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Herpes Simplex Virus Immunoglobulin G Fc Receptor Activity Depends on a Complex of Two Viral Glycoproteins, gE and gI

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Evidence was recently presented that herpes simplex virus type 1 (HSV-1) immunoglobulin G (IgG) Fc receptors are composed of a complex containing a previously described glycoprotein, gE, and a novel virus-induced polypeptide, provisionally named g70 (D. C. Johnson and V. Feenstra, J. Virol. 61:2208–2216, 1987). Using a monoclonal antibody designated 3104, which recognizes g70, in conjunction with antipeptide sera and virus mutants unable to express g70 or gE, we have mapped the gene encoding g70 to the US7 open reading frame of HSV-1 adjacent to the gE gene. Therefore, g70 appears to be identical to a recently described polypeptide which was named gI (R. Longnecker, S. Chatterjee, R. J. Whitley, and B. Roizman, Proc. Natl. Acad. Sci. USA 84:147–151, 1987). Under mildly denaturing conditions, monoclonal antibody 3104 precipitated both gI and gE from extracts of HSV-1-infected cells. In addition, rabbit IgG precipitated the gE-gI complex from extracts of cells transfected with a fragment of HSV-1 DNA containing the gI, gE, and US9 genes. Cells infected with mutant viruses which were unable to express gE or gI did not bind radiolabeled IgG; however, cells coinfected with two viruses, one unable to express gE and the other unable to express gI, bound levels of IgG approaching those observed with wild-type viruses. These results further support the hypothesis that gE and gI form a complex which binds IgG by the Fc domain and that neither polypeptide alone can bind IgG.

Receptors which have affinity for the Fc domain of immunoglobulin G (IgG) are expressed on the surfaces of cells infected with a number of human herpesviruses (2, 17, 26, 29, 34, 38–40). It is not clear what physiological role Fc receptors play in the replicative cycles of herpesviruses, although it has been suggested that they influence events in the infected cell (5, 20, 40) or play a role in reducing immune cytolysis of virus-infected cells (1, 20). Fc receptors may act to coat virus-infected cells with IgG so as to reduce exposure of viral antigens to immune effector cells, a role suggested for Fc receptors expressed by malignant cells (18). Alternatively, these receptors may bind polypeptides which share structural homology with immunoglobulin Fc receptors, for example, histocompatibility antigens (30).

Baucke and Spear (2) identified a group of electrophoretically similar polypeptides in extracts of herpes simplex virus type 1 (HSV-1)-infected cells which bound to IgG affinity columns. These polypeptides were thought to be derived by posttranslational modification from the same gene product, designated gE. The gene for HSV-1 gE was subsequently mapped to a region of the short unique (Us) component (map coordinates 0.924 to 0.951) of the HSV-1 genome (12, 19, 32). Nucleotide sequence analysis of this region placed the HSV-1 gE gene in the US8 open reading frame (24). A polypeptide analogous to HSV-1 gE was identified in extracts from HSV-2-infected cells (32), and a homologous gene is found in the HSV-2 genome (25). There is also evidence that gE is essential for IgG Fc binding activity because Fc receptors are not expressed on cells infected with a mutant HSV-1 unable to express gE (27).

Recently, we detected a novel HSV-1-induced glycoprotein, electrophoretically and structurally distinct from gE, which binds IgG (13). This polypeptide, which we provisionally named g70, was detected on the surfaces of infected cells and was coprecipitated with gE by using rabbit or human IgG or anti-gE antibody. We presented evidence suggesting that gE and g70 form a complex which binds IgG and that gE in isolation does not bind IgG. In this report, we describe a monoclonal antibody (MAb), 3104, which recognizes g70, and our use of MAb 3104, viral mutants, and antipeptide sera to demonstrate that g70 is encoded by the US7 gene, which is adjacent to the gE gene. Therefore, g70 appears to be identical to a polypeptide recently named gI (21). In addition, we show that cells infected with viruses containing mutations in either the gE gene or the gI gene do not express Fc receptors, supporting the hypothesis that the Fc receptor is composed of gE and gI and that both gE and gI are required for Fc receptor activity.

MATERIALS AND METHODS

Cells and viruses. Vero cells, human R970 cells (35), and mouse LTA cells (9) were grown in minimum essential medium supplemented as previously described (13). IE43 cells were selected from a number of G418-resistant colonies after transfection of Z4 cells with pIE5 as described previously (15, 36) and were grown in medium supplemented with hypoxanthine-aminopterin-thymidine and G418 (150 μg/ml; GIBCO, Burlington, Ontario, Canada). HSV-1 strains F and HFEMsyn (obtained from P. G. Spear, University of Chicago) and F-US7kan were propagated and assayed by plaque formation on Vero cells. HSV-1 strains 17syn+ (4) and *in*1404 were grown on BHK-21 clone 13 cells (23) or Vero cells.

Antibodies. MAb II-481, which recognizes HSV-1 gE (16), was a gift of P. G. Spear. MAb 15βB2, which recognizes HSV-1 or HSV-2 gB, was a gift of S. Bacchetti (McMaster

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University). MAbs 3104, specific for gI, and 3114, specific for HSV-1 gE, were prepared as described previously (6). Rabbit IgG was purchased from Sigma Chemical Co. (St.Louis, Mo.). Synthetic oligopeptides corresponding to residues 133–144 and 251–261 of the predicted amino acid sequence of the US7 open reading frame (24) were prepared by Cambridge Research Biochemicals (Cambridge, England). Antisera to these peptides were prepared as previously described (7). An equal-parts mixture of the two antipeptide sera, designated A/US7, was used in immunoprecipitation experiments.

Labeling of cells with [^{35}S]methionine, immunoprecipitations, and gel electrophoresis. R970 cells were infected with HSV-1(F) or HSV-1(F-US7kan), using 10 PFU/cell, and the virus was removed after 1.5 or 2 h. The cells were labeled with [^{35}S]methionine (25 to 50 μCi/ml; Amersham, Oakville, Ontario, Canada) in medium containing no methionine and 1% fetal calf serum from 3 h until 7 h after infection. IE43 cells or LTA cells, either infected with 20 PFU of HSV-1(F) per cell or left uninfected, were labeled for 4 h with [^{35}S]methionine (100 μCi/ml), using medium containing no methionine and supplemented with 1% fetal calf serum. BHK cells were labeled with [^{35}S]methionine (see Fig. 6) as previously described (7). Cells were pulse-labeled with [^{35}S]methionine 7 h after infection by washing the monolayer three times with medium containing no methionine and then incubating the cells with [^{35}S]methionine (100 to 200 μCi/ml) for 12 min in medium lacking methionine. The cells were immediately extracted with Nonidet P-40–deoxycholate (NP40-DOC) extraction buffer (13). Extracts were sonicated and clarified by centrifugation, and then 0.3 to 0.5 ml of the extract (from 5×10^5 cells) was incubated with 1.5 to 3 μl of mouse ascites fluid (MAb) or 5 to 10 μl of rabbit IgG (10 mg/ml) for 1 to 1.5 h, followed by the addition of protein A-Sepharose (Pharmacia, Dorval, Quebec, Canada) for 1.5 to 2 h at 4°C on a rotating wheel. When MAb II-481 was used, 0.1% sodium dodecyl sulfate (SDS) and 50 mM NaCl were added to the lysates and they were heated to 55°C for 5 min and cooled before ascites fluid or rabbit IgG was added. Protein A-Sepharose beads were washed three times with NP40-DOC extraction buffer, and precipitated proteins were eluted with twofold-concentrated sample buffer (100 mM Tris hydrochloride [pH 6.8], 4% SDS, 4% 2-mercaptoethanol, 20% glycerol, bromophenol blue). Samples were immunoprecipitated (see Fig. 6) as described by Frame et al. (7). Samples were analyzed on 8.5% *N,N'*-diallyltartardiamide-cross-linked SDS-polyacrylamide gels as described (11, 13) except for the samples shown in Fig. 6, which were analyzed on 10% SDS-polyacrylamide gels cross-linked with *N,N'*-methylenebisacrylamide. Gels were infused with 2,5-diphenyloxazole by the procedure of Bonner and Laskey (3), dried, and exposed to Kodak XAR film.

Construction of plasmid pUS7kan and HSV-1 mutant F-US7kan. Plasmid pUS7kan was constructed from pSS17, which contains the *Bam*HI J fragment (map units 0.89–0.94) of HSV-1(KOS) cloned into pUC19, by inserting a kanamycin resistance gene cassette derived from pUC4-KIXX (Pharmacia) into a unique *Bal*I site in the US7 gene. VD60 cell monolayers (50% confluent 100-mm dishes) were transfected with 10 μg of pUS7kan and viral DNA which had been extracted from VD60 cells infected with F-gDβ as previously described (14). Progeny viruses were plaque purified three times on Vero cells, and the viral DNA was then analyzed using Southern blots (14).

Construction of cells expressing gI and gE. Z4 cells (33) in 60-mm dishes were transfected, using the $CaPO_4$ precipitation technique (10, 15), with 5 μg of pIE5, which was derived by subcloning an *Fsp*I fragment from pSG25 (8) into the *Eco*RI site of pSV2neo. The cells were trypsinized 24 h after transfection into five 60-mm dishes containing medium with 400 μg of G418 per ml. The medium was changed every 3 or 4 days, and colonies arising after 17 to 22 days were trypsinized using cloning cylinders and expanded in medium containing G418 (400 μg/ml). The cell lines were initially screened by labeling monolayers with [^{35}S]methionine and immunoprecipitating gE with MAb II-481. One of the lines, IE43, producing the highest levels of gE was selected for further study.

Cleveland partial proteolysis of glycoproteins. Rabbit IgG was used to immunoprecipitate pgE and pgI, or MAb 3104 was used to precipitate gI from pulse-labeled cell extracts of HSV-1-infected cells. The glycoproteins were eluted and electrophoresed on preparative gels, and bands corresponding to the 60-kilodalton (kDa) polypeptide (pgI) were located by using X-ray film, cut out, and rehydrated as described previously (13). Samples were treated with 2, 10, or 50 μg of V8 protease per ml and analyzed on 18% SDS-polyacrylamide gels (13).

Preparation and binding to cells of ^{125}I-labeled IgG. Rabbit IgG was iodinated as previously described (13) or with chloramine T as described (2). Radioiodinated IgG (2×10^5 cpm, 10 μg) was incubated with Vero or R970 cells (2×10^6) infected with HSV-1 strains F, HFEMsyn, 17syn+, F-US7kan, or *in*1404 (10 PFU/cell) or with both F-US7kan and *in*1404 (10 PFU of each virus per cell) 14 h after infection as previously described (13), except that the ^{125}I-labeled IgG was diluted in phosphate-buffered saline (PBS) containing 2.5% fetal calf serum. The monolayers were incubated at 37°C for 2 h; they were then washed twice with PBS containing 2.5% fetal calf serum and solubilized with PBS containing 0.5% SDS, and extracts were counted using aqueous counting scintillation fluid and a scintillation counter.

RESULTS

MAb 3104 recognizes a polypeptide structurally identical to g70 precipitated by rabbit IgG. We recently described a polypeptide, provisionally named g70, which was precipitated in addition to gE by rabbit IgG and protein A-Sepharose (13). However, at that time, our only means of detecting g70 was by coprecipitating the gE-g70 Fc binding complex by using rabbit IgG. Preliminary experiments suggested that an MAb designated 3104, which had initially been thought to immunoprecipitate gE, actually recognized g70. To compare g70 with the polypeptide precipitated by MAb 3104, cells were infected with HSV-1 strain F and then labeled with [^{35}S]methionine for 4 h or pulse-labeled for 12 min. Cell extracts were then immunoprecipitated with MAb 3104, rabbit IgG, or anti-gE MAb II-481. Using extracts from pulse-labeled cells, a polypeptide was precipitated with MAb 3104, which had an electrophoretic mobility identical to that of the smaller polypeptide precipitated using rabbit IgG (Fig. 1). This polypeptide had been previously designated pg70 (the immature form of g70) and rapidly chases into g70, which has an electrophoretic mobility very similar to that of pgE, the immature form of gE, which is recognized by MAb II-481 (13). Similarly, MAb 3104 precipitated g70 and a small amount of pg70 from extracts of cells labeled for 4 h. Here it is clear that much of the diffuse g70 band comigrates with pgE precipitated with MAb II-481, while a portion of the g70 band migrates faster than pgE. Very little pg70 was detected

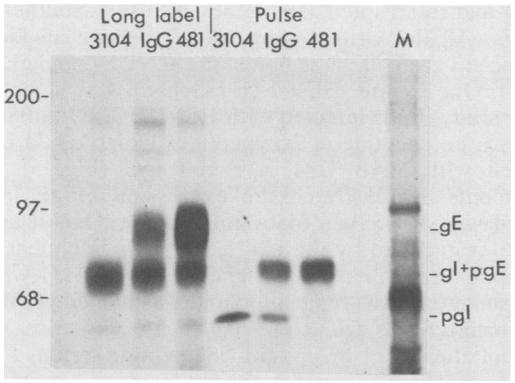

FIG. 1. Viral polypeptides immunoprecipitated using MAb 3104, rabbit IgG, or anti-gE MAb II-481. R970 cells were infected with HSV-1(F) and labeled with [^{35}S]methionine from 3 to 7 h postinfection (Long label) or for 12 min at 7 h postinfection (Pulse). Cell extracts were mixed with MAb 3104, rabbit IgG, or MAb II-481 (481) and then with protein A-Sepharose beads. The precipitated proteins were eluted from the protein A-Sepharose beads and analyzed on 8.5% *N,N'*-diallyltartardiamide-cross-linked polyacrylamide gels. The positions of marker proteins of 200, 97, and 68 kDa and of gE, pgE, g70 (gI; see below), and pg70 (pgI) are indicated.

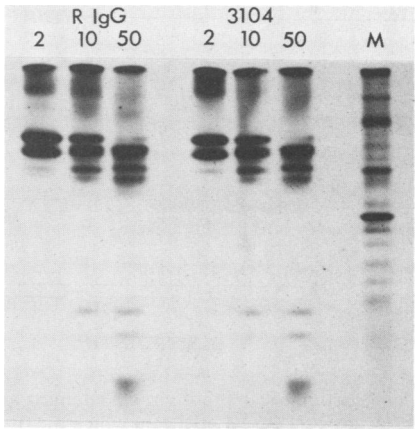

FIG. 2. Comparison of the Cleveland partial proteolytic fragments derived from g70 and the polypeptide precipitated by MAb 3104. HSV-1-infected R970 cells were pulse-labeled with [^{35}S] methionine, and cell extracts were mixed with rabbit IgG (R IgG) or MAb 3104 and protein A-Sepharose. The precipitated proteins were eluted from the protein A-Sepharose and electrophoresed on preparative SDS-polyacrylamide gels. Gel slices containing the faster migrating species precipitated with rabbit IgG (pg70) or the single species precipitated with MAb 3104 were excised from the gels, and samples were digested with 2, 10, or 50 μg of V8 protease per ml and then subjected to electrophoresis on 18% SDS-polyacrylamide gels as previously described (13).

by using a long labeling period, suggesting that the processing of pg70 to mature g70 is more rapid than is the processing of pgE to gE.

Cleveland peptide analysis was carried out to determine whether the polypeptide precipitated by MAb 3104 was identical to the smaller (60-kDa) polypeptide precipitated by rabbit IgG (g70 or pg70). Cells were pulse-labeled with [^{35}S]methionine, and either the pgE-pg70 complex was immunoprecipitated with rabbit IgG or the 60-kDa polypeptide was precipitated with MAb 3104. The 60-kDa polypeptides precipitated by either IgG or MAb 3104 were further purified on preparative polyacrylamide gels and digested with various concentrations of V8 protease. An identical pattern of peptide bands was generated from pg70 precipitated by rabbit IgG and from the 60-kDa polypeptide precipitated with MAb 3104 (Fig. 2). Therefore, pg70 precipitated by rabbit IgG is identical to the polypeptide precipitated by MAb 3104.

Complex of gE and g70 precipitated by MAb 3104 as well as by rabbit IgG. Previously, we showed that g70 and gE form a complex which is precipitated by rabbit IgG and, to a lesser extent, by MAb II-481, which recognizes gE (13). Since MAb 3104 apparently recognizes gI, it was of interest to determine whether MAb 3104 also coprecipitates gE and g70 under certain nondenaturing conditions. Cells were pulse-labeled with [^{35}S]methionine so that pg70 and pgE could be easily resolved, and cell extracts were mixed with rabbit IgG or MAb 3104 under various conditions of detergent and salt. Rabbit IgG clearly precipitated both pgE and pg70 from extracts of cells when NP40-DOC extraction buffer was used (Fig. 3). The precipitation of both proteins was reduced in extraction buffers supplemented with 0.1% SDS and 50 mM NaCl, and very little of both was precipitated if the extracts were heated to 55°C. Similarly, MAb 3104 precipitated pg70 (pgI; see below) and a smaller amount of pgE under mild conditions (NP40-DOC extraction buffer). When the buffer was supplemented with 0.1% SDS and 50 mM NaCl, the amount of pgE precipitated was reduced slightly, and when the extracts were heated to 55°C, pgE was not precipitated, although pg70 (pgI; see below) was efficiently precipitated. Thus, as we suggested previously (13), the complex of gE and g70 appears to be disrupted by SDS and heat, and MAb 3104 precipitates gE poorly if the g70-gE complex is disrupted. It appears that only a small fraction of the total gE present in the cell extract is complexed with g70 when it is precipitated with MAb 3104 or, possibly, that MAb 3104 disrupts the gE-g70 complex. We found that the coprecipitation of gE with the MAb 3104-gI antibody-antigen complex was variable from experiment to experiment.

Evidence that g70 is encoded by the US7 gene and is identical to a polypeptide designated gI. We have previously

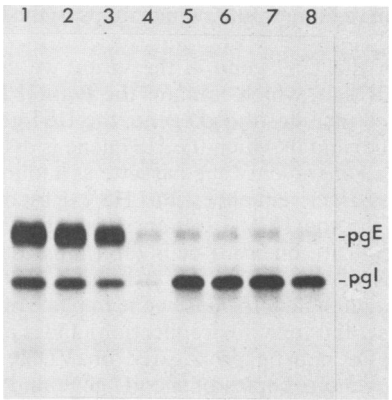

FIG. 3. Complex of g70 and gE precipitated with rabbit IgG and MAb 3104. HSV-1-infected cells were pulse-labeled with [^{35}S] methionine, and then cell extracts were made using NP40-DOC extraction buffer. Extracts were not supplemented (lanes 1, 5) or were supplemented with 0.1% SDS (lanes 2 and 6), 0.1% SDS and 50 mM NaCl (lanes 3 and 7), or 0.1% SDS and 50 mM NaCl and then heated at 55°C for 5 min (lanes 4 and 8). The extracts were mixed with rabbit IgG (lanes 1 through 4) or MAb 3104 (lanes 5 through 8) and protein A-Sepharose. Precipitated proteins were eluted and analyzed on SDS-polyacrylamide gels. The positions of the immature form of gE, pgE, and the immature form of g70, pg70 (here designated pgI; see below), are indicated.

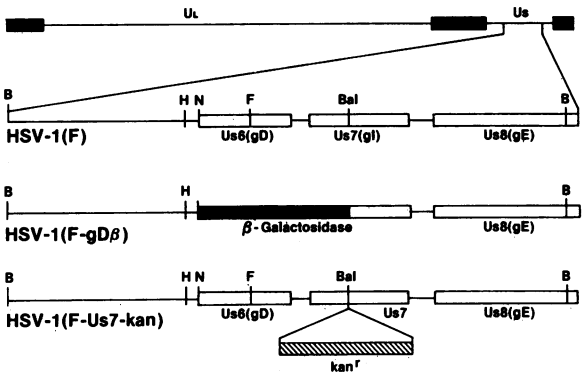

FIG. 4. Construction of a mutant virus containing an insertion in the US7 gene. A virus, HSV-1(F-gDβ), in which the β-galactosidase gene replaces the gD (US6) gene and 42% of the US7 gene, was constructed by transfecting VD60 cells, which express HSV-1 gD, with wild-type HSV-1(F) DNA and a plasmid, pDGAL11ZK, which contains the *Bam*HI J fragment of HSV-1 into which was inserted the β-galactosidase gene of *E. coli*, resulting in the loss of gD and the partial loss of gI coding sequences. The construction and properties of VD60 cells and HSV-1(F-gDβ) will be described in more detail (Ligas and Johnson, in press). VD60 cells were cotransfected with F-gDβ DNA (extracted from VD60 cells) and plasmid pUS7kan, which contains the *Bam*HI J fragment of HSV-1 and an insert of a kanamycin resistance gene cassette at a unique *Bal*I site in the US7 gene. A virus isolate, designated HSV-1(F-US7kan), was selected on Vero cells and plaque purified three times. Southern blot analysis of viral DNA confirmed that F-US7kan had the genomic structure in the region of the gD, US7, and gE genes depicted here (blot results not shown).

suggested that the gene encoding g70 might map in the Us component of HSV-1 (13). This suggestion was based on two observations, (i) that g70 could be detected in cells infected with HSV-1 × HSV-2 intertypic recombinants containing an HSV-1 Us component, and (ii) that the deglycosylated form of g70 had a mobility similar to that of a polypeptide previously described by Lee et al. (19) which maps near the US8 (gE) gene. It appeared likely that g70 was encoded in the US7 open reading frame, which maps to the left of the gE gene (24).

To disrupt the US7 gene in the virus, we constructed a plasmid, pUS7kan, which contains the *Bam*HI J fragment of HSV-1 (which includes the gD gene, the US7 gene, and part of the gE gene) and in which the US7 gene is disrupted by an insertion of a kanamycin gene cassette at a unique *Bal*I site. To insert pUS7kan sequences into HSV-1 by marker transfer, we utilized the HSV-1 mutant strain F-gDβ. F-gDβ is unable to replicate on Vero cells (and most other cultured cells) because it possesses a substitution mutation of the *Escherichia coli* β-galactosidase gene in place of the gD gene (which is essential for virus replication) and a portion of the gI gene. F-gDβ can be propagated on VD60 cells, which contain endogenous copies of the gD gene and can complement the mutant virus. The construction and characterization of F-gDβ and VD60 cells will be described in more detail in another manuscript (M. W. Ligas and D. C. Johnson, J. Virol., in press). The structure of the F-gDβ DNA in the region of the US6 (gD), US7, and US8 (gE) genes is shown in Fig. 4. VD60 cells were cotransfected with F-gDβ DNA and plasmid pUS7kan. Viruses which acquired an intact gD gene and the disrupted US7 gene by homologous recombination were selected by plaquing the virus stock on Vero cells, which do not support the replication of F-gDβ. A number of virus isolates were screened for the presence of the gD and interrupted US7 genes by using Southern blot analysis. All the viruses were found to have an identical genomic structure in the region of the gD, US7, and gE genes (Fig. 4). One isolate, HSV-1 (F-US7kan), was selected for further study. Cells infected with HSV-1(F-US7kan) did not express a polypeptide or polypeptides which could be precipitated with MAb 3104 or rabbit IgG (Fig. 5). Other experiments demonstrated the expression of gE in cells infected with F-US7kan (data not shown). Thus it appears that g70 is encoded by the US7 gene and is identical to the polypeptide described by Longnecker et al. (21) which is dispensable for virus replication and was named gI. We will henceforth refer to g70 as gI.

Precipitation of gI by an anti-US7-peptide serum. Further evidence that gI is encoded in the US7 gene was provided by experiments involving antisera directed to synthetic oligopeptides. Antisera to the synthetic oligopeptides which correspond to residues 133–144 and 251–261 of the predicted amino acid sequence of the US7 open reading frame (24) were generated in rabbits as described previously (7). An equal-parts mixture of the two sera, designated A/US7, was used in immunoprecipitation experiments. It had been found previously that nonspecific rabbit serum or IgG precipitated both gI and gE (13; M. Frame, unpublished results); therefore, to test the antipeptide sera we utilized extracts from cells infected with an HSV-1 mutant which is unable to induce synthesis of gE. A recombinant virus, designated *in*1404, which does not express gE was derived from HSV-1(17syn+) by inserting an 8-base-pair *Xba*I linker into a *Hpa*I site of the gE gene so that a termination codon was generated after codon 124 of the coding sequences. Details of the construction and phenotype of this virus will be published elsewhere (M. C. Frame, A. M. Cross, and N. D. Stow, submitted for publication). The mixed anti-US7 peptide sera, A/US7, precipitated a polypeptide from extracts of cells infected with *in*1404 which had an electrophoretic mobility identical to that of the polypeptide precipitated by MAb 3104 (Fig. 6). It should be noted that the electrophoretic mobility of gI in this gel system, which utilizes bisacrylamide cross-linking, is slightly faster than in the *N,N'*-diallyltartardiamide-cross-linked polyacrylamide gel system used for Fig. 1. The finding that anti-US7 peptide sera and

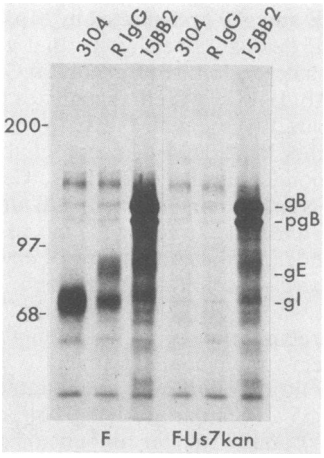

FIG. 5. Expression of g70 or gI in cells infected with HSV-1(F-US7kan). R970 cells were infected with HSV-1(F) or HSV-1(F-US7kan) and labeled for 4 h with [^{35}S]methionine. Cell extracts were prepared and immunoprecipitated using anti-gI MAb 3104, rabbit IgG (RIgG), or anti-gB MAb 15βB2. The positions of gI, gE, and gB/pgB and marker proteins of 200, 97, and 68 kDa are indicated.

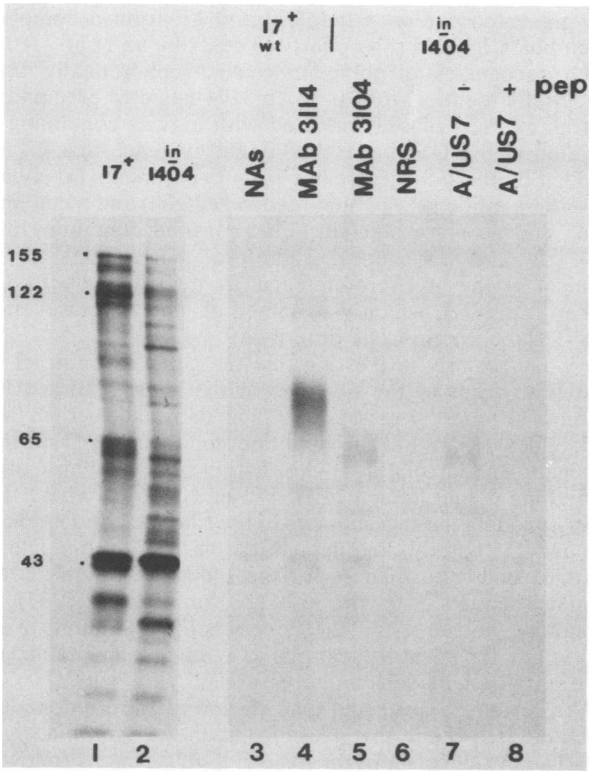

FIG. 6. US7 gene antipeptide sera precipitate a polypeptide with an electrophoretic mobility identical to that of gI. BHK cells were infected with HSV-1(17syn+) or mutant *in*1404, which does not express gE; cells were then labeled with [^{35}S]methionine. Cell extracts were precipitated with normal ascites fluids (NAs), gE-specific MAb 3114, gI-specific MAb 3104, normal rabbit serum (NRS), rabbit anti-US7 peptide serum mixture (A/US7), or A/US7 in the presence of 50 μg of the two oligopeptides used to inoculate the rabbits (A/US7 + pep). Lanes 1 and 2 are marker tracks showing HSV-1(17syn+) (17$^+$)-infected and mutant *in*1404-infected cell polypeptides.

MAb 3104 precipitate polypeptides with identical electrophoretic mobility confirms the mapping of the polypeptide recognized by MAb 3104 or gI to the US7 gene. In addition, we found that rabbit IgG did not precipitate gI or gE from extracts of *in*1404-infected cells, suggesting that gI, in the absence of gE, does not bind IgG (Fig. 7).

Expression of gI and gE by a cell line transfected with the US7 and US8 genes. A 5.5-kilobase DNA fragment, containing part of the US6 (gD) and US10 genes and all of the US7, US8 (gE), and US9 genes, was excised from pSG25 (8) by using the restriction enzyme *Fsp*I and was inserted into the *Eco*RI site of pSV2neo by using *Eco*RI linkers. The resulting plasmid, pIE5, was transfected into Z4 cells, which constitutively express ICP4 (33), and G418-resistant colonies were selected as described previously (15, 36). G418-resistant cell lines were screened for expression of HSV-1 gE by using MAb II-481, and several, including one designated IE43, were found to express gE. IE43 cells and LTA cells, the parental cells from which Z4 cells were derived, were infected with HSV-1 or left uninfected and were labeled with [^{35}S]methionine for 4 h. Cell extracts were then mixed with MAb 3104, rabbit IgG, or anti-gE MAb II-481 and then bound to protein A-Sepharose. IE43 cells expressed gI, which was precipitated with MAb 3104, and the gE-gI complex, which was precipitated using rabbit IgG, although

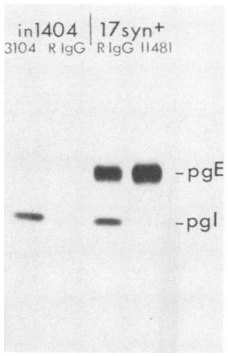

FIG. 7. Glycoproteins gE and gI are not precipitated with IgG from extracts of cells infected with mutant *in*1404. R970 cells were infected with wild-type HSV-1(17syn$^+$) or mutant *in*1404 and pulse-labeled for 12 min with [^{35}S]methionine. Cell extracts were prepared and immunoprecipitated using MAb3104, MAb II-481, or rabbit IgG (RIgG). The positions of pgI and pgI are indicated.

the expression of both polypeptides was lower than in HSV-1-infected LTA cells (Fig. 8). In contrast to these results, rabbit IgG did not precipitate gE from the E4/13, E4/17, or E4/18 cell lines, which were transfected with a plasmid containing the gE gene and not the gI gene (13). Therefore, these observations further support the conclusions that rabbit IgG precipitates a complex of gE and gI and that both proteins are required for IgG binding activity. However, in other experiments we were unable to detect Fc receptors on the surfaces of IE43 cells by incubating the cells with radioiodinated rabbit IgG as previously described (13). We do not fully understand this result, although it may be related to the observation that HSV-1-infected mouse cells express low levels of IgG binding activity as compared with

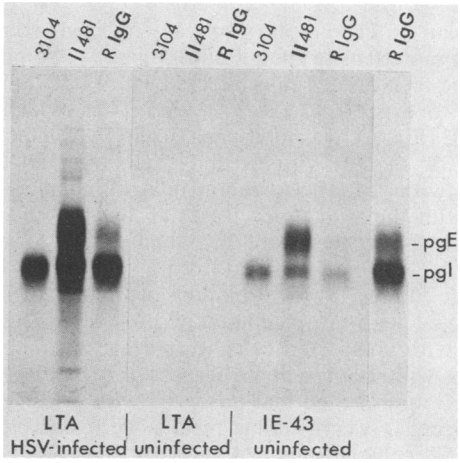

FIG. 8. A cell line transfected with the US7, US8, and US9 genes expresses polypeptides which are precipitated with rabbit IgG and MAb 3104. Z4 cells were transfected with plasmid pIE5, and a cell line, IE43, which expresses HSV-1 gE, was selected. LTA cells infected with HSV-1(F), uninfected LTA cells, or uninfected IE43 cells were labeled for 4 h with [^{35}S]methionine, and cell extracts were immunoprecipitated with anti-gI MAb 3104, anti-gE MAb II-481, or rabbit IgG (R IgG). The exposures of all the lanes to film, except the lane at the far right, were the same. The far right lane, showing immunoprecipitation of IE43 cell extracts with rabbit IgG, was exposed four times longer than the other lanes so that the weaker pgE band would be evident. The positions of the immature forms of gI (pgI) and gE (pgE) are indicated.

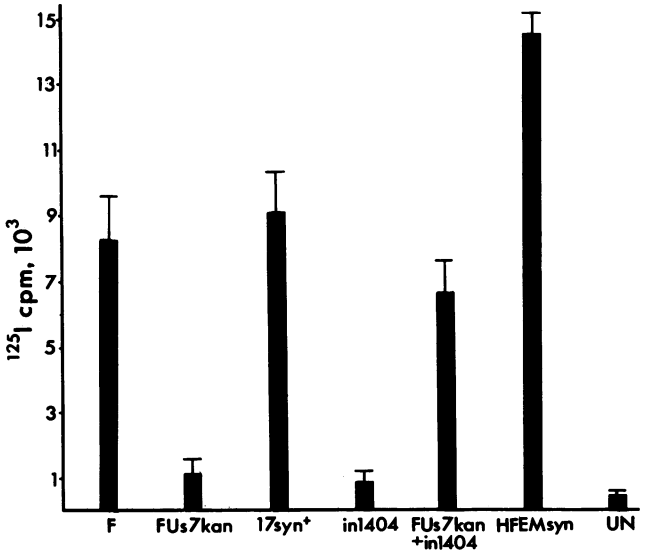

FIG. 9. Fc receptors on cells infected with mutant viruses unable to express gI or gE. Monolayers of Vero cells were infected with HSV-1 strains F, F-US7kan, 17syn+, *in*1404, or HFEMsyn or were coinfected with F-US7kan and *in*1404. Some of the monolayers were left uninfected (UN). After 14 h the cells were washed once in PBS containing 2.5% fetal bovine serum and incubated with ^{125}I-labeled rabbit IgG diluted in PBS containing 2.5% fetal bovine serum for 2 h at 37°C. The cells were washed twice, and extracts of the cells were counted using a scintillation counter. The results shown are the mean of three determinations.

infected human or monkey cells (D. C. Johnson, unpublished results). In previous work, Fc receptors could only be detected on mouse cells late after infection with HSV-1, and the binding of ^{125}I-labeled IgG was at the limits of detection (13).

Expression of Fc receptors by cells infected with viruses containing mutations in the gE and gI genes. The binding of ^{125}I-labeled IgG to Vero cells infected with F-US7kan, which is unable to express gI (Fig. 4), or *in*1404, which cannot express gE (Fig. 6), was measured. Cells were infected with wild-type or mutant viruses and then, after 14 h, were incubated with ^{125}I-labeled rabbit IgG. Monolayers of cells infected with the wild-type viruses, F and 17syn+, bound significantly higher levels of IgG than did monolayers infected with F-US7kan (derived from F) and *in*1404 (derived from 17syn+) (Fig. 9). The binding of radiolabeled IgG to cells infected with either mutant virus was similar to that observed with uninfected cells. However, when cells were coinfected with both mutant viruses the binding of ^{125}I-labeled IgG approached that observed with wild-type HSV-1-infected cells. Very similar results were obtained using human R970 cells (results not shown). Therefore, both gE and gI are essential for Fc receptor activity on the surfaces of cells. We consistently observed much higher binding of radiolabeled IgG to cells infected with HSV-1 strain HFEMsyn, which was the strain of virus used by Spear and co-workers (2, 31, 32), than with strains F and 17syn+.

DISCUSSION

In a previous report (13) we described a novel cell surface polypeptide, induced by infection of cells with HSV-1, which binds human or rabbit IgG, in conjunction with the previously described viral glycoprotein gE (2, 31, 32). We also presented evidence that g70 and gE form a complex which binds IgG. In this report we describe an MAb, 3104, which recognizes a polypeptide electrophoretically and structurally identical to g70. MAb 3104 failed to precipitate gI from extracts of cells infected with a virus containing a mutation in the US7 gene. Additional evidence that g70 is encoded by the US7 gene came from experiments involving anti-US7-peptide sera (which recognized g70) and a cell line which had been transfected with a plasmid containing the US7, gE, and US9 genes. Therefore, g70 appears to be identical to the glycoprotein recently described by Longnecker et al. (21), which was named gI, and is the seventh HSV-1 glycoprotein to be described.

It is particularly interesting that the gene encoding gI is located adjacent to the gE gene in the short unique (Us) region of the HSV-1 genome. In fact, there is less than 300 nucleotides separating the initiation codon of gE from the termination codon of gI (25). A situation similar to this exists in the long unique region of HSV-1 and HSV-2, where the genes encoding the two subunits of the ribonucleotide reductase enzyme partially overlap. In the case of the reductase subunits, coding sequences for the 140-kDa subunit act as promoter sequences for the 35-kDa subunit gene (28, 37). It is, perhaps, not surprising that genes encoding subunits of an active complex might be acquired as a unit and maintained in close proximity by selective pressure.

We previously suggested that gE and gI form a complex which binds IgG and that gE in isolation does not bind IgG (13). The evidence supporting this hypothesis is as follows: (i) both polypeptides were precipitated with rabbit or human IgG and with an MAb specific for gE; (ii) a fraction of gE did not bind to rabbit IgG during sequential precipitations; and (iii) rabbit IgG did not precipitate gE expressed in stably transformed cell lines which had been transfected with a plasmid containing the gE gene but not the gI gene. Here, we extend the evidence that gE and gI form a complex and that both polypeptides are required for Fc binding activity: (i) anti-gI MAb 3104 precipitates gI as well as a fraction of the gE in cell extracts; (ii) rabbit IgG precipitated the gE-gI complex from cells transfected with a plasmid containing an HSV-1 DNA fragment including the gI, gE, and US9 genes; and (iii) rabbit IgG did not bind to cells infected with mutant viruses unable to express gI or gE, although when the cells were coinfected with both viruses, the binding of IgG approached that observed with cells infected with wild-type HSV-1. Although we have not exhaustively excluded the participation of other viral or host polypeptides in Fc receptor activity, it appears that neither gE or gI alone can bind IgG and that together they form an Fc binding complex.

On the surface, it is perhaps troubling that we were unable to detect Fc receptors on mouse L cells expressing gE and gI. However, we also found that HSV-1-infected mouse cells bind very low levels of IgG as compared with HSV-1-infected human or monkey cells, possibly because HSV-1 replicates poorly in mouse cells. In addition, the transfected IE43 cells express lower levels of gE and gI than do infected parental L cells. Thus our inability to detect IgG Fc binding activity with these cells most probably relates to the low level of expression of these polypeptides. We plan to transfect primate cells with plasmids containing the gE and gI genes to establish whether expression of gE and gI is sufficient to confer Fc receptors on the cells.

We still have little information on the role played by Fc receptors during HSV infection in humans. It is now clear that viruses unable to express Fc receptors can replicate efficiently in a variety of cultured cells (21, 22, 27; results

presented here). However, the widespread prevalence of Fc receptors among members of the herpesvirus family suggests that these receptors play an important role in the viral replicative cycles, possibly by shielding virus-infected cells from the host's immune response. Adler et al. (2) found that preincubation of HSV-infected cells with nonimmune IgG markedly reduced complement- and cell-mediated lysis of the cells. We favor a model in which IgG Fc receptors act to reduce complement-mediated immune cytolysis by binding to the Fc domains of antibody molecules and sterically hindering the binding of complement molecules. We plan to test this model by using mutant viruses which are unable to express Fc receptors.

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