TRANSACTIONS OF THE AMERICAN CLINICAL AND CLIMATOLOGICAL ASSOCIATION, VOL. 114, 2003

IMMUNE EVASION BY HERPES SIMPLEX VIRUS TYPE 1, STRATEGIES FOR VIRUS SURVIVAL

HARVEY M. FRIEDMAN

PHILADELPHIA, PENNSYLVANIA

ABSTRACT

Many viruses capable of persistent or recurrent infections have evolved strategies to evade host immunity. Viral evasion molecules target components of innate and acquired immunity, including complement proteins, natural killer cells, MHC Class I or Class II molecules and antibody. Our work focuses on HSV-1 glycoproteins gC and gE that impair antibody and complement responses. gC inhibits complement activation by binding C3b and blocking activities mediated by this pivotal complement protein, while gE binds the IgG Fc domain, blocking Fc-mediated activities, including complement activation and antibody-dependent cellular cytotoxicity. HSV-1 mutant viruses that lack the ability to bind C3b, IgG Fc, or both are much less virulent than wild-type virus in a murine model. These HSV-1 immunoevasins help explain the virus' ability to produce recurrent infections despite intact immunity. Strategies to prevent immune evasion may be required to develop successful HSV vaccines.

INTRODUCTION

In the past two decades, studies of viral pathogenesis have revealed fascinating and wily mechanisms used by microorganisms to evade host immunity. Viral proteins have been identified that interfere with many steps in innate and adaptive immune responses. Immune evasion appears to be particularly important as a strategy to escape from immune attack for viruses that cause recurrent infections, including herpes viruses, which are common human pathogens. Herpes simplex virus types 1 and 2 (HSV-1 and -2) produce recurrent episodes of fever blisters and genital disease, varicella zoster virus causes shingles, while human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and human herpesvirus type 8 (HHV-8) frequently cause relapsing infections in immunocompromised subjects. Each of these viruses encodes

Harvey M Friedman, M.D., 502 Johnson Pavilion, University of Pennsylvania, Philadelphia, PA 19104-6073, Phone: 215 662-3557, Fax: 215 349-5111, Email: hfriedma@mail.med.upenn.edu

immunoevasins that likely contribute to the virus' ability to cause recurrent infections (1).

Viral immunoevasins that inhibit complement activation are described in Table 1. HIV, HCMV and vaccinia virus incorporate cellular complement regulatory proteins into the viral envelop as they egress from cells. These regulatory proteins protect the virus from complement-mediated injury. Murine γ herpesvirus and herpesvirus saimiri encode viral proteins homologous to mammalian complement regulatory proteins, suggesting that these viruses have incorporated mammalian DNA into their genome. HSV-1 and -2, bovine herpes virus type 1, pseudorabies virus and equine herpes virus type 1 bind complement component C3b, despite the proteins involved having no apparent sequence similarities to mammalian C3b-binding proteins. This observation suggests that some viruses have acquired complement regulatory functions by convergent evolution.

Many viruses have evolved strategies to inhibit MHC Class I antigen expression, which is required to induce cytotoxic CD8 T cell responses. Down-regulation of MHC Class I triggers natural killer (NK) cell cytotoxicity. Therefore, the possibility exists that cells harboring viruses that down-regulate Class I expression will be recognized and killed by NK cells. HCMV and HHV-8 have devised strategies to prevent this from occurring (Table 1). HCMV encodes UL18, which is homologous to the MHC Class I α chain and foils NK cells by mimicking MHC Class I expression on infected cells (2, 3). The HHV-8 K5

TABLE 1

Viral Strategies to Evade Innate Immunity Mediated by Complement and NK Cells (1)

Viral Mechanism	Virus (Gene) Involved
Evasion of complement	
1. Virus incorporates cellular C-regulatory proteins into envelope: CD46, CD55, CD59	HCMV, HIV, Vaccinia, Smallpox
2. Viral protein has SCR sequences similar to mammalian C control proteins	Murine γ herpesvirus (RCA), HVS (HVSCD59, CCPH), Vaccinia (VCP)
3. Viral protein interacts with C, despite having no sequence homology	PRV (gC), EHV-1 (gC), BHV-1 (gC), HSV-1, -2 (gC), HIV-1 (gp120, gp41)
Evasion of NK cells	
1. Viral protein acts as decoy–class I homolog	HCMV (UL18)
2. Down-regulate NK cell ligands ICAM-1 & B7-2	HHV-8 (K5)

Abbreviations: SCR, short consensus repeats; HVS, herpesvirus saimiri; C, complement; PRV, pseudorabies virus; EHV-1, equine herpesvirus type 1; BHV-1, bovine herpesvirus type 1.

protein down-regulates ICAM-1 and B7-2, which are required for efficient NK cell killing (4).

Viral strategies to evade MHC Class I antigen presentation are listed in Table 2. Certain viral proteins, such as adenovirus E3 and HCMV US3, retain Class I molecules in the endoplasmic reticulum, thereby blocking trafficking to the cell surface. Some viruses encode proteins that degrade the MHC Class I complex, stimulate endocytosis of the complex thereby removing it from the cell surface, inhibit antigen presentation by interfering with the peptide antigen transporter system, or inhibit proteolysis by the proteosome, which prevents peptide production (1). Viral strategies to evade MHC Class II CD4 T-helper cell responses have also been described (Table 2). These include viral proteins that block cytokine-mediated upregulation of Class II antigens, and proteins that degrade the Class II complex (5, 6).

Many human and veterinary herpes viruses encode proteins that bind the IgG Fc domain, which are referred to as FcγR proteins (Table 2). Glycoproteins gE and gI form a heterodimer complex that functions as an FcγR for HSV-1. During HSV infection, antibodies are produced to HSV antigens, including those expressed on the virion or infected cell surface. The Fab domain of the IgG antibody binds to its target antigen, while the Fc region of the same antibody molecule binds to the gE-gI complex, which blocks activities mediated by the IgG Fc domain, including complement activation and antibody-dependent cellular cytotoxicity (7, 8) (Figure 1).

Complement is activated on the virus or infected cell surface either in the presence or absence of antibody, which leads to cleavage of C3

TABLE 2

Viral Strategies to Evade Acquired Immunity Mediated by B and T Cells (1, 18)

Viral Mechanism	Virus (Gene) Involved
Evasion of MHC Class I	
1. ER retention	Adeno (E3), HCMV (US3)
2. Degradation	HCMV (US2, US11), HIV (VPU)
3. Endocytosis	HIV (Nef)
4. Prevent proteolysis	EBV (EBNA1)
5. Inhibit TAP	HCMV (US6), HSV (ICP47)
Evasion of MHC Class II	
1. Block IFN-γ up-regulation of Class II	Adeno (E1A), HCMV (IE/E)
2. Degradation	HCMV (US2)
Evasion of IgG Fc	
1. Block activities of IgG Fc domain	HSV-1, -2 (gE), VZV (gE), PRV (gE), HCMV (TRL111)

Abbreviations: Adeno, adenovirus; IFN-γ, interferon-γ; VZV, varicella zoster virus.

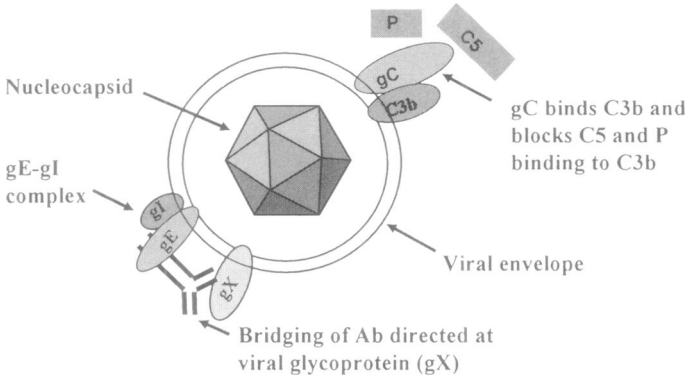

FIG. 1. Cartoon of immune evasion strategies mediated by HSV-1 glycoproteins gC and gE. An antibody molecule binds by the Fab domain to its target, shown as glycoprotein gX, while the Fc domain of the same antibody molecule binds to glycoprotein gE, which forms a heterodimer complex with glycoprotein gI. By binding the IgG Fc domain, gE blocks activities mediated by Fc, such as complement activation and antibody-dependent cellular cytotoxicity. Complement is activated by the virus in the absence or presence of antibody. C3 is cleaved to C3a and C3b. The latter covalently attaches to the virus surface. gC has a domain that binds to C3b and blocks the ability of C3b to participate in activation of other complement proteins. gC also interferes with C5 and properdin (P) binding to C3b. The net effect is that gC inhibits the complement cascade. Abbreviations: Ab, antibody; C, complement.

into C3a and C3b. The latter becomes covalently bound to the activating surface. gC has a C3b-interacting domain, which enables gC to bind to C3b and block activities mediated by this pivotal complement protein (9–11). gC also has a domain at its N-terminus that blocks the interactions of properdin (P) and C5 with C3b (12) (Figure 1). The net effect is that gC is a potent inhibitor of the complement cascade.

Our studies focus on two immunoevasins encoded by HSV-1, gC and gE. Glycoproteins gC and gE are expressed on the virus envelop and at the infected cell surface where they can readily interact with antibody and complement. Below, we describe studies that address the role of these HSV-1 immunoevasins in vitro and in a murine model. We demonstrate that these glycoproteins are major contributors to pathogenesis because they function as immunoevasins.

MATERIALS AND METHODS

Preparation of gC and gE Mutant HSV-1 Strains

To evaluate the biologic relevance of gC and gE as immunoevasins, a panel of HSV-1 mutant viruses were constructed, each derived from

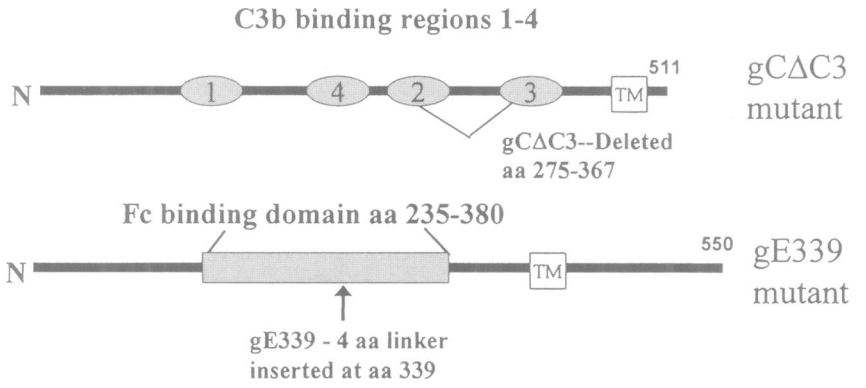

FIG. 2. Stick figure showing features of HSV-1 glycoproteins gC and gE. gC is a 511 amino acid glycoprotein that has 4 domains involved in C3b binding (19). Domains two and three were deleted (amino acids 275–367), and the mutant gene was recombined into virus to replace wild-type gC. The mutant virus, referred to as NS-gCΔC3, no longer binds complement component C3b (13). gE is a 550 amino acid glycoprotein that has an IgG Fc binding domain extending from amino acids 235–380. Four amino acids were inserted after gE amino acid 339, which disrupts the ability of the glycoprotein to bind IgG Fc (14). The mutant gene was recombined into virus to replace wild-type gE and create a mutant strain NS-gE339 (14). A third mutant virus, NS-gCΔC3,gE339 was created by replacing both wild-type gC and gE with the respective mutant genes (15). Abbreviations: N, amino terminus; TM, transmembrane domain.

wild-type strain NS (Figure 2). Strain NS-gCΔC3 has a deletion of gC amino acids 275–367, which eliminates the ability of the gC protein to bind C3b (13). Strain NS-gE339 has four amino acids inserted after gE amino acid 339, which eliminates the ability of gE or the gE-gI complex to bind the IgG Fc domain (14). NS-gCΔC3,gE339 contains both gC and gE mutations described above. The gC/gE double-mutant virus fails to bind C3b and IgG Fc (15).

Virus Neutralization Assays

Approximately 10^6 plaque forming units (PFU) of wild-type, gC mutant, gE mutant, or gC/gE double-mutant virus was incubated for 1 hour at 37°C with PBS as a control, or with anti-HSV IgG (100 μg/ml) and 10% human complement obtained from an HSV seronegative donor. The amount of neutralization was determined by plaque assay on Vero cells and was calculated by subtracting the titer when virus was incubated with antibody and complement from the titer when virus was incubated with PBS.

Murine Studies With Antibody Passive Transfer in Complement-Intact Mice (Antibody and Complement Present)

Five to six week old female BALB/c mice were passively immunized intraperitoneally with 100 μg of pooled human IgG (HSV antibody). Passive transfer of human HSV antibodies are used to assess the role of the HSV-1 FcγR in immune evasion because the Fc domain of human IgG binds to the HSV-1 FcγR, while murine IgG Fc does not (16). Human antibody activates mouse complement, and murine C3b binds to gC; therefore, mice serve as the source of complement for these studies (17). Sixteen hours after antibody passive transfer, mice were infected on the denuded flank with wild-type, gC or gE single-mutant or gC/gE double-mutant virus. Disease at the inoculation site was calculated by counting the number of lesions that developed. One point was assigned for each lesion up to a maximum daily score of 5 points, which is the upper limit of the number of distinct lesions that can be readily counted. Results are presented as the cumulative lesion scores from days 3–7. If lesions coalesced such that individual lesions could not be distinguished from one another, scores were based on the surface area of skin involved (13).

Murine Studies Without Antibody Passive Transfer in C3-Deficient Mice (Antibody and Complement Absent)

Experiments were performed as described above, except that non-immune human IgG was used for passive transfer instead of pooled human IgG, and infection was performed in C3 knockout mice (15).

RESULTS

Virus neutralization experiments were performed comparing wild-type virus, NS, gC mutant virus, NS-gCΔC3, gE mutant virus, NS-gE339, or gC/gE double-mutant virus, NS-gCΔC3,gE339 (15). 10^6 PFU of virus was incubated for 1 hour at 37°C with PBS, as control, or with antibody (100 μg/ml pooled human IgG) and complement (10% HSV seronegative human serum), and the amount of virus that resisted neutralization determined by plaque assay on Vero cells. Results represent the mean and standard deviation of 5–7 separate determinations (Table 3). Antibody and complement neutralized 0.8 $\log_{10}$ of wild-type virus, 2.1 $\log_{10}$ gC single-mutant virus, 1.5 $\log_{10}$ gE single-mutant virus, and 4.5 $\log_{10}$ gC/gE double-mutant virus. Therefore, the gC/gE double-mutant virus was far more susceptible to antibody and complement neutralization than wild-type virus or either single-mutant virus ($P < 0.001$ for each comparison).

TABLE 3
Neutralization of Wild-Type, gC Mutant, gE Mutant, or gC/gE Double-Mutant Virus by Antibody and Complement (15)

Virus strain	Titer (log_{10}) When Incubated With PBS	Titer (log_{10}) When Incubated With Ab & C	Amount of Virus Neutralized (log_{10})
Wild-type	6.1 ± 0.4	5.3 ± 0.7	0.8 ± 0.4
gC mutant	5.7 ± 0.6	3.6 ± 1.3	2.1 ± 0.8
gE mutant	5.9 ± 0.7	4.4 ± 1.5	1.5 ± 0.8
gC/gE double-mutant	6.0 ± 0.5	1.5 ± 0.4	4.5 ± 0.4*

* $P < 0.0001$ compared with neutralization of wild-type, gC or gE mutant virus. Abbreviations: Ab, antibody; C, complement.

In vivo studies were performed using the mouse flank model. Disease scores produced by wild-type, gC single, gE single, or gC/gE double-mutant virus at the inoculation site were evaluated in BALB/c mice passively immunized with anti-HSV IgG (antibody and complement present) (15) (Table 4). Each result is the mean and standard error of infection in 4–8 mice. The maximum cumulative score for days 3 through 7 is 25. At 5×10^3 PFU, the wild-type virus score was 21.8, which is similar to the gC/gE double-mutant virus score of 21 at 5×10^6 PFU (1,000-fold higher inoculation titer) (compare numbers marked by * in Table 4). At 5×10^4 PFU, wild-type virus score was 23.9, which is similar to the gC mutant virus score of 24.6 at 5×10^5 PFU (10-fold higher inoculation titer), and gE mutant virus score of 24

TABLE 4
Disease Scores at the Inoculation Site in Mice Infected With Wild-Type HSV-1, gC Single-Mutant, gE Single-Mutant, or gC/gE Double-Mutant Strains (15)

Experimental Conditions	Wild-Type Virus	gC Mutant Virus	gE Mutant Virus	gC/gE Double-Mutant Virus
Ab & C Present				
Virus Inoculum				
1. 5×10^3	21.8 ± 1.9*	12.1 ± 1.2	10 ± 0.8	7.8 ± 1.4
2. 5×10^4	23.9 ± 0.7#	16.1 ± 2.7	11.9 ± 2.2	6.6 ± 1
3. 5×10^5	25 ± 0	24.6 ± 0.4#	18.6 ± 0.6	11 ± 2.1
4. 5×10^6	24.8 ± 0.2	24.8 ± 0.1	24 ± 0.4#	21 ± 1.1*
Ab & C Absent				
Virus Inoculum				
1. 5×10^4	25 ± 0	ND	ND	25 ± 0
2. 5×10^6	25 ± 0	ND	ND	25 ± 0

* Used to denote similar disease scores for wild-type virus at 5×10^3 and gC/gE double-mutant virus at 5×10^6; # used to denote similar disease scores for wild-type virus at 5×10^4, gC mutant virus at 5×10^5 and gE mutant virus at 5×10^6. Abbreviations: Ab, antibody; C, complement; ND, not done.

at 5×10^6 PFU (100-fold higher inoculation titer) (compare numbers marked by # in Table 4). Differences between wild-type and gC/gE double-mutant virus are significant at each virus inoculum, $P \leq 0.01$.

We postulated that if differences in disease scores comparing wild-type and gC/gE double-mutant viruses are attributable to immune evasion, then disease scores should be similar in C3 knockout mice that are not passively immunized with HSV IgG (antibody and complement absent). Therefore, C3 knockout mice were passively immunized with nonimmune human IgG and infected with wild-type or gC/gE double mutant virus at 5×10^4 PFU or 5×10^6 PFU. No differences in disease scores were observed (15) (Table 4). Therefore, differences between the two virus strains in the presence of antibody and complement are related to immune evasion and not caused by other functions that may be mediated by gC and gE.

DISCUSSION

Viral evasion strategies are highly adapted to permit a symbiotic relationship between pathogen and host. Small mutations within critical domains of HSV-1 glycoproteins gC and gE eliminate the ability of the virus to bind C3b or IgG Fc, respectively, and the mutant strains are more susceptible to antibody and complement neutralization and less virulent in a murine model (13–15). The most impressive differences are those comparing the gC/gE double-mutant with wild-type virus. These differences are no longer detected when infection is performed in the absence of immune mediators (absence of HSV IgG and complement). Approximately 10- to 100-fold more gC or gE mutant virus than wild-type virus is required to cause comparable disease, while 1,000-fold more gC/gE double-mutant virus is required. The results support an important role for gC and gE immunoevasins in pathogenesis.

It is unknown whether immune evasion is more important to prevent virus neutralization by antibody and complement or to protect virus infected cells from antibody and complement attack. Glycoproteins gC and gE are expressed on the virion envelope and at the infected cell surface; therefore, these immunoevasins are likely active at both sites. Since these immunoevasins are surface-expressed molecules, they are potentially accessible to antibodies that can bind and block their function. Efforts to develop antibodies to gC and gE immune evasion domains seems warranted as a possible vaccine strategy. If immune evasion domains on wild-type virus can be blocked in vivo,

then virulence of wild-type virus may be greatly reduced to approach that of the gC/gE double-mutant virus.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants from the National Institutes of Health AI 33063, DE 14152, and HL 28220. We thank John Lubinski, Ph.D. for performing the animal experiments and Ming Jiang for the virus neutralization studies.

REFERENCES

1. Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL. Viral subversion of the immune system. Annual Review of Immunology 2000;18:861–926.
2. Farrell HE, Degli-Esposti MA, Davis-Poynter NJ. Cytomegalovirus evasion of natural killer cell responses. Immunological Reviews. 1999;168:187–97.
3. Reyburn HT, Mandelboim O, Vales-Gomez M, Davis DM, Pazmany L, Strominger JL. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells. Nature 1997;386:514–17.
4. Ishido S, Choi JK, Lee BS, Wang C, DeMaria M, Johnson RP, Cohen GB, Jung JU. Inhibition of natural killer cell-mediated cytotoxicity by Kaposi's sarcoma-associated herpesvirus K5 protein. Immunity 2000;13:365–74.
5. Tomazin R, Boname J, Hegde NR, Lewinsohn DM, Altschuler Y, Jones TR, Cresswell P, Nelson JA, Riddell SR, Johnson DC. Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by CD4+ T cells. Nature Medicine 1999;5:1039–43.
6. Miller DM, Rahill BM, Boss JM, Lairmore MD, Durbin JE, Waldman JW, Sedmak DD. Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway. Journal of Experimental Medicine 1998;187:675–83.
7. Frank I, Friedman HM. A novel function of the herpes simplex virus type 1 Fc receptor: participation in bipolar bridging of antiviral immunoglobulin G. Journal of Virology 1989;63:4479–88.
8. Dubin G, Socolof E, Frank I, Friedman HM. Herpes simplex virus type 1 Fc receptor protects infected cells from antibody-dependent cellular cytotoxicity. Journal of Virology 1991;65:7046–50.
9. Friedman HM, Cohen GH, Eisenberg RJ, Seidel CA, Cines DB. Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. Nature 1984;309:633–5.
10. Friedman HM, Wang L, Fishman NO, Lambris JD, Eisenberg RJ, Cohen GH, Lubinski J. Immune evasion properties of herpes simplex virus type 1 glycoprotein gC. Journal of Virology 1996;70:4253–60.
11. Harris SL, Frank I, Yee A, Cohen GH, Eisenberg RJ, Friedman HM. Glycoprotein C of herpes simplex virus type 1 prevents complement-mediated cell lysis and virus neutralization. Journal of Infectious Diseases 1990;162:331–7.
12. Kostavasili I, Sahu A, Friedman HM, Eisenberg RJ, Cohen GH, Lambris JD. Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. Journal of Immunology 1997;158:1763–71.
13. Lubinski J, Wang L, Mastellos D, Sahu A, Lambris JD, Friedman HM. In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. Journal of Experimental Medicine 1999;190:1637–46.

14. Nagashunmugam T, Lubinski J, Wang L, Goldstein LT, Weeks BS, Sundaresan P, Kang EH, Dubin G, Friedman HM. In vivo immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. Journal of Virology 1998;72:5351–9.
15. Lubinski JM, Jiang M, Hook L, Chang Y, Sarver C, Mastellos D, Lambris JD, Cohen GH, Eisenberg RJ, Friedman HM. Herpes simplex virus type 1 evades the effects of antibody and complement in vivo. Journal of Virology 2002;76:9232–41.
16. Johansson PJ, Myhre EB, Blomberg J. Specificity of Fc receptors induced by herpes simplex virus type 1: comparison of immunoglobulin G from different animal species. Journal of Virology 1985;56:489–94.
17. Huemer HP, Larcher C, van Drunen Littel-van den Hurk S, Babiuk LA. Species selective interaction of Alphaherpesvirinae with the "unspecific" immune system of the host. Archives of Virology 1993;130:353–64.
18. Lubinski J, Nagashunmugam T, Friedman HM. Viral interference with antibody and complement. Seminars in Cell & Developmental Biology 1998;9:329–37.
19. Hung SL, Srinivasan S, Friedman HM, Eisenberg RJ, Cohen GH. Structural basis of C3b binding by glycoprotein C of herpes simplex virus. Journal of Virology 1992;66:4013–27.

DISCUSSION

Lawley, Atlanta: Does the gC and gE mediated immune evasion work when there's also an IgM response, because IgM activates complement somewhat more efficiently, if I remember my complement biology?

Friedman, Philadelphia: You are correct about IgM being a more efficient activator of complement. gC protects the virus or infected cell against the effects of complement independent of whether IgG or IgM activates the complement cascade. gE binds the Fc domain of IgG, but does not bind IgM; therefore, if IgM is activating complement, the virus has to rely only on gC to protect itself against complement-mediated injury.

Bardondess, New York: Are there analogies between what you have discovered in these viral systems and microbial persistence in infections such as tuberculosis?

Friedman: We don't know whether mycobacterium tuberculosis uses similar evasion strategies, but evasion strategies comparable to those that I discussed for herpes are widely expressed on many microbes. They are detected on viruses, parasites, fungi and bacteria. For example, protein G and protein A are IgG Fc binding proteins found on staphylococci and streptococci and are virulence factors. These proteins are used in many research laboratories to purify IgG or other Fc binding proteins. I suspect that these Fc-binding proteins function in ways similar to those discussed for herpes simplex virus. Microbial Fc-binding proteins block activities mediated by the IgG Fc domain and are probably widely used as immuno-evasins to establish acute, chronic and recurrent infections.