the efficiency of complement-mediated lysis by competing with C5 or C5b for binding sites on C3b (L. H. Fries, H. M. Friedman, G. H. Cohen, R. J. Eisenberg, C. H. Hammer, and M. M. Frank, submitted for publication).

The results of this study indicate that as antibody binds to gC it alters the ability of the glycoprotein to react with C3b. Recently, Ross et al. showed that serum from patients with recurrent herpes labialis blocks the binding of antibody C16 to gC (36). This indicates that human antibody recognizes a similar site on gC as antibody C16. Since C16 inhibits C3b binding (Table 1), this implies that following HSV-1 infection the host's humoral immune response to gC is likely to block the binding of C3b. Whether sufficient quantities of anti-gC antibody are produced in vivo to occupy all available gC sites is currently unknown. However, it seems reasonable to conclude from our results that, once the host mounts an antibody response to gC, the C3b-binding property of the glycoprotein will be diminished. An implication of this conclusion is that any selective advantage gC offers in survival of the virus is likely to occur early in infection, before anti-gC antibody is made. Alternatively, C3b may compete with antibody for binding sites on gC, thereby decreasing the efficiency of antibody-directed attack on virus-infected cells.

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