A Novel Function of the Herpes Simplex Virus Type 1 Fc Receptor: Participation in Bipolar Bridging of Antiviral Immunoglobulin G

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We describe a novel function of the Fc receptor of herpes simplex virus type 1 (HSV-1), its ability to participate in antibody bipolar bridging. This refers to the binding of a single immunoglobulin G (IgG) molecule by its Fab end to its antigenic target and by its Fc end to an Fc receptor (FcR). We demonstrate that various immune IgG antibodies, including polyclonal rabbit antibodies to HSV-1 glycoproteins gC1 and gD1 and monoclonal human antibody to gD1 blocked rosetting of IgG-coated erythrocytes at IgG concentrations 100- to 2,000-fold lower than required for rosette inhibition with nonimmune IgG. Steric hindrance did not account for the observed differences between immune and nonimmune IgG since rabbit anti-gC1 F(ab')₂ fragments did not block rosetting. Murine anti-gC1 or anti-gD1 IgG, a species of IgG incapable of binding by its Fc end to the HSV-1 FcR, also did not block rosetting. When cells were infected with a gC1-deficient mutant, anti-gC1 IgG inhibited rosetting to the same extent as nonimmune IgG. This indicates that binding by the Fab end of the IgG molecule was required for maximum inhibition of rosetting. Bipolar bridging was shown to occur even when small concentrations of immune IgG were present in physiologic concentrations of nonimmune IgG. The biologic relevance of antibody bipolar bridging was evaluated by comparing antibody- and complement-dependent virus neutralization of an FcR-negative mutant and its parent HSV-1 strain. By engaging the Fc end of antiviral IgG, the parent strain resisted neutralization mediated by the classical complement pathway. These observations provide insight into the role of the HSV-1 FcR in pathogenesis and may help explain the function of FcR detected on other microorganisms.

Receptors for the Fc domain of immunoglobulin G (IgG) are expressed on the surface of herpes simplex virus (HSV)-infected cells and on the virion envelope (2, 54). Recent work suggests that the HSV-1 Fc receptor (FcR) is formed by a complex of glycoproteins gE and gI, which are the products of genes mapped to the unique short open reading frames US8 and US7, respectively (27, 28, 34, 39, 43, 44). These genes are not essential for virus replication *in vitro*; however, mutants lacking FcR function have not been isolated from patients (34, 35, 41). This suggests a possible important role for the viral FcR *in vivo*.

Several studies have addressed the functions of the HSV FcR in pathogenesis. These studies have focused on the binding of nonimmune IgG to the FcR. Aggregated nonimmune IgG molecules bound to the FcR on HSV-1-infected cells and protected cells against complement-dependent and cell-mediated immune lysis (1). Purified nonimmune IgG and Fc fragments also protected HSV-2 virions from neutralization by specific HSV-2 antiserum (15). The protection presumably occurred by nonimmune IgG sterically hindering access of immune IgG or Fc-dependent effector cells to the virus or virus-infected cell. Therefore, the current hypothesis is that the HSV FcR offers a survival advantage to the virus by binding nonimmune IgG.

We postulated that the HSV FcR may also play a role in pathogenesis by binding the Fc end of immune IgG. The Fc end of IgG is the region of the molecule responsible for mediating important effector functions, such as complement activation and antibody-dependent cellular cytotoxicity. Therefore, binding of the Fc end of immune IgG could modify the effectiveness of IgG-mediated attack.

Immune IgG potentially can bind to the virus or virus-

infected cell in several ways: (i) binding by its Fab end to its antigenic target; (ii) binding by its Fc end to the HSV FcR; and (iii) simultaneous binding by both its Fab and Fc ends to the antigenic target and the FcR, respectively. For this third possibility to occur, an IgG molecule must bind in a bipolar bridging fashion. Antibody bipolar bridging refers to the molecular bridge formed on a cell's surface by the simultaneous binding of the Fab end of the molecule to its antigen target and by the Fc end to an FcR. Antibody bipolar bridging has been demonstrated on several FcR-bearing leukocytes, including murine mast cells, guinea pig neutrophils, alveolar macrophages, and U937 cells, a promonocytelike cell line (3, 11, 31, 33).

We now report that immune IgG binds in a bipolar fashion on an infected cell, its Fab end binding to a viral glycoprotein and its Fc end binding to the HSV FcR. In addition, we show that bipolar bridging of immune IgG offers a survival advantage to the virus. By binding the Fc end of immune IgG, the FcR protects the virus from antibody- and complement-dependent neutralization.

MATERIALS AND METHODS

Cell cultures and virus strains. HEP-2 cells, African green monkey kidney cells (Vero), and human umbilical vein endothelial cells were propagated as described before (7, 36). Wild-type HSV-1 strain NS was used to select for a gE-negative mutant virus by twice plaque-purifying isolates of NS which resisted neutralization with monoclonal anti-gE antibody 1E3 and complement (7, 20). The gE1-negative mutant strain selected was called 1-ENS-3, 8 and is referred to as -ENS. Strain gC-551, a mutant of HSV-1 derived from strain KOS 321, makes a truncated form of gC1 which is not expressed on the surface of infected cells (21). All virus

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strains were grown on MRC-5 cells and quantified by plaque formation on Vero cells.

Antibodies. Murine IgG monoclonal anti-gC1 (1C8) anti-gD1 (1D3) and anti-gE1 (1E3 and 1BA10) antibodies were prepared as described before (20). Additional monoclonal antibodies used in these studies included murine IgG anti-gC1, -C7, C13, and -C14; murine IgG anti-gI1 (1304); and human IgG monoclonal anti-gD1 antibody (28, 37, 50). Rabbit polyclonal IgG antibodies to gB1, gC1, and gD1 were prepared by immunizing rabbits with purified glycoproteins (16). Affinity-purified nonimmune rabbit IgG was obtained from Calbiochem (San Diego, Calif.). Human nonimmune serum was taken from a single donor whose serum was tested for HSV antibody by anticomplement immunofluorescence and by neutralization assay. Rabbit IgG from polyclonal antisera and nonimmune human IgG were purified by DEAE-Affi-Gel blue ion-exchange chromatography (Bio-Rad Laboratories, Richmond, Calif.). IgG preparations were cleared of immune aggregates by centrifugation at $50,000 \times g$ at 4°C for 2 h. Rabbit anti-gC1 F(ab')₂ fragments were made by incubating 14.5 mg of purified rabbit anti-gC1 IgG and 0.45 mg of crystallized pepsin overnight at 37°C as described before (22). F(ab')₂ fragments were separated from uncleaved IgG by passing the mixture over a protein A-Sepharose C4B column. The effluent was concentrated and passed over a Sephadex G-100 column, and the first peak was collected. The sample was devoid of intact IgG as determined by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis run under denaturing and nondenaturing conditions and by failure to bind ¹²⁵I-protein A when tested by immunoblotting. Anti-gC1 F(ab')₂ retained antigen binding activity, as tested by immunofluorescent staining of HSV-1-infected cells.

Western immunoblot of infected-cell extracts. NS and mutant -ENS were grown in Hep-2 cells, and infected-cell extracts were electrophoresed in 10% N,N'-diallyltartardiamide cross-linked polyacrylamide gels, transferred to nitrocellulose, and probed with rabbit polyclonal anti-gB1, anti-gC1, or anti-gD1 or murine monoclonal anti-gE1 (1BA10) antibodies as described before (9). Bound antibody was detected by using ¹²⁵I-protein A (Amersham Corp., Arlington Heights, Ill.).

Detection of Fc receptors by erythrocyte-binding assay. Human umbilical vein endothelial cells were grown in 24-well plates coated with purified human plasma fibronectin. Cells were infected with HSV-1 strain NS, -ENS, or gC-551 at a multiplicity of infection of 3 (7). When virtually all cells showed cytopathology, which was 18 h postinfection for NS and -ENS and 42 h for gC-551, a rosetting assay was performed to test for the availability of the viral FcR (7). Sheep erythrocytes were labeled with 100 µCi of Na₂⁵¹CrO₄ per 10⁹ cells and sensitized with subagglutinating concentrations of goat anti-sheep erythrocyte IgG (Cordis Laboratories, Miami, Fla.). IgG-coated erythrocytes (EAIgG) were added to wells for 2 h at 37°C. Monolayers were washed to remove unbound erythrocytes and observed for rosettes by light microscopy. A rosette was defined as four or more erythrocytes bound per endothelial cell. Bound erythrocytes were lysed with distilled water, and the lysate was counted in an LKB 1275 mini-gamma counter. Percent ⁵¹Cr-labeled EAIgG bound was calculated as follows: (⁵¹Cr-EAIgG bound divided by total ⁵¹Cr-EAIgG added) × 100.

Blocking of Fc receptors. HSV-1-infected endothelial cells were incubated with 200 µl of various antibodies or with minimal essential medium as a control for 1 h at 37°C. The antibodies were diluted in phosphate-buffered saline contain-

ing 1% bovine serum albumin. After the 1-h incubation, the antibodies were removed by washing, and ⁵¹Cr-EAIgG were added. The effects of various antibodies on blocking rosetting was calculated as follows: $1 - [(^{51}\text{Cr-EAIgG bound to infected cells incubated with blocking antibody} - ^{51}\text{Cr-EAIgG bound to uninfected cells incubated with medium}) / (^{51}\text{Cr-EAIgG bound to infected cells incubated with medium} - ^{51}\text{Cr-EAIgG bound to uninfected cells incubated with medium})] \times 100$. The paired Student's *t* test was used for statistical analysis.

Virus neutralization assay. Virus neutralization was determined by a modified plaque reduction assay (48). Briefly, the neutralization mixture contained 100 µl of virus at 10⁵, 10⁶, or 10⁷ PFU/ml, 100 µl of antibody at the indicated dilution, and 2.5 or 10% human agammaglobulinemic serum as a source of complement. All dilutions were performed in phosphate-buffered saline containing 1% bovine serum albumin, except for experiments with 10% complement, which were diluted in 0.02 M Mg₂SO₄ and 0.01 M EGTA (ethylene glycol tetraacetic acid) in gelatin-Veronal-buffered saline or 0.01 M EDTA in gelatin-Veronal-buffered saline. We found that 10% serum treated with Mg and EGTA retained greater than 90% alternative-complement-pathway hemolytic activity and lacked classical complement activity (45, 47). Virus, antibody, and complement mixtures were incubated at 37°C for 1 h, and then serial 10-fold dilutions were titrated in duplicate to determine whether virus neutralization had occurred.

RESULTS

Comparison of binding of immune and nonimmune IgG to the HSV-1 FcR. Experiments were performed to compare binding of immune and nonimmune IgG to the HSV-1 FcR. ⁵¹Cr-labeled sheep erythrocytes were sensitized with anti-erythrocyte IgG (EAIgG) and used in a rosetting assay to quantitate binding to the HSV-1 FcR expressed on infected cells. These studies were performed at 18 h postinfection, at a point when gC1, gD1, and the FcR are abundantly expressed on the cell surface (24). Figure 1a demonstrates the typical rosetting pattern observed when human umbilical vein endothelial cells were infected with a clinical isolate of HSV-1, strain NS. Rosetting was significantly blocked when infected cells were incubated with a low concentration of purified rabbit IgG prepared against glycoprotein gC1 (anti-gC1) of HSV-1 (Fig. 1b). However, when an identical concentration of nonimmune rabbit IgG was used, no appreciable decrease in rosetting was detected (Fig. 1c). These results indicate that immune IgG blocked access to the HSV FcR more efficiently than nonimmune IgG, raising the possibility that the former bound to the HSV-infected cells in a bipolar bridging fashion.

Figure 2 schematically depicts antibody gC1 or gD1 binding to HSV by bipolar bridging on an infected cell. The Fab end of IgG binds to the viral glycoprotein, while the Fc end of the same molecule binds to the HSV FcR.

Evidence for antibody bipolar bridging on infected cells. (i) Inhibition of rosetting by immune IgG. To determine whether anti-HSV-1 IgG binds by bipolar bridging, several antibodies and antibody fragments were tested for their ability to block rosetting of EAIgG to the HSV-1 FcR on infected cells (Fig. 3). Rabbit, human, and murine antibodies were used. Based upon species-specific differences, the Fc end of murine IgG does not bind to the HSV-1 FcR, while rabbit and human IgGs do bind (25). Therefore, antibodies could be grouped into three categories: (i) those capable of binding by their

FIG. 1. Rosetting of EA1gG to endothelial cells infected with HSV-1 strain NS. (a) Typical rosettes demonstrating the HSV FcR on infected cells. (b) Incubation of infected cells with rabbit anti-gC1 IgG (160 μ g/ml) inhibited rosetting. (c) Incubation of infected cells with a similar concentration of nonimmune rabbit IgG (160 μ g/ml) did not block rosetting. All photomicrographs, 100 $\times$ magnification.

FIG. 2. Schematic representation of an antibody molecule directed against HSV-1 gC or gD binding by bipolar bridging. The Fab end of the molecule binds to gC or gD, while the Fc end of the same molecule binds to the HSV-1 FcR, gE/gI. V_H , Variable region of heavy chain; V_L , variable region of light chain; H, hinge region; C_H1 , C_H2 , and C_H3 , constant regions 1, 2, and 3, respectively, of IgG heavy chain; C_L1 , C_L2 , and C_L3 , constant regions 1, 2, and 3, respectively, of IgG light chain; gC, gD, gE, and gI, HSV-1 glycoproteins; S-S, disulfide bridge between the heavy chains of an IgG molecule.

Fab end, their Fc end, or both ends simultaneously (bipolar bridging); (ii) those capable of binding by their Fab ends only; and (iii) those capable of binding by their Fc ends only. Antibodies capable of bipolar bridging included rabbit polyclonal anti-gC1 and anti-gD1 IgG and human monoclonal anti-gD1 IgG. Each of these antibodies significantly inhibited rosetting ($P < 0.001$ for each condition compared with medium, Fig. 3a).

(ii) Unipolar Fab binding does not block EA IgG access to the FcR. Infected cells were incubated with antibodies or antibody fragments capable of binding by their Fab ends only, which included rabbit anti-gC1 $F(ab')_2$ fragments and murine monoclonal anti-gC1 or anti-gD1 IgG (Fig. 3a). These antibodies failed to inhibit rosetting significantly ($P > 0.05$ compared with medium). This indicates that unipolar binding of IgG by its Fab end did not sterically hinder rosetting to the HSV FcR. As further proof of this point, additional experiments were performed with a panel of four murine monoclonal antibodies to gC1 (C7, C13, C14, and 1C8). Combined, these antibodies bind to the two antigenic sites described for gC1, sites I and II (37). When virus-infected cells were incubated with this panel of antibodies, no inhibition of rosetting occurred. Therefore, IgG binding by its Fab end to several antigenic sites on gC1 was not sufficient to block the HSV-1 FcR. In addition, experiments were performed with murine monoclonal anti-gD1 IgG antibody 11S. This antibody and the human monoclonal anti-gD1 antibody used in Fig. 3a recognize the same epitope within antigenic site III of HSV gD1 (G. Cohen and R. Eisenberg, personal communication). Murine monoclonal antibody 11S failed to inhibit rosetting when used at 160 $\mu\text{g/ml}$ (result not shown), whereas human monoclonal IgG anti-gD1 antibody significantly inhibited rosetting at 33 $\mu\text{g/ml}$ (Fig. 3a).

(iii) Requirement of Fab binding for maximum inhibition of rosetting. Fab binding of immune IgG to the infected cell was required for maximum binding of the Fc portion of the molecule to the HSV-1 FcR. To demonstrate this, antibodies were used which were capable of binding only by their Fc

end. In the first set of experiments, infected cells were incubated with nonimmune rabbit IgG (Fig. 3a). Nonimmune rabbit IgG failed to inhibit rosetting when added at concentrations similar to rabbit anti-gC1 IgG and rabbit anti-gD1 IgG. In an additional set of experiments, IgG binding to the HSV-1 FcR was studied by using a gC mutant strain of HSV-1, gC-551 (Fig. 3b). Strain gC-551 makes a truncated form of gC1 that is not expressed on the infected-cell surface (21). On gC-551-infected cells, rabbit anti-gC1 IgG had the same binding potential as nonimmune IgG; that is, it bound by its Fc end only. On these cells, rabbit anti-gC1 IgG no longer inhibited rosetting. This is in marked contrast to the results when this antibody was used to block rosettes on cells infected with wild-type NS virus (Fig. 3a). Rabbit anti-gD1 IgG was then examined for inhibition of rosettes on gC-551-infected cells. This antibody potentially can bind by bipolar bridging on gC-551-infected cells, as it can on NS-infected cells. On gC-551-infected cells, rabbit anti-gD1 IgG extensively inhibited rosetting ($P < 0.001$ compared with medium, Fig. 3b). These experiments confirm that an antibody inhibited rosetting most effectively if it bound by its Fab end.

(iv) Inhibition of rosetting at various IgG concentrations. Next, we compared the ability of immune and nonimmune IgG to inhibit rosetting at various concentrations of IgG. Cells infected with wild-type virus (strain NS) were exposed to human monoclonal anti-gD1 IgG antibody, an antibody that potentially can bind by bipolar bridging, and nonimmune human IgG, which can bind by its Fc end only (Fig. 4). Human monoclonal anti-gD1 IgG inhibited rosetting by 79% at a concentration of 25 $\mu\text{g/ml}$ (Fig. 4A). In contrast, nonimmune human IgG inhibited rosetting by only 41% at a concentration of 8,000 $\mu\text{g/ml}$ (Fig. 4B). At 1 $\mu\text{g/ml}$, human monoclonal anti-gD1 IgG inhibited rosetting to the same extent as did nonimmune IgG at 2,000 $\mu\text{g/ml}$ (compare Fig. 4A with B). Therefore, more IgG bound to the HSV-1 FcR if the Fab end of the molecule also bound to the infected cell.

Rosette inhibition comparing various concentrations of rabbit immune and nonimmune IgG was performed. At a concentration of 2,000 $\mu\text{g/ml}$, nonimmune IgG inhibited rosetting by 30%; anti-gC1 IgG at 20 $\mu\text{g/ml}$ or anti-gD1 IgG at 10 $\mu\text{g/ml}$ caused similar inhibition (results not shown). Therefore, polyclonal antisera (polyclonal anti-gC1 or anti-gD1), which contained a mixture of immune and nonimmune IgG molecules, blocked rosetting at concentrations 100- to 200-fold lower than nonimmune IgG.

To further define the ability of immune IgG to inhibit rosetting, we performed admixture experiments, diluting small quantities of immune IgG (25 $\mu\text{g/ml}$) in high concentrations of nonimmune IgG (2,000 to 8,000 $\mu\text{g/ml}$, Fig. 4C). Despite these concentrations of nonimmune IgG, human monoclonal anti-gD IgG effectively inhibited rosette formation. These results support the conclusion that bipolar bridging of immune IgG occurs and indicate that physiologic concentrations of nonimmune IgG do not prevent the reaction.

Effect of bipolar bridging on virus neutralization. We examined whether bipolar bridging confers a biological advantage to the virus. The HSV FcR binds a region of IgG containing the C_H2 and C_H3 domains (26). The C_H2 domain is also the binding site for complement component C1q (17, 30). Complement enhances HSV neutralization by antibody (12, 53, 56, 57). The HSV FcR could protect the virus from neutralization by interfering with access of C1q to IgG, thereby limiting subsequent activation of the classical complement pathway. To test this possibility, we compared the

FIG. 3. Blocking of rosetting by immune or nonimmune IgG incubated with endothelial cells infected with (a) wild-type HSV-1 strain NS or (b) HSV-1 gC mutant strain gC-551. Antibodies were used at the following concentrations: mouse monoclonal anti-gC1 1C8 (IgG2a), 190 μ g/ml, and anti-gD1 1D3 (IgG2a), 154 μ g/ml; human monoclonal anti-gD1 IgG1, 33 μ g/ml; rabbit polyclonal anti-gC1 IgG, 160 μ g/ml; rabbit polyclonal anti-gC1 F(ab')₂, 160 μ g/ml; rabbit polyclonal anti-gD1 IgG, 160 μ g/ml; nonimmune rabbit serum, 270 μ g of IgG per ml, or nonimmune rabbit IgG, 160 μ g/ml.

ability of antibody and complement to neutralize wild-type virus and an FcR-deficient strain of HSV 1, -ENS.

Strain -ENS was selected by plaque-purifying NS strain virus that resisted neutralization by anti-gE1 monoclonal antibody 1E3 and complement. Strain -ENS lacked a func-

tional FcR as tested by rosetting. Figure 5 is a Western blot of -ENS- and NS-infected cell extracts run under denaturing conditions. It demonstrates the lack of gE1 on the mutant strain. The mutant was also shown to lack gE1 by immunofluorescence and immunoprecipitation of [³⁵S]methionine-

FIG. 4. Comparison of rosette inhibition by human monoclonal anti-gD1 IgG1 antibody and nonimmune human IgG. (A) From 0.2 to 25 μg of human monoclonal anti-gD1 IgG per ml was added to inhibit rosettes; (B) 500 to 8,000 μg of human nonimmune IgG per ml was added to inhibit rosettes; (C) 25 μg of human monoclonal anti-gD1 IgG per ml was added to 2,000, 4,000, or 8,000 μg of human nonimmune IgG per ml to inhibit rosettes. Each point is the mean of two experiments.

FIG. 5. Western blot of extracts of cells infected with wild-type strain NS and mutant strain -ENS. The blot was probed with rabbit polyclonal antisera to gB1, gC1, and gD1 and murine monoclonal anti-gE1 antibody 1BA10. 1BA10 recognizes a different antigenic site on gE1 than 1E3, which was the monoclonal antibody used to select -ENS. Sizes are shown to the right (in kilodaltons).

labeled infected-cell extracts (results not shown). Immunofluorescence performed with monoclonal anti-gI1 antibody 1304 showed that the mutant made gI1.

For neutralization assays, various concentrations of virus were incubated with constant concentrations of antibody. Agammaglobulinemic human serum was used as a source of complement. Results with virus titers of 10^5 PFU/ml are shown in Fig. 6; similar results were obtained with 10^6 and 10^7 PFU/ml. Rabbit anti-gC1 IgG and complement neutralized approximately 100-fold more -ENS than NS, while rabbit anti-gD1 IgG and complement neutralized approximately 10-fold more -ENS than NS (Fig. 6a). Rabbit anti-gC1 and anti-gD1 IgG can bind to NS by bipolar bridging but to -ENS by their Fab ends only, leaving their Fc ends available to activate complement. Murine anti-gC1 IgG neutralized both viruses to a similar extent; it can bind to both viruses by Fab ends only. Thus, the HSV FcR-bearing virus NS was protected from neutralization if the neutralizing antibody was capable of bipolar bridging. This effect required a functional classical complement pathway, as demonstrated by experiments in which human serum was either omitted or modified by Mg^{2+} -EGTA or EDTA (Fig. 6b). Incubated in Mg^{2+} -EGTA, the classical complement pathway is inactivated, while the alternative pathway remains intact; in EDTA, both classical and alternative pathways are inactivated (45, 47). With the classical complement pathway inactivated, no differences were detected in neutralization of NS and -ENS by rabbit anti-gC1 IgG. Therefore, the HSV-1 FcR protected the virus by engaging the Fc end of antibody through bipolar bridging, preventing activation of the classical complement pathway.

DISCUSSION

This report describes a novel function of the HSV-1 FcR, its participation in antibody bipolar bridging. This concept was first hypothesized by Lehrner and colleagues (32). Evidence to support bipolar bridging is as follows. (i) Antibodies capable of binding by their Fab end to HSV-1 glycoproteins and by their Fc end to the HSV-1 FcR blocked rosetting at IgG concentrations 100 to 2,000 times lower than those required for nonimmune IgG. (ii) Antibodies capable of binding to HSV-1 glycoproteins by their Fab end but incapable of binding by their Fc end to the HSV-1 FcR did not block rosetting. This indicates that IgG binding by its Fab end did not sterically hinder rosetting. (iii) Rabbit

FIG. 6. Reduction in viral titers of HSV-1 strains NS and -ENS by neutralizing antibody and complement. (a) Comparison of neutralizing effects of antibodies capable of bipolar bridging (rabbit anti-gC1 IgG and rabbit anti-gD1 IgG) with antibody that binds by its Fab end only (mouse monoclonal anti-gC1 IgG antibody 1C8). (b) Effects of complement on virus neutralization. Human agammaglobulinemic serum at 2.5 or 10% was used as a complement (C') source.

polyclonal antibody to gC1 blocked rosetting at low concentrations of IgG when added to cells expressing gC1. However, this antibody did not block rosetting at similar concentrations when exposed to cells infected with a gC1-negative mutant virus. This indicates that binding of IgG by its Fab end was required for maximum inhibition of rosetting. (iv) Admixture experiments were performed in which 25 μ g of

human monoclonal immune IgG antibody per ml was added to physiologic concentrations of human nonimmune IgG (8,000 μ g/ml). The high concentrations of nonimmune IgG did not prevent binding of immune IgG to the FcR. Considered together, these results indicate that some IgG molecules directed against viral antigens expressed on cells bind by bipolar bridging.

Several possible explanations can account for the increased binding of immune IgG to the FcR. First, the high-affinity binding between the Fab end of antibody and its target antigen may increase the amount of IgG in proximity to the HSV FcR. A second explanation involves the inherent flexibility of antibodies (4). It is possible that binding by the Fab end induces conformational changes in the antibody which facilitate or stabilize the interaction of the Fc end with an FcR. Third, conformational changes in the structure of the viral glycoprotein may occur following antibody binding by the Fab end, which may bring the Fc end in closer proximity to the FcR. Support for altered flexibility of antigen or antibody is provided by experiments examining the three-dimensional structure of influenza virus neuraminidase bound by an Fab antibody fragment. Structural changes in the virus antigen and in the light and heavy chains of the antibody occurred following binding (10).

In addition to demonstrating antibody bipolar bridging, we showed that the HSV-1 FcR offers a survival advantage to the virus, preventing antibody- and complement-dependent neutralization. This protective effect was observed with anti-gC1 and anti-gD1 IgG from rabbits but not with IgG from mice. The rabbit antibodies are capable of bipolar binding, while murine IgG does not bind to the HSV-1 FcR (25). By binding the Fc end of IgG, the HSV-1 FcR diminished the effectiveness of antibody- and complement-dependent neutralization by 10- to 100-fold. This protection was mediated by interfering with activation of the classical complement pathway.

Our study focuses on the binding of immune IgG to the FcR and demonstrates a protective effect of this binding for the virus. Previous studies have explored the role of the FcR in binding nonimmune IgG, which also protects the virus or virus-infected cell from antibody or immune effector cell attack, presumably by steric hindrance (1, 15). Whether binding of immune IgG or nonimmune IgG is more important for virus survival cannot be determined from our studies, since no direct comparisons were made. However, we showed that even in the presence of physiologic concentrations of nonimmune IgG, bipolar bridging occurred.

In vivo, the Fc portion of antiviral IgG has been shown to be important in defense against HSV infection. Mice that received a lethal inoculum of HSV-1 and then were passively immunized with whole rabbit or human antiviral IgG were protected from illness, while mice immunized with antiviral F(ab')₂ fragments were not similarly protected (23, 38, 42). Bipolar bridging is a mechanism by which the virus, in effect, can convert intact IgG into F(ab')₂ fragments. Therefore, these animal studies offer indirect evidence that bipolar bridging may have in vivo significance.

Several other microorganisms have proteins that function as FcRs, including HSV-2, cytomegalovirus, *Staphylococcus aureus*, group A, C, and G streptococci, several species of *Trypanosoma* and *Leishmania*, and *Schistosoma mansoni* (6, 18, 19, 29, 44, 46, 51, 52, 55). The FcR of some of these organisms, including *Staphylococcus aureus*, group A streptococci, and members of the *Trypanosomatidae* are thought to be virulence factors (5, 14, 18, 49). The FcR of *S. aureus* has been shown to impair antibody- and complement-depen-

dent killing of organisms by interfering with complement fixation to antibody (49). Staphylococcal protein A binds the C γ 2 and C γ 3 domains of IgG (13). This is the same region of the IgG molecule bound by the HSV FcR. Therefore, in a fashion analogous to the HSV FcR, protein A may bind the Fc end of immune IgG, thereby protecting the microbe from antibody attack. Similar properties may also apply to the FcR found on other microbes and tumor cells.

Studies are in progress to determine whether the HSV-1 FcR protects the virus by interfering with other Fc-dependent IgG clearance mechanisms, such as antibody-dependent cellular cytotoxicity. It is of interest that HSV-1 has evolved to express C3b and Fc receptors (20, 40). Both of these virally encoded receptors protect the virus or virus-infected cell from complement-mediated attack and may be important in permitting the virus to survive during reactivation infection despite intact humoral and cellular immunity.

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