Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

seminars in **CELL & DEVELOPMENTAL BIOLOGY,** Vol 9, 1998: pp 329–337 *Article Number: sr980242*

Viral interference with antibody and complement

*John Lubinski, Thandavarayan Nagashunmugam and Harvey M. Friedman**

Viruses have evolved strategies to evade immunity mediated by antibody and complement. Herpesviruses and coronaviruses encode IgG Fc binding proteins that inhibit IgG activity, enabling the virus or infected cell to escape antibody attack. Herpesviruses, vaccinia virus and HIV-1 have the capacity to interfere with complement, either by incorporation of cellular complement regulatory proteins into the virion envelope or cell membrane, or by expression of viral molecules that mimic functions of complement regulatory proteins. The structure and biological activities of herpes simplex virus type 1 (HSV-1) glycoproteins gE, gI and gC are described. These glycoproteins protect HSV from immune attack; HSV-1 gE/gI form a complex that binds the Fc domain of IgG while gC is a C3b binding complement regulatory protein, providing a survival advantage to the virus in vitro *and* in vivo *by inhibiting immune functions.*

Key words: Complement / Complement regulatory proteins / Fc receptors / HSV-1 / Immune evasion

Viruses interfere with antibody by expressing IgG Fc receptors

IMMUNOGLOBULIN G (IgG) Fc receptors (FcγRs) are present on many mammalian hematopoietic cells, including monocytes, macrophages, neutrophils, eosinophils, platelets, B cells and certain classes of T cells.[1] FcγRs contain multiple globular extracellular domains characteristic of the immunoglobulin gene superfamily[2] and are divided into three groups based on structure.[3] FcγRI (CD64) is a trimer consisting of one α chain subunit (with three extracellular domains) and two γ chain subunits. It functions as a high affinity FcγR that binds IgG monomers with a K_A of 10^8. FcγRII (CD32) is a monomer with an α chain that has two extracellular domains. FcγRIII (CD16) is a dimer with one α chain subunit (two extracellular domains) and one ζ chain subunit. FcγRII and FcγRIII are low affinity FcγRs that bind IgG aggregates at a K_A of 2×10^6 and 5×10^5, respectively, but do not bind IgG monomers. All mammalian FcγRs are membrane spanning proteins, except for FcγRIIIB, a variant of FcγRIII that is anchored by a glycophosphatidylinositol (GPI) tail.[3] FcγRs provide an important link between the humoral and cellular immune systems, since interaction of the IgG Fc domain with FcγRs triggers effector activities, including phagocytosis, cell proliferation, cell activation or inhibition, antibody-dependent cellular cytotoxicity (ADCC), and release of cytokines and inflammatory mediators.

HSV-dependent FcγR activity was shown when IgG-coated erythrocytes were added to HSV-infected cells and found to form rosettes, indicating that HSV induces formation of an IgG binding protein[4] which was found to interact with the Fc domain of IgG.[5] Virus proteins binding Fc were identified by passing virus-infected cell and virion extracts through an IgG affinity column. Three HSV polypeptides were identified, corresponding to precursor and mature forms of glycoprotein E (gE) and a second glycoprotein gI.[6–8] gE and gI (encoded by HSV genes US8 and US7, respectively[9]) form a hetero-oligomer complex that binds the Fc domain of IgG in both IgG aggregates and monomers.[7,10–12] gE alone functions as a lower affinity FcγR, binding IgG aggregates but not IgG monomers.[12] These results suggest that mammalian and HSV FcγRs share certain structural similarities in that FcγRI and gE/gI are complexes (hetero-trimer and -oligomer respectively) that form higher affinity FcγRs, while FcγRII and gE are monomeric proteins that form lower affinity FcγRs.

HSV-1 is classified as an alphaherpesvirus. Other human alphaherpesviruses express FcγRs, including HSV-2,[13] and varicella-zoster virus (VZV).[14,15] HSV-2 gE was identified as the Fc binding glycoprotein by IgG affinity chromatography.[16] Similarly, the gE ho-

*From the * Division of Infectious Diseases, Department of Medicine, University of Pennsylvania School of Medicine, 536 Johnson Pavilion, Philadelphia, PA 19104-6073, USA*

1084-9521/98/030329+09 $30.00/0

molog in VZV functions as an IgG Fc binding glycoprotein.[15] VZV gI alone does not bind IgG; however, whether gI interacts with gE to modify FcγR activity is unknown. Human cytomegalovirus (HCMV) is a betaherpesvirus that induces an FcγR on infected cells,[17] although whether a viral protein mediates this activity has not been determined. The closely related murine cytomegalovirus (MCMV) also induces FcγR activity on infected cells which has been ascribed to a viral protein (designated fcr1) encoded by a gene m138;[18] a homolog of MCMV m138 is not encoded by HCMV.

In addition to herpesviruses, coronaviruses including mouse hepatitis virus, bovine coronavirus and porcine transmissible gastroenteritis virus, express IgG Fc binding proteins on infected cells.[19,20] This was shown using rabbit, mouse or rat non-immune IgG to immunoprecipitate the coronavirus S peplomer protein.[20] Antigenic domains on the S peplomer protein cross-react with sites on mammalian FcγRs, demonstrating conserved structural features between and viral and cellular proteins.[20] Of the viral FcγRs identified to date, the HSV-1 gE/gI complex is the best characterized as described below.

HSV-1 gE structure

HSV-1 gE is a 550 amino acid type I transmembrane glycoprotein that has two potential N-linked glycosylation sites and contains nine cysteines in the extracellular domain, one cysteine in the cleaved signal sequence and another in the transmembrane domain. (Figure 1)[9] Seven of the cysteine residues are in the central portion of the molecule. The disulfide bonding pattern of the cysteines has not been determined; therefore it is unknown whether gE forms globular domains characteristic of members of the immunoglobulin superfamily. gE has a large cytoplasmic tail of 106 amino acids that undergoes serine phosphorylation.[21]

Several studies have reported divergent results when mapping gE domains that bind IgG Fc. In one study, using overlapping gE peptides of 7–13 amino acids, five non-contiguous peptides exhibited Fc binding activity when reacted with nonimmune IgG in an enzyme immunoassay (22). Four of these peptides plus two additional peptides demonstrated Fc binding activity when tested in a rosette inhibition assay. These results suggest that many domains on gE

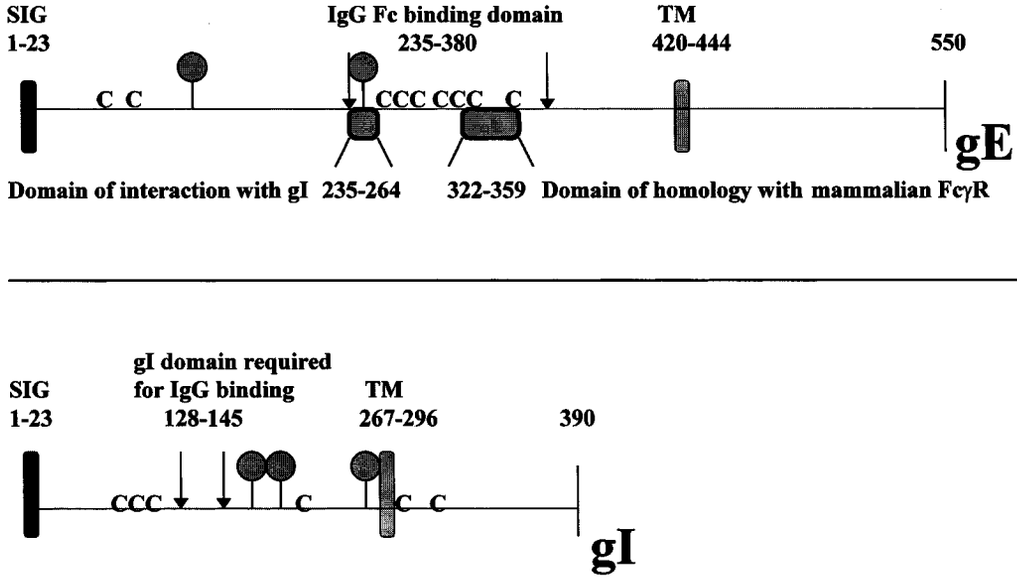

Figure 1. Schematic figures of HSV-1 gE (top) and gI (bottom) showing positions of the signal peptide (SIG), transmembrane domain (TM), potential N-linked glycosylation sites (gray balloons), and cysteines (C).[9] Arrows at gE amino acids 235 and 380 indicate the margins of IgG Fc binding domain.[23] Within this domain, the shaded rectangles indicate the gE region from amino acid 235-264 that forms a complex with gI,[24] and the gE domain from amino acid 322-359 that shares sequence homology with mammalian FcγRs.[23] Arrows at gI amino acids 128 and 145 indicate the gI domain that interacts with gE to form the higher affinity FcγR.[26]

contribute to Fc binding; however, the peptide approach is limited in that it does not take into consideration the effects of tertiary structure. In another study, gE fragments were cloned into the ectodomain of HSV-1 glycoprotein gD,[23] demonstrating that gE amino acids 183–402 bound IgG Fc. Further studies were performed using in-frame, four amino acid, linker insertion mutants.[23] Ten of twenty-one gE linker insertion mutants failed to bind IgG Fc, indicating that a region from amino acids 235–380 contributes to Fc binding (Figure 1). Each of these ten mutants failed to rosette IgG-coated erythrocytes or, when co-expressed with gI, to bind IgG monomers, indicating that this region is required for activity of both the lower and higher affinity FcγRs.[23,24] Comparisons of the ability of each of these mutants to form a hetero-oligomer with gI enabled a gE region required for complex formation to be localized between amino acids 235–264. These results were supported by experiments using gD/gE fusion proteins,[24] which demonstrated that a gE peptide spanning amino acids 183–288 was sufficient for interaction with gI. A region within the gE IgG Fc binding domain (amino acids 322–359) was noted to show strong similarity with human FcγRII extracellular domain 2 (amino acids 142–187),[23] the region of the mammalian FcγR that mediates Fc binding.[25]

HSV-1 gI structure

HSV-1 gI is a 390 amino acid type I transmembrane glycoprotein that contains three potential N-linked glycosylation sites and a cluster of four cysteine residues in the extracellular domain. (Figure 1)[9] gD/gI fusion proteins and gI linker insertion mutants were used to define gI domains required for IgG binding.[26] gD/gI fusion genes were transfected into cells infected with gE^+/gI^- virus, enabling expression of the gD/gI fusion proteins in the presence of gE but in the absence of wild-type gI. These studies demonstrated that a region of gI (amino acids 43–192) was sufficient for interaction with gE and formation of the high affinity IgG Fc binding complex. Linker insertion studies indicated that gI amino acids 128–145 are required for monomeric IgG Fc binding. (Figure 1) How this gI region contributes to IgG Fc binding has not been established. Possibilities include that sequences from both gE (amino acids 235–380) and gI (amino acids 128–145) combine to form the higher affinity FcγR, or that this region of gI changes gE conformation such that gE becomes a higher affinity FcγR.

HSV-1 FcγR functions

While early work focused on the role of the HSV-1 FcγR in binding non-immune IgG,[27,28] more recent studies from our laboratory addressed whether the HSV-1 FcγR preferentially binds the Fc domain of immune IgG,[29] thereby inhibiting antiviral effector functions including complement activation and ADCC. We demonstrated that anti-HSV IgG binds to its target antigen by its Fab end and to the HSV FcγR by its Fc end, a process called antibody bipolar bridging.[29] (Figure 2) Bipolar bridging was proposed since efficient binding of IgG to the FcγR depends on two conditions: (1) the IgG must be from a species whose Fc domain is capable of binding to the HSV FcγR and (2) antibodies must bind by their Fab domain to HSV antigens. The former condition was demonstrated by comparing the efficacy of various antibodies in blocking rosetting of IgG-coated erythrocytes to the HSV-1 infected cells.[29] Whereas human anti-gD MAb and rabbit anti-gC IgG efficiently blocked rosetting, murine anti-gD MAb did not block FcγR activity[29] because the Fc domain of murine IgG does not bind to the HSV-1 FcγR.[30] The latter condition was demonstrated by two observations.

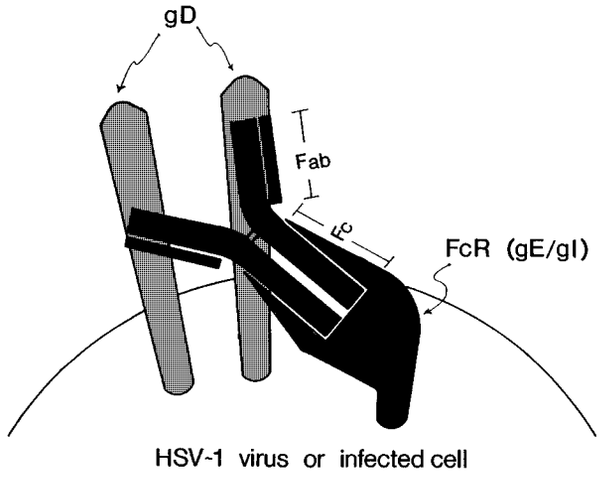

Figure 2. Model of antibody bipolar bridging: an IgG molecule (centre) binds by its Fab domain to its target antigen, shown here as HSV-1 glycoprotein gD (gray), and the Fc domain of the same IgG molecule binds to the HSV-1 FcγR which consists of glycoproteins gE and gI (black, 'U-shaped' structure). The FcγR blocks activities mediated by the Fc domain of IgG.

Firstly, rabbit IgG directed against viral gC or gD blocked rosetting 100–2000 fold more efficiently than non-immune IgG. Secondly, FcγR activity on cells infected with an HSV-1 gC null virus could be efficiently blocked with anti-gD IgG but not anti-gC IgG.[29] Thus, the HSV-1 FcγR effectively binds the IgG Fc domain when the IgG Fab domain binds to HSV antigens.

In vitro studies were performed to evaluate the importance of the FcγR in modifying IgG Fc-mediated functions. As predicted, antibody bipolar bridging inhibits C1q binding,[31] complement activation by the IgG Fc domain,[29] and ADCC.[31] These *in vitro* results suggest a biologically significant role of the HSV-1 FcγR and form the rationale for pursuing *in vivo* experiments.

Studies to define the role of the HSV-1 FcγR in immune evasion *in vivo*

A factor that complicates studies of the role of the HSV-1 FcR *in vivo* is that gE and gI are multifunctional proteins, exhibiting both FcγR activity and mediating cell-to-cell virus spread, since gE and gI null viruses produce small plaques in certain cell types *in vitro*.[32–34] The latter property appears separate from FcγR activity, since small plaques occur in absence of IgG.[33] Consequently, attenuation of HSV-1 gE or gI null viruses *in vivo* is probably due to defective spread in addition to any immune mediated effect.[32–34] It has also been noted that gE and gI of PRV are involved in spread, particularly transneuronal spread,[35] suggesting that this may be a conserved feature of alphaherpervirus gE/gI homologs.

We have developed an HSV-1 mutant virus that is $gE^+/gI^+/Fc\gamma R^-$ and capable of normal cell-to-cell spread in an animal model. On the basis of linker scanning data, a recombinant HSV-1 was constructed ($NS\text{-}gE_{339}$) carrying an insertion at gE amino acid position 339, such that the gE/gI complex is still formed, but inactive for FcγR activity.[23,36] Plaque size was normal in human epidermal keratinocyte cells, which indicated that cell-to-cell spread of the mutant virus remained intact.[36] This recombinant proved useful for *in vivo* studies to address the role of the FcγR in immune evasion independent of the function of gE/gI in cell-to-cell spread. Experiments performed in the murine flank model indicate that the HSV-1 FcγR provides significant protection to the virus against antibody mediated immunity.[37]

Viruses that interfere with complement activation by expressing complement regulatory proteins

The complement cascade is activated on contact with microorganisms and serves as one of the initial lines of host defense against infection. Activation of the cascade occurs by the classical, alternative or lectin complement pathways,[38] resulting in deposition of complement components on microbial surfaces. Injury to bystanders is prevented by cell-surface expression of proteins which down-regulate complement activity.[39] These include complement regulatory proteins 1, 2, 3, (CR1 [CD35], CR2 [CD21], CR3 [CD11b/CD18]) membrane cofactor protein (MCP, [CD46]) and decay accelerating factor (DAF [CD55]). These regulatory proteins are characterized by the presence of short consensus repeat sequences (SCRs): motifs of approximately 58 to 66 amino acids with four invariant cysteine residues in which cysteine 1 is disulfide linked to cysteine 3, and cysteine 2 is disulfide linked to cysteine 4. SCRs have 30–40% identity at the amino acid level with one another and they occur as a variable number of repeats, for example MCP and DAF have four SCRs while CR1 has thirty.[39] In addition to the above proteins that inhibit complement activation, cells are also able to inhibit the membrane attack complex (MAC) via a C9 binding protein, CD59.

Depositon of activated complement on virus surfaces may coat the virus and block attachment to cell receptors. Alternatively, activated complement may aggregate viruses and facilitate phagocytosis, or C3b and iC3b bound to the virus may promote opsonization via CR1 and CR3 receptors on monocytes, macrophages or granulocytes.[40–42] Complement may also lyse virus through generation of the MAC, which creates pores in the virus envelope.[42]

Viruses have evolved strategies for protection against complement activation. These can be classified into three general categories: (1) virus proteins which are homologous to mammalian complement regulatory proteins; (2) virus proteins which have no sequence homology, but share functional characteristics with complement regulatory proteins and (3) viruses that incorporate host complement regulatory proteins into their envelope during virus maturation. Examples from the first category include vaccinia complement-control protein (VCP) and herpesvirus saimiri (HVS) complement control-protein homolog (CCPH), since these proteins exhibit SCR sequences.[43] The C3b binding proteins of HSV-1, HSV-2, PRV-2,

BHV-1 and EHV-1 are examples of functional homologs within the second category.[44-46] HIV and CMV exemplify viruses within the third category, since the virions incorporate MCP (CD46), DAF (CD55) and the MAC inhibitor, CD59.[47,48]

Vaccinia virus complement-control protein

Vaccinia virus encodes a complement-control protein, VCP (C3L gene),[49] which is secreted from infected cells and protects the virus from antibody-dependent complement neutralization.[50] VCP binds complement components C4b and C3b, functioning as a cofactor with Factor I in cleaving C4b and C3b leading to inhibition of the complement cascade by accelerated decay of both the alternative and classical pathway C3-convertases.[51] Virus lacking VCP causes less skin disease in rabbits, suggesting a role for VCP in virulence.[50] A second vaccinia gene (B5R) encodes a glycoprotein with four SCRs that appears on the virion envelope and the membrane of infected cells,[52-54] although no complement regulatory or binding function has yet been described for this protein.

Complement-control proteins of HIV-1

HIV-1 activates complement by the classical and alternative complement pathways, mediated by viral proteins gp120 and gp41 in the absence of antibody and by naturally occurring cross-reactive IgM antibodies that recognize asialo-oligosaccharides on the virus.[55] Despite complement activation, complement-mediated viral lysis does not occur, which is linked to the presence of complement regulatory molecules on the viral envelope that prevent generation of the membrane attack complex.[48,56,57] These regulatory proteins include MCP, DAF and CD59, which are incorporated into the virus as it buds from T cell lines or peripheral blood mononuclear cells.[48,56,58]

Complement-control proteins of herpesviruses

A number of different complement inhibiton strategies are utilized by herpesviruses. Herpesvirus saimiri (HVS) encodes a complement-control protein homolog (CCPH) that inhibits C3 convertase activity, thereby decreasing deposition of the membrane attack complex (MAC) on infected cell surfaces and inhibiting complement mediated cell lysis.[59,60] CCPH is 52% identical to C4 binding protein[59,60] and is also homologous to MCP, DAF and VCP. Through alternative splicing, the gene encoding CCPH encodes two different forms of the protein, one anchored to the cell surface by a hydrophobic tail and the other secreted from the cell. A second HVS protein, HVSCD59, has functional homology and 48% amino acid sequence identity with human CD59.[61] Like human CD59, HVSCD59 is a GPI anchored membrane protein and it shows species specificity in that it most effectively inhibits the activity of C9 from primate serum.[62] Thus, HVS encodes proteins that can inhibit the complement cascade at two different control points, namely the C3 convertase and assembly of the MAC. Epstein–Barr virus (EBV), in the presence of factor I, possesses cofactor activity since it cleaves C3b, iC3b, C4b, and iC4b.[63] EBV also accelerates the decay of C3bBb, the alternative pathway C3 convertase.[63] HCMV up-regulates MCP and DAF on the surface of infected cells, protecting them from complement-dependent lysis. Furthermore, MCP, DAF and CD59 are present on the HCMV envelope, which may protect virions from complement-mediated damage.[64] A number of alphaherpesviruses encode a protein with functional homology to complement-control proteins as described below.

Structure of HSV-1 and -2 glycoprotein C (gC), a complement regulatory protein

gC of HSV-1 and -2 (gC-1 and gC-2, respectively) and gC homologs of PRV, BHV-1, and EHV-1 bind complement component C3b,[46,65-68] a critical protein in the complement cascade. These viral proteins share little homology with mammalian complement regulatory proteins; however, at the carboxy-terminal half of the molecule they are similar to one another.[68,69] possessing six carboxyl-terminal cysteines, as well as conserved spacing between the first two cysteines and conserved amino acids adjacent to the cysteines. The ability of various gC homologs to bind species-specific C3b suggests that they may have similar functions *in vivo*.

Our work focuses on gC-1 and gC-2. gC-1 is a 511 amino acid protein encoded by the HSV-1 UL44 gene. Four distinct gC-1 domains mediate C3b binding: (Figure 3).[44] These domains were mapped by site-directed and linker insertion mutagenesis and then testing for binding in rosetting assays using C3b-coated erythrocytes.[44] gC-1 has eight cysteine

residues that form four internal disulfide bonds. (Figure 3)[70] gC-2 is a 480 amino acid protein, encoded by the HSV-2 UL44 gene and has three domains that mediate C3b binding: (Figure 3).[45] The disulfide bonding pattern of gC-2 has not yet been defined, but the cysteine positions are highly conserved with gC-1 and gC homologs of other alphaherpesviruses.[69]

gC-1 and gC-2 function as inhibitors of the complement cascade

Purified glycoproteins gC-1 and gC-2 bind C3b but only gC-1 accelerates the decay of the alternative pathway C3 convertase, C3bBb.[71,72] This difference is probably due to the ability of the amino-terminal region of gC-1 (but not gC-2) to inhibit properdin binding to C3b.[71,73] The amino-terminal domain of gC-1 also inhibits the interaction of C5 with C3b, decreasing activation of the terminal components of the complement cascade.[71,72] gC-1 binds native C3 and its enzymatic cleavage products C3b, iC3b and C3c, but not C3d suggesting that the binding site on C3 is located in the C3c fragment.[71,74] On infected cells, gC-1 but not gC-2 binds C3b,[65] while on transfected cells both gC-1 and gC-2 bind C3b.[75] The basis for this difference is not defined, but may be due to other HSV-2 protein(s) interfering with C3b binding. On transfected and infected cells, gC-1 protects the cell from complement-mediated lysis; similar studies have yet to be performed for gC-2. On the virion, both gC-1 and gC-2 inhibit complement-mediated neutralization.[76–78]

The effects of gC-1 in preventing complement-mediated neutralization and cell lysis were tested using HSV-1 mutants expressing no gC (gC-null) or mutated gC (gC-mut) which does not bind C3b. In the absence of antibody, complement lysed cells infected with gC-mut while having little effect on cells infected with wild-type virus.[79] Complement cytolysis was mediated by activation of the alternative complement pathway.[79] In the absence of antibody, complement neutralized gC-null and gC-mut viruses approximately 50-fold more efficiently than wild-type or gC-rescued viruses.[78] In contrast to cytolysis, virus neutralization was mediated by components of the classical complement pathway, since C4 deficient serum failed to neutralize gC mutant viruses.[78]

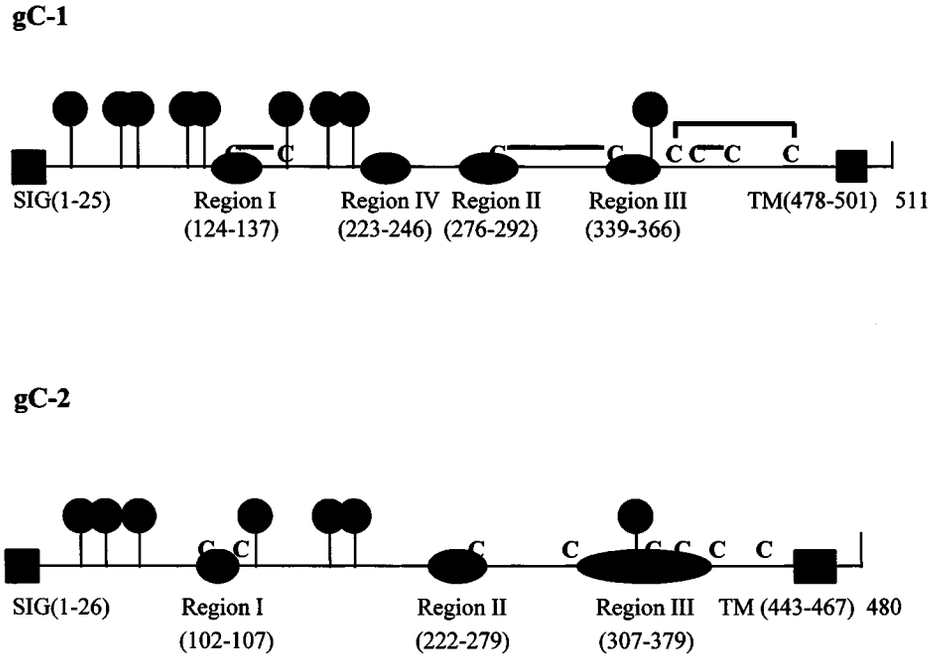

Figure 3. Schematic figures of HSV gC-1 (top)[44] and gC-2 (bottom)[45] showing positions of the signal sequence (SIG), transmembrane domain (TM), potential N-linked glycosylation sites (balloons), and cysteines (C). Intrachain disulfide bonding pattern has been defined for gC-1 and is represented by lines joining two cysteines.[70] The C3b binding regions are indicated by ovals and the corresponding amino acid positions are noted below each oval.

Studies of gC-mediated immune evasion *in vivo*

We initiated studies to define the importance of gC-complement interactions *in vivo* using a guinea pig vaginal model of infection. gC-1 is a multifunctional protein which, in addition to inhibiting complement activation, mediates virus binding to cell surface heparan sulfate, the initial step in virus attachment to cells.[80] Several regions of gC-1 appear to be involved in the latter activity, including amino acids 33–123[81] and an arginine-rich, polycationic domain around amino acid 150.[82] The fact that gC-1 has at least two functions, heparan binding and complement regulatory activity, has complicated attempts to study the significance of gC-mediated complement inhibition *in vivo*.

We have now identified a gC mutant virus that *in vitro* is defective for C3 binding but intact for virus attachment,[83] which should facilitate interpretation of *in vivo* experiments. Guinea pigs were infected intravaginally with this gC-1 mutant virus or a rescued strain. Vaginal titers were 20–30 fold higher in animals infected with the gC-1 rescued virus compared with the mutant.[83] To evaluate the role of complement, virus was inoculated into C3 deficient guinea pigs that have serum C3 levels approximately 6% of normal and total hemolytic complement activity 15% of normal. The vaginal titers of the gC mutant virus were higher in C3 deficient guinea pigs, while titers of wild-type virus showed little change.[83] These results support the importance of gC-complement interaction *in vivo*.

Conclusions

Complement and antibody represent two important lines of defense against virus infection. As described above, a number of viruses have the capacity to interfere with each of these mechanisms as exemplified by HSV-1 gC and gE/gI. gC modifies antibody-independent complement activation; therefore, we postulate that gC may be particularly important early in infection before antibodies develop. gE/gI block later events that require the Fc domain of anti-HSV IgG to activate complement and mediate ADCC. Do these immune evasion activities act in synergy to enable virus to escape host attack? Construction of mutant viruses defective in both complement regulatory and Fc binding activities are required to address this question. Studies of gC, gE, and gI immune evasion may have broad implications in microbial pathogenesis because FcγRs and complement regulatory proteins are expressed on many microorganisms.

Acknowledgements

Liyang Wang contributed to gC and gE/gI studies; Gary Cohen, Roselyn Eisenberg, John Lambris and their students and post-doctoral fellows to gC studies; Gary Dubin, Ian Frank, Saswata Basu, Periasamy Sundaresan, Lester Goldstein and Benjamin Weeks contributed to gE/gI studies, and Stuart Isaacs helped edit the manuscript. This work was supported by NIH grants AI 33063 and HL 28220.

References

1. Anderson CL, Looney RJ (1986) Human leukocyte IgG Fc receptors. Immunol Today 7:264–266
2. Kinet J-P (1989) Antibody-cell interactions: Fc receptors. Cell 57:351–354
3. Ravetch JV (1994) Fc receptors: rubor redux. Cell 78:553–560
4. Watkins JF (1964) Adsorption of sensitized sheep erythrocytes to HeLa cells infected with herpes simplex virus. Nature 202:1364–1365
5. Westmoreland D, Watkins JF (1974) The IgG receptor induced by herpes simplex virus: studies using radioiodinated IgG. J Gen Virol 24:167–178
6. Baucke RB, Spear PG (1979) Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J Virol 32:779–789
7. Johnson DC, Feenstra V (1987) Identification of a novel herpes simplex virus type 1-induced glycoprotein which complexes with gE and binds immunoglobulin. J Virol 61:2208–2216
8. Johnson DC, Frame MC, Ligas MW, Cross AM, and Stow ND (1988) Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI. J Virol 62:134–1354
9. McGeoch DJ, Dolan JA, Donald S, Rixon FJ (1985) Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J Mol Biol 181:1–13
10. Bell S, Cranage M, Borysiewicz L, Minson T (1990) Induction of immunoglobulin G Fc receptors by recombinant vaccinia viruses expressing glycoproteins E and I of herpes simplex virus type 1. J Virol 64:2181–2186
11. Hanke T, Graham FL, Lulitanond V, Johnson DC (1990) Herpes simplex virus IgG Fc receptors induced using recombinant adenovirus vectors expressing glycoproteins E and I. Virology 177:437–444
12. Dubin G, Frank I, Friedman HM (1990) Herpes simplex virus type 1 encodes two Fc receptors which have different binding characteristics for monomeric immunoglobulin G (IgG) and IgG complexes. J Virol 64:2725–2731
13. Costa J, Yee C, Nakamura Y, Rabson A (1978) Characteristics of the Fc receptor induced by herpes simplex virus. Intervirology 10:32–39
14. Litwin V, Sandor M, Grose C (1990) Cell surface expression of the varicella-zoster virus glycoprotein and Fc receptor. Virology 178:263–272

15. Litwin V, Grose C (1992) Herpes viral Fc receptors and their relationship to the human Fc receptors. Immun Res 11:226–238
16. Para MF, Goldstein L, Spear PG (1982) Similarities and differences in the Fc-binding glycoprotein (gE) of herpes simplex virus types 1 and 2 and tentative mapping of the viral gene for this glycoprotein. J Virol 41:137–144
17. MacCormac LP, Grundy JE (1996) Human cytomegalovirus induces and Fc γ receptor (FcγR) in endothelial cells and fibroblasts that is distinct from the human cellular FcγRs. J Infect Dis 174:1151–1161
18. Thale R, Lucin P, Schneider K, Eggers M, Koszinowski UH (1994) Identification and expression of a murine cytomegalovirus early gene coding for an Fc receptor. J Virol 68:7757–7765
19. Oleszak EL, Leibowitz JL (1990) Immunoglobulin Fc binding activity is associated with the Mouse Hepatitis Virus E2 peplomer protein. Virology 176:70–80
20. Oleszak EL, Kuzmak J, Hogue B, Parr R, Collisson EW, Rodkey LS, Leibowitz JL (1995) Molecular mimicry between Fc receptor and S peplomer protein of mouse hepatitis virus, bovine corona virus, and transmissible gastroenteritis virus. Hybridoma 14:1–8
21. Edson CM, Hosler BA, Waters DJ (1987) Varicella-zoster virus gpI and herpes simplex virus gE: Phosphorylation and Fc binding. Virology 161:599–602
22. Williams RC, Kievit E, Tsuchiya N, Malone C, Hutt-Fletcher L (1992) Differential mapping of Fc-binding and monoclonal antibody reactive epitopes on gE, the Fc-binding glycoprotein of herpes simplex virus type 1. J Immunol 149:2415–2427
23. Dubin G, Basu S, Mallory DL, Basu M, Tal-Singer R, Friedman, HM (1994) Characterization of domains of herpes simplex virus type 1 glycoprotein E involved in Fc binding activity for immunoglobulin G aggregates. J Virol 68:2478–2485
24. Basu S, Dubin G, Basu M, Nguyen V, Friedman HM (1995) Characterization of regions of herpes simplex virus type 1 glycoprotein E involved in binding the Fc domain of monomeric IgG and in forming a complex with glycoprotein I. J Immunol 154:260–267
25. Hulett MD, Wifurt E, Brinkworth RI, McKenzie IFC, Hogarth PM (1994) Identification of the IgG binding site of the low affinity receptor for IgG FcγRII: enhancement and ablation of binding by site-directed mutagenesis. J Biol Chem 269:15287–15293
26. Basu S, Dubin G, Nagashunmugam T, Basu M, Goldstein LT, Wang L, Weeks B, Friedman HM (1997) Mapping regions of herpes simplex virus type 1 glycoprotein I required for formation of the virus Fc receptor for monomeric IgG. J Immunol 158:209–215
27. Adler R, Glorioso JC, Cossman J, Levine M (1978) Possible role of Fc receptors on cells infected and transformed by herpesvirus: escape from immune cytolysis. Infect Immun 21:442–447
28. Dowler KW, Veltri RW (1984) *In vitro* neutralization of HSV-2: inhibition by binding of normal IgG and purified Fc to virion Fc receptor (FcR). J Med Virol 13:251–259
29. Frank I, Friedman HM (1989) A novel function of the herpes simplex type 1 Fc receptor: participation in bipolar bridging of antiviral immunoglobulin G. J Virol 63:4479–4488
30. Johansson PJH, Myhre EB, Blomberg J (1985) Specificity of Fc receptors induced by herpes simplex virus type 1: comparison of immunoglobulin G from different animal species. J Virol 56:489–494
31. Dubin G, Socolof E, Frank I, Friedman HM (1991) Herpes simplex virus type 1 Fc receptor protects infected cells from antibody-dependent cellular cytotoxicity. J Virol 65: 7046–7050
32. Balan P, Davis-Poynter N, Bell S, Atkinson H, Browne H, Minson T (1994) An analysis of the *in vitro* and *in vivo* phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or putative gJ. J Gen Virol 75: 1245–1258
33. Dingwell KS, Doering LC, Johnson DC (1995) Glycoprotein E and I facilitate neuron-to-neuron spread of herpes simplex virus. J Virol 69:7087–7098
34. Dingwell KS, Brunetti CR, Hendricks RL, Tang Q, Tang M, Rainbow AJ, Johnson DC (1994) Herpes simplex virus glycoprotein E and I facilitate cell-to-cell spread *in vivo* and across junctions of cultured cells. J Virol 68:834–845
35. Enquist LW, Dubin J, Whealy ME, Card JP (1994) Complementation analysis of pseudorabies virus gE and gI mutants in retinal ganglion cell neurotropism. J Virol 68:5275–5279
36. Weeks BS, Sundaresan P, Nagashunmugam T, Kang E, Friedman HM (1997) The herpes simplex virus-1 glycoprotein E (gE) mediates IgG binding and cell-to-cell spread through distinct gE domains. Biochem Biophys Res Commun 235: 31–35
37. Nagashunmugam T, Lubinski J, Wang L, Goldstein LT, Weeks BS, Sundaresan P, Kang EH, Dubin G, Friedman HM (1998) *In vivo* immune evasion mediated by herpes simplex virus type 1 IgG Fc receptor. J Virol (in press)
38. Reid KBM, Turner MW (1994) Mammalian lectins in activation and clearance mechanisms involving the complement system. Springer Semin Immunopathol 15:307–325
39. Morgan BP, Meri S (1994) Membrane proteins that protect against complement lysis. Springer Semin Immunopathol 15:369–396
40. Van Strijp JAG, van Kessel KPM, Miltenburg LAM (1989) Attachment of human polymorphonuclear leukocytes to herpes simplex-infected fibroblasts mediated by antibody-independent complement activation. J Virol 62:847–850
41. Van Strijp JAG, van Kessel KPM, van der Rol ME (1989) Complement mediated phagocytosis of herpes simplex virus by granulocytes: binding or ingestation. J Clin Invest 84:107–112
42. Cooper NR, Nemerow GR (1986) Complement-dependent mechanisms of virus neutralization, in Immunobiology of the Complement System. An Introduction for Research and Clinical Medicine. (Ross GD, ed.) pp 139–162. Academic Press, New York
43. Fishelson Z (1994) Complement-related proteins in pathogenic organisms. Springer Semin Immunopathol 15: 345–368
44. Hung S-L, Sriniivasan S, Friedman HM, Eisenberg RJ, Cohen GH (1992) Structural basis of C3b binding by glycoprotein C of herpes simplex virus. J Virol 66:4013–4027
45. Seidel-Dugan C, Ponce de Leon M, Friedman HM, Eisenberg RJ, Cohen GH (1990) Identification of C3b-binding regions on herpes simplex virus type 2 glycoprotein C. J Virol 64:1897–1906
46. Huemer HP, Larcher C, den Hurk S, Babiuk LA (1993) Species selective interaction of Alphaherpesvirinae with the 'unspecific' immune system of the host. Arch Virol 130:353–364
47. Spiller OB, Hanna SM, Devine DV, Tufaro F (1997) Neutralizations of cytomegalovirus virions: the role of complement. J Infect Dis 176:339–347
48. Montefiori DC, Cornell RJ, Zhou JY, Hirsch VM, Johnson PR (1994) Complement control proteins, CD46, CD55, CD59 as common surface constituents of human and simian immunodeficiency viruses and possible targets for vaccine protection. Virology 205:82–92
49. Kotwal GJ, Isaacs SN, McKenzie R, Frank MM, Moss B. (1990) Inhibition of the complement cascade by the major secretory protein of vaccinia virus. Science 250:827–830
50. Isaacs SN, Kotwal GJ, Moss B (1992) Vaccinia virus complement-control protein prevents antibody-dependent comple-

ment-enhanced neutralization of infectivity and contributes to virulence. Proc Natl Acad Sci USA 89:628–632
51. McKenzie R, Kotwal GJ, Moss B, Hammer CH, Frank MM. (1992) Regulation of complement activity by vaccinia virus complement-control protein. J Infect Dis 166:1245–1250
52. Isaacs SN, Wolffe EJ, Payne LG, Moss B (1992) Characterization of a vaccinia virus-encoded 42-kilodalton class I membrane glycoprotein component of the extracellular virus envelope. J Virol 66:7217–7224
53. Englestad M, Howard ST, Smith GL (1992) A constitutively expressed vaccinia gene encodes a 42-kDa glycoprotein that forms part of the extracellular virus envelope. Virology 188:801–810
54. Takashi-Nishimaki F, Funahashi S-I, Miki K, Hashizume S, Sugimoto M (1991) Regulation of plaque size and host range by a vaccinia virus gene related to complement system proteins. Virology 181:158–164
55. Montefiori DC (1997) Role of complement and Fc receptors in the pathogenesis of HIV-1 infection. Springer Semin Immunopathol 18:371–390
56. Saifuddin M, Parker CJ, Peeples ME, Gorny MK, Zola-Passner S, Ghassemi M, Rooney IA, Atkinson JP, Spear GT (1995) Role of virion associated glycosylphosphatidylinositol-linked proteins CD55 and CD59 in complement resistance of cell-line derived and primary isolates of HIV-1. J Exp Med 182:501–509
57. Schmitz J, Zimmer JP, Kluxen B, Aries S, Bogel M, Gigli I, Schmitz H (1995) Antibody-dependent complement-mediated cytotoxicity in sera from patients with HIV-1 infection is controlled by CD55 and CD59. J Clin Invest 96:1520–1526
58. Marschang P, Sodroski J, Wurzner R, Dierich MP (1995) Decay-accelerating factor (CD55) protects human immunodeficiency virus type 1 from inactivation by human complement. Eur J Immunol 25:285–290
59. Fodor WL, Rollins SA, Bianco-Caron S, Rother RP, Guilmette ER, Burton WV, Albrecht J-C, Fleckenstein B, Squinto, SP (1995) The complement control protein homolog of herpesvirus saimiri regulates serum complement by inhibiting C3 convertase activity. J Virol 69:3889–3892
60. Albrecht J-C, Fleckenstein B (1992) New member of the multigene family of complement control proteins in herpes virus saimiri. J Virol 66:3937–3940
61. Rother RP, Rollins SA, Fodor WL, Albrecht J-C, Setter E, Fleckenstein B, Squinto SP (1994) Inhibition of complement-mediated lysis by the terminal complement inhibitor of herpesvirus saimiri. J Virol 68:730–737
62. Rollins SA, Zhao J, Ninomiya H, Sims PJ (1991) Inhibition of homologous complement by CD59 is mediated by a species-selective recognition conferred through binding to C8 within C5b-8 or C9 within C5b-9. J Immunol 146:2345–2351
63. Mold C, Bradt BM, Nemerow GR, Cooper NR (1988) Epstein-Barr virus regulates activation and processing of the third component of complement. J Exp Med 168:949–969
64. Spiller OB, Morgan BP, Tufaro F, Devine DV (1996) Altered expression of host-encoded complement regulators on human cytomegalovirus-infected cells. Eur J Immunol 26:1532–1538
65. Friedman HM, Cohen GH, Eisenberg RJ, Seidel CA, Cines DB (1984) Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. Nature 309:633–635
66. Eisenberg RJ, Ponce de Leon M, Friedman HM, Fries LF, Frank MM, Hastings J, Cohen GH (1987) Complement component C3b binds directly to purified glycoprotein C of herpes simplex virus types 1 and 2. Microb Pathog 3:423–435
67. Heumer HP, Larcher C, Coe NE (1992) Pseudorabies virus glycoprotein III derived from virions and infected cells binds to the third component of complement. Virus Res 23:271–280
68. Allen G, Coogle L (1988) Characterization of an equine herpesvirus type 1 gene encoding a glycoprotein (gp13) with homology to herpes simplex virus glycoprotein C. J Virol 62:2850–2858
69. Fitzpatrick DR, Babiuk LA, Zamb TJ (1989) Nucleotide sequence of bovine herpesvirus type 1 glycoprotein III, a structural model for gIII as a new member of the immunoglobulin superfamily, and implications for the homologous proteins of other herpesviruses. Virology 173:46–57
70. Rux AH, Moore WT, Lambris JD, Abrams WR, Peng C, Friedman HM, Cohen GH, Eisenberg RJ (1996) Disulfide bond structure determination and biochemical analysis of glycoprotein C from herpes simplex virus. J Virol 70:5455–5465
71. Kostavasili I, Sahu A, Friedman HM, Eisenberg RJ, Cohen GH, Lambris JD (1997) Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. J Immunol 158:1763–1771
72. Fries LF, Friedman HM, Cohen GH, Eisenberg RJ, Hammer CH, Frank MM (1986) Glycoprotein C of herpes simplex virus 1 is an inhibitor of the complement cascade. J Immunol 137:1636–1641
73. Hung S-L, Peng C, Kostavasili I, Friedman HM. Lambris JD, Eisenberg RJ, Cohen GH (1994) The interaction of glycoprotein C of herpes simplex virus types 1 and 2 with the alternative complement pathway. Virology 203:299–312
74. Tal-Singer R, Seidel-Dugan C, Fries L, Huemer HP, Eisenberg RJ, Cohen GH, Friedman HM (1991) Herpes simplex virus glycoprotein C is a receptor for the complement component iC3b. J Infect Dis 164:750–753
75. Seidel-Dugan C, de Leon M, Friedman HM, Fries LF, Frank MM, Cohen GH, Eisenberg RJ (1988) C3b receptor activity on transfected cells expressing glycoprotein C of herpes simplex virus types 1 and 2. J Virol 62:4027–4036
76. Harris SL, Frank I, Yee A, Cohen GH, Eisenberg RJ, Friedman HM (1990) Glycoprotein C of herpes simplex virus type 1 prevents complement-mediated cell lysis and virus neutralization. J Infect Dis 162:331–337
77. McNearney TA, Odell C, Holers VM, Spear PG, Atkinson JP (1987) Herpes simplex virus glycoproteins gC-1 and gC-2 bind the third component of complement and provide protection against complement-mediated neutralization of viral infectivity. J Exp Med 166:1525–1535
78. Gerber SI, Belval BJ, Herold BC (1995) Differences in the role of glycoprotein C of HSV-1 and HSV-2 in viral binding may contribute to serotype differences in cell tropism. Virology 214:29–39
79. Friedman HM, Wang L, Fishman NO, Lambris JD, Eisenberg RJ, Cohen GH, Lubinski JM (1996) Immune evasion properties of herpes simplex virus type 1 glycoprotein gC. J Virol 70:4253–4260
80. Herold BC, WuDunn D, Soltys N, Spear PG (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 65:1090–1098
81. Tal-Singer R, Peng C, de Leon M, Abrams WR, Banfield BW, Tufaro F, Cohen GH, Eisenberg RJ (1995) The interaction of herpes simplex glycoprotein C with mammalian cell surface molecules. J Virol 69:4471–4483
82. Trybala E, Bergstrom T, Svennerholm B, Jeansson S, Glorioso JC, Olofsson S (1994) Localization of a functional site on herpes simplex virus type 1 glycoprotein C involved in binding to cell surface heparan sulfate. J Gen Virol 75:743–752
83. Friedman HM, Wang L, Lambris J, Eisenberg RJ Cohen GH, Burger R, Frank MM, Lubinski J (1997) Immune evasion by HSV-1 glycoprotein gC. Presented at the 22nd International Herpesvirus Workshop, San Diego, CA, Abstract 417