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Membrane Proteins Specified by Herpes Simplex Viruses

V. Identification of an Fc-Binding Glycoprotein

ROBERT B. BAUCKE AND PATRICIA G. SPEAR*
Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

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A glycoprotein with affinity for the Fc region of immunoglobulin was isolated from extracts of cultured cells infected with herpes simplex virus type 1, and experiments were done to characterize its properties and to investigate whether it could account for the Fc-binding activity previously demonstrated on the surfaces of intact herpes simplex virus-infected cells. The technique of affinity chromatography was used to identify and isolate the Fc-binding glycoprotein and to demonstrate the specificity of its interaction with immunoglobulin G-Fc. Although three electrophoretically distinguishable Fc-binding polypeptides were identified by affinity chromatography, these three species appear to be different forms of the same translation product, based on comparisons of proteolytic digestion products and on the kinetics of appearance of each form after a brief pulse with radioactive amino acids. The results suggest that one polypeptide, designated pE, is processed to yield gE_1, which is in turn processed to yield gE_2. Both gE_1 and gE_2 are glycosylated membrane proteins and both can be labeled by the lactoperoxidase-catalyzed radioiodination of intact infected cells, indicating the presence of these proteins in surface membranes of the cells. Increases in the amounts of gE_1 and gE_2 at the cell surface were found to parallel the increase in Fc-binding activity of intact infected cells.

Cells infected with either serotype of herpes simplex virus (HSV type 1 [HSV-1] or HSV-2) display membrane receptors that have affinity for the Fc region of immunoglobulin G (IgG) (11, 19, 26, 28, 29, 31, 32). These viruses induce the expression of the receptors in a variety of different cell types that normally do not display such binding characteristics, i.e., cultured epithelioid or fibroblastic cells. The increase in number of receptors parallels the production of HSV proteins and, in fact, has been shown to depend on viral gene expression (9, 29, 31). More recently, it has been reported that two other human herpesviruses, cytomegalovirus (15, 21, 23, 30) and varicella-zoster virus [M. Ogata and S. Shigeta, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S(H)59, p. 304], also induce receptors capable of binding to the Fc region of IgG.

Immunoglobulins from several mammalian species, including man, have been shown to bind to HSV-infected cells (31, 32). Assuming that all these immunoglobulins bind via their Fc regions to the same virus-induced receptor (which has not been proven in every case), one could infer that the receptor has affinity for a highly conserved portion of the antibody molecule. The fact that identification and characterization of the virus-induced receptor has so far depended on its affinity for IgG should not obscure the possibility, however, that it may have evolved to interact with some other molecule, related in structure to IgG.

Speculations about the function(s) of this herpesvirus-induced cell surface receptor have been based on the assumption that binding of IgG is pertinent to its role in viral replication or pathogenesis. It has been suggested that binding of IgG to the receptors on the surfaces of infected cells could interfere with immune cytolysis (7, 16), and evidence has recently been presented that aggregated rabbit IgG can inhibit both antibody-dependent, complement-mediated and antibody-independent, cell-mediated cytolysis of HSV-infected cells in vitro (1). In addition, it has been suggested that the binding of IgG to the herpesvirus-induced receptors may influence the expression of viral gene products in infected cells and could perhaps lead to malignant transformation or the establishment of latency (8, 16, 31). With regard to this hypothesis it is of interest that incubation of HSV-infected cells with relatively high concentrations of nonimmune rabbit IgG can reduce the yield of infectious progeny recovered from the cells (8). It remains to be determined, however, whether these observed effects of immunoglobulin are directly

relevant to the primary function of the herpesvirus-induced receptor.

Regardless of the function of this receptor, its affinity for IgG can be exploited for the isolation and characterization of the molecule(s) responsible for the Fc-binding activity. Sakuma et al. (23) recently presented evidence that cytomegalovirus receptor-IgG complexes can be precipitated by anti-IgG and that the cytomegalovirus Fc-binding receptor is a glycoprotein with an apparent molecular weight of 43,000.

In this report we describe the identification and isolation, by affinity chromatography, of an HSV-1-induced Fc-binding glycoprotein and also provide evidence that it could account for at least some, and possibly all, of the Fc-binding activity detected on the surfaces of infected cells. This Fc-binding glycoprotein accumulates in two electrophoretically distinguishable forms, whose apparent molecular weights are approximately 80,000 and 65,000, and is distinct from the HSV-1 glycoproteins that have been previously characterized (6, 12, 17, 22, 24, 25, 27).

MATERIALS AND METHODS

Cells and viruses. The cell lines used in these studies were HEp-2 and BHK-21, clone 13, obtained from Flow laboratories (Rockville, Md.). Both cell types were grown in the Dulbecco modification of Eagle minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum. The virus strains used were HSV-1(HFEM)syn, a syncytial plaque variant that we isolated from a nonmutagenized stock of HSV-1(HFEM) provided by A. Buchan (University of Birmingham, Birmingham, England), and HSV-2(GP), a syncytial plaque variant isolated from HSV-2(G) by Cassai et al. (4). Although HSV-1(HFEM)syn causes extensive fusion of BHK cells, it exhibits much less fusion-inducing activity in HEp-2 cells. HSV-2(GP), on the other hand, induces extensive fusion of HEp-2 cells. Both virus strains were propagated in HEp-2 cells and, from their initial isolation, have been passaged only a few times at low multiplicities of infection.

Chemicals and radioactive precursors. Reagents for electrophoresis, including acrylamide and the cross-linker *N,N*-diallyltartardiamide, were obtained from Bio-Rad Laboratories (Richmond, Calif.). The nonionic detergent Nonidet P-40 (NP40) was obtained from Gallard-Schlesinger Manufacturing Corp. (Carle Place, N.Y.); cyanogen bromide-activated Sepharose 4B was from Pharmacia Fine Chemicals (Piscataway, N.J.); bovine serum albumin (BSA), chicken egg albumin (OV), and protease V8 of *Staphylococcus aureus* were from Miles Laboratories (Elkhart, Ind.); rabbit anti-BSA and anti-OV IgG (each containing 4 to 6 mg of specific antibody per ml) were from Cappel Laboratories (Downington, Pa.); pepsin, glucose oxidase, and lactoperoxidase were from Worthington Biochemicals Corp. (Freehold, N.J.); chloramine-T was from Sigma (St. Louis, Mo.); and [^{35}S]methionine (500 to 600 Ci/mmol), [^{14}C]glucosamine (45 to 60 mCi/mmol), and [^{125}I]Na (carrier-free, 17 Ci/mg) were from New England Nuclear Corp. (Boston, Mass.).

Infection or mock infection of cells and incubation with radioactive precursors. Monolayer cultures of cells were infected with virus at an input multiplicity of 5 to 10 PFU/cell. After adsorption for 2 h at 37°C, the inoculum was removed, and the cells were incubated at 37°C in medium 199 supplemented with 1% fetal bovine serum (199-V). When continuously labeled extracts were desired, medium containing [^{35}S]methionine (3 μCi/ml in 199-V prepared with one-tenth the usual concentration of unlabeled methionine) or [^{14}C]glucosamine (1 μCi/ml in 199-V) was added at 3 h after infection, and incubation was continued at 37°C until 18 h after infection, at which time the cells were harvested. For pulse-labeled extracts, infected cells were incubated for 5 min at 6 h after infection with [^{35}S]methionine (10 μCi/ml in 199-V lacking unlabeled methionine), and the cells were either harvested immediately or after continued incubation in nonradioactive 199-V.

Cell surface iodination. Cell surface proteins were iodinated with ^{125}I according to the lactoperoxidase-catalyzed procedure described by Hubbard and Cohn (13). Briefly, cell monolayers were overlaid with phosphate-buffered saline (PBS; 10 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.14 M NaCl, 3 mM KCl, 0.5 mM $MgCl_2 \cdot 6H_2O$, 1 mM $CaCl_2$), pH 7.4, containing 5 mM glucose and [^{125}I]Na at 400 μCi/ml. Lactoperoxidase and glucose oxidase were added to give final concentrations of 20 μg/ml and 0.1 U/ml, respectively. After 10 min at room temperature on a shaker, unreacted ^{125}I was poured off and the cells were washed and harvested as usual.

Preparation of cell extracts. After cell monolayers were washed with PBS, the cells were scraped with a rubber policeman and collected by centrifugation. For extraction of proteins the cells were suspended in PBS containing 1% NP40 (1.0 ml/2 × 10^7 cells) and kept on ice, with occasional mixing, for 15 min. Nuclei were then removed by low-speed centrifugation, and the cytoplasmic extracts were stored at −70°C. Immediately before use for affinity chromatography, the extracts were thawed and centrifuged at 25,000 rpm in an SW 27.1 rotor for 2 h to remove insoluble material.

Preparation and use of Fc affinity columns. BSA or OV was covalently linked to cyanogen bromide-activated Sepharose 4B according to procedures supplied by Pharmacia. Typically, 15 g of cyanogen bromide-activated Sepharose 4B was rehydrated and washed with 1 mM HCl on a sintered-glass filter and then equilibrated with coupling buffer (0.1 M $NaHCO_3$, pH 8.3, containing 0.5 M NaCl). BSA or OV dissolved in coupling buffer was added to give a final concentration of 5 to 10 mg of protein per ml, and coupling was allowed to proceed at room temperature for 2 h with shaking of the mixture. Unreacted protein was then washed out with coupling buffer, and the remaining active groups on the Sepharose were blocked with 1 M ethanolamine, pH 8.5 (2 h at room temperature). The Sepharose was washed several times with coupling buffer, alternating with 0.1 M sodium acetate buffer (pH 4.0) containing 0.5 M NaCl,

to remove proteins that were adsorbed but not covalently coupled to the beads. Finally, the protein-conjugated Sepharose was equilibrated with PBS and stored at 4°C with 0.02% sodium azide.

Affinity columns were prepared by pouring sufficient BSA- or OV-conjugated Sepharose into 5-ml polypropylene columns to yield a bed volume of approximately 1 ml. This was washed and equilibrated with PBS-0.5% NP40 by passing at least 25 ml of this buffer slowly through the column, and then 0.4 to 0.6 mg of specific anti-BSA or anti-OV IgG (1.0 to 3.0 mg of total IgG) in 1.0 ml of PBS-0.5% NP40 was added. This solution was allowed to enter the bed slowly over a period of 1 h at room temperature, and the unbound immunoglobulin was then washed out with excess PBS-0.5% NP40. The columns were equilibrated at 37°C, and NP40-solubilized cell extracts were added (3 ml/column). Every 15 min approximately 0.5 ml was allowed to enter the bed until all had been applied, after which the columns were washed extensively with PBS-0.5% NP40. Finally, column-bound proteins (including immunoglobulin) were eluted with 2 to 3 ml of 3 M potassium thiocyanate in PBS-0.5% NP40. The eluted macromolecules were precipitated with 5% trichloroacetic acid, and the precipitates were washed sequentially with ethanol and acetone and then solubilized for analysis by sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis (12). Greater than 70% of trichloroacetic acid-precipitable radioactivity was recovered for the analysis.

Preparation of $F(ab')_2$ fragments from IgG. Rabbit anti-BSA IgG was treated with pepsin to yield $F(ab')_2$ fragments according to the procedure described by Nisonoff (20). After dialysis of the IgG (7 mg/ml) against several changes of 0.1 M sodium acetate, pH 7.8, the pH was adjusted to pH 4.5 with 1 M acetic acid and pepsin was added to yield a 1:50 (wt/wt) ratio of pepsin to IgG. This mixture was incubated overnight at 37°C and then neutralized with NaOH to inactivate the pepsin. As a control, an equal amount of IgG was treated identically except that no pepsin was added ("mock-treated" IgG). Ouchterlony double-diffusion precipitation tests indicated that the pepsin-treated, mock-treated, and untreated IgG had comparable precipitating activity with BSA (data not shown).

Preparation and use of radiodinated immunoglobulin. Rabbit IgG was iodinated with ^{125}I according to the chloramine-T method described by Byrt and Ada (3) and modified by Jensenius and Williams (14). A 10-µl amount of $[^{125}I]Na$ (5 mCi) was added to 5 ml of chilled rabbit IgG solution (1.5 mg/ml in 0.05 M sodium phosphate buffer, pH 7), followed by the addition and rapid mixing of 100 µl of chloramine-T solution (1 mg/ml). After 10 min on ice the reaction was stopped by addition of excess tyrosine (100 µl at 0.4 mg/ml). The IgG was isolated from the reaction mixture by chromatography on Sephadex G-50 and had a specific activity of approximately 10^8 cpm/mg of IgG. This preparation was diluted 10^{-2} in PBS containing 5% heat-inactivated fetal bovine serum for incubation with intact cell monolayers to monitor the appearance of Fc-binding receptors at various times after infection. Incubation was for 2 h with shaking at 37°C, after which the cells were washed extensively with PBS-5% heat-inactivated fetal bovine serum, solubilized in 1% SDS, and sonicated briefly; small samples were taken for quantitation of bound radioactivity.

Isolation and partial proteolysis of the Fc-binding polypeptides. Pulse-labeled or continuously labeled forms of the Fc-binding polypeptides were isolated by affinity chromatography, followed by preparative acrylamide gel electrophoresis, for partial proteolysis and analysis of the peptides. For each analysis extracts were prepared from approximately 1.2×10^8 cells and applied to a single affinity column. The polypeptides eluted from the column were concentrated by trichloroacetic acid precipitation as described above and fractionated by electrophoresis on SDS-acrylamide gel slabs (1.5 mm thick). Wide sample wells (4 cm) were used to accommodate the large amount of material. Both the fast-migrating and more slowly migrating Fc-binding polypeptides were localized in the gel by either punching out segments of the gel for quantitation of radioactivity or estimating their positions relative to BSA (the location of BSA was determined by staining one or two vertical strips of the gel; the remainder of the gel was not stained or fixed).

A horizontal strip of gel containing the desired Fc-binding polypeptide was cut from the gel slab and divided into 5-mm segments for the peptide-mapping procedure described by Cleveland et al. (5). Briefly, the gel segments were placed in sample wells of a second acrylamide gel (15% separating gel prepared with a *N,N*-diallyltartardiamide cross-linker instead of bisacrylamide) and overlaid with buffer containing various concentrations of the staphylococcal V8 protease. The resulting proteolytic fragments were separated by electrophoresis at constant current (12 mA/gel) for about 6 h. The gels were fixed and stained according to the procedure of Fairbanks et al. (10), and the radioactive bands were located by fluorography (2).

RESULTS

Detection of an IgG-binding protein in extracts from infected cells. The technique of affinity chromatography was applied to the isolation of a molecule(s) responsible for HSV-1-induced Fc-binding activity. The affinity columns consisted of rabbit antibody bound to antigen which itself was covalently coupled to Sepharose 4B. In all experiments reported here, the antigen was either OV or BSA and the antibodies had been prepared against OV or BSA, respectively. In the experiment shown in Fig. 1, extracts prepared from $[^{35}S]$methionine-labeled infected cells, by use of the detergent NP40 as described in Materials and Methods, were applied to a pair of OV-conjugated columns, only one of which contained bound IgG. After being extensively washed to remove unbound proteins, the columns were sequentially eluted with 2-ml aliquots of potassium thiocyanate solution, ranging in concentration from 0.25

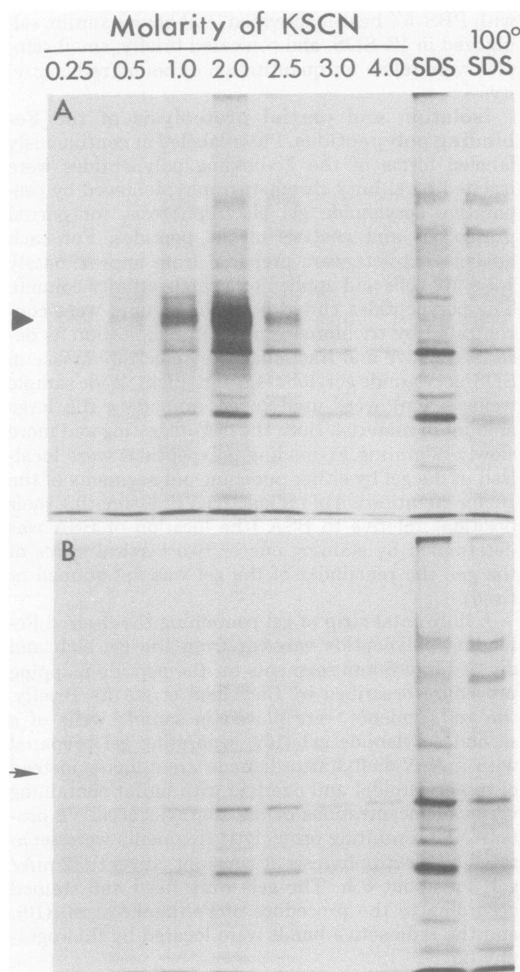

FIG. 1. *Elution of polypeptides bound to IgG-OV-Sepharose (A) or to control OV-Sepharose (B). Extracts were prepared from HEp-2 cells infected with HSV-1(HFEM)syn and incubated with [^{35}S]methionine from 3 to 18 h after infection. Samples of the extracts were applied to columns A and B, and, after extensive washing to remove unbound proteins, the columns were eluted sequentially with 2-ml aliquots of the solutions indicated, as described in the text. The labeled polypeptides present in each eluted fraction were analyzed by electrophoresis on SDS-acrylamide gel slabs, autoradiograms of which are shown.*

to 4 M, and then with 1% SDS. Finally, the Sepharose was suspended in a second aliquot of 1% SDS and heated to 100°C for 2 min, and the supernatant was recovered for analysis. The eluted proteins in each of these fractions were precipitated by trichloroacetic acid, solubilized, and analyzed by SDS-acrylamide electrophoresis. The autoradiogram shown in Fig. 1 demonstrates the specific binding of at least one polypeptide (indicated by the filled triangle) to the IgG-OV-Sepharose column (panel A) but not to the control OV-Sepharose column (panel B). This protein was optimally eluted by potassium thiocyanate in the range of 1.0 to 2.5 M. Small quantities of a protein with similar electrophoretic mobility had bound to the control column (indicated by the arrow), but its elution at a lower molarity of potassium thiocyanate suggests that it is either a different protein of approximately the same molecular weight or the same protein capable of interacting in a different fashion with the control column.

An experiment was done to determine whether the IgG-binding protein was produced only after infection or could also be detected in extracts from uninfected cells. Extracts were prepared from HSV-1-infected and from mock-infected cells labeled with [^{35}S]methionine for 15 h (from 3 to 18 h after infection), and aliquots were applied either to IgG-BSA-Sepharose columns or to control BSA-Sepharose columns. After being washed, the column-bound proteins were eluted in a single step with 3 M potassium thiocyanate and prepared for electrophoresis. Figure 2 shows that the IgG-binding protein (indicated by the filled triangle) could be isolated by affinity chromatography from extracts of the infected cells but not from those of the uninfected cells. It should be noted that this IgG-binding protein is a minor component of the infected cell extracts and is barely detectable in electropherograms of unfractionated extracts. For the electrophoretic analysis shown in Fig. 2, the IgG-binding protein eluted from the affinity column and applied to the gel was obtained from approximately 80 times as much extract as was also applied to the gel for comparative purposes. Results indistinguishable from those shown in Fig. 2 were obtained whether OV or BSA was used as the covalently coupled antigen (data not shown), indicating that interaction of the IgG-binding protein with the affinity column was independent of the antigen used.

In preliminary studies, we have also identified an IgG-binding protein induced after infection of HEp-2 cells by an HSV-2 strain [HSV-2(GP)]. The results obtained by affinity chromatography were very similar to those shown in Fig. 2, and the IgG-binding protein induced by HSV-2(GP) was indistinguishable by electrophoretic mobility from the protein induced by HSV-1(HFEM)syn.

Glycosylation of the IgG-binding protein. To determine whether the IgG-binding protein is glycosylated, extracts were also prepared from HSV-1-infected cells labeled with [^{14}C]glucosamine. Column-bound proteins from these extracts are compared with [^{35}S]methionine-labeled, column-bound proteins in Fig. 3. The

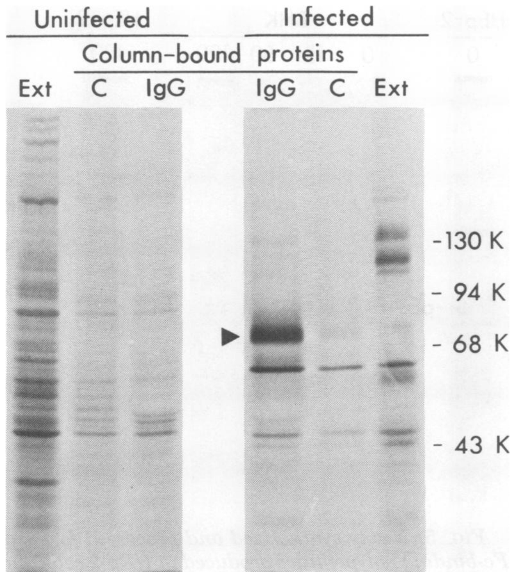

FIG. 2. *Specific binding to IgG-BSA-Sepharose of a polypeptide produced by HSV-1(HFEM)syn-infected HEp-2 cells, but not by uninfected cells. Extracts were prepared from infected or mock-infected HEp-2 cells that had been incubated with [^{35}S]methionine from 3 to 18 h after the addition of virus. This autoradiogram of an acrylamide gel slab shows the labeled polypeptides that were bound to and eluted from either IgG-BSA-Sepharose (IgG) or control BSA-Sepharose (C) and also the labeled polypeptides present in unfractionated extracts (Ext) from the uninfected or infected cells (the column-bound proteins were obtained from approximately 80 times as much extract as was present in the unfractionated sample applied to the acrylamide gel). In this and all subsequent figures elution was achieved in a single step by the application of 3 M potassium thiocyanate, after extensive washing of the affinity columns to remove unbound proteins. The numbers along the right edge indicate the positions of unlabeled polypeptides used as molecular weight standards (β-galactosidase, 130,000; phosphorylase b, 94,000; BSA, 68,000; OV, 43,000).*

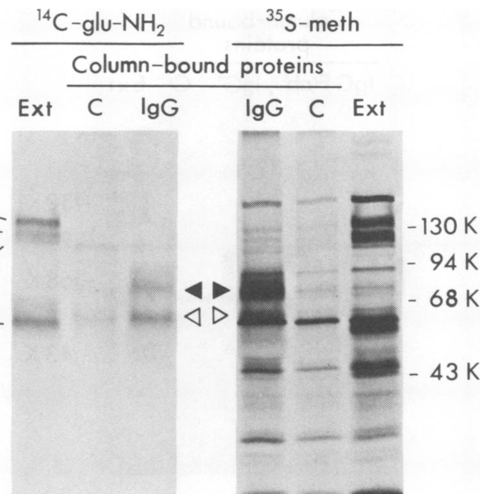

FIG. 3. *Presence of radiolabel from [^{14}C]glucosamine, as well as from [^{35}S]methionine, in HSV-1-induced polypeptides that bound specifically to IgG-BSA-Sepharose. This autoradiogram of an SDS-acrylamide gel slab shows the labeled polypeptides present in unfractionated extracts (Ext) from HSV-1(HFEM)syn-infected HEp-2 cells and the polypeptides bound to and eluted from either IgG-BSA-Sepharose (IgG) or control BSA-Sepharose (C). The polypeptide indicated by the filled triangles has the same electrophoretic mobility as the major IgG-binding species shown in Fig. 1 and 2. In different experiments, variable quantities of the polypeptide indicated by the open triangles were also found to bind specifically to the IgG-containing affinity columns. The symbols shown on the left edge are designations of the major HSV-1 glycoproteins, which have previously been described (6, 27).*

results show that a glucosamine-containing polypeptide binds specifically to IgG-BSA-Sepharose columns and that its electrophoretic mobility is the same as that of the [^{35}S]methionine-labeled, IgG-binding protein (filled triangles). Another glycosylated polypeptide of lower apparent molecular weight (open triangles) also appears to bind specifically to the IgG-containing column. Variable quantities of this species were detected in replicate experiments. Evidence will be presented below which suggests that these two IgG-binding species are related in amino acid sequence.

Demonstration that the IgG-binding protein interacts with the Fc region of IgG. To determine whether binding of the glycosylated polypeptides to IgG depends upon an intact Fc region, rabbit anti-BSA IgG was treated with pepsin to yield F(ab')$_2$ fragments, as described in Materials and Methods. The F(ab')$_2$ fragments were demonstrated to have retained antigen-binding activity and divalency by Ouchterlony gel diffusion tests with BSA (data not shown). Extracts from [^{35}S]methionine-labeled, HSV-1-infected cells were applied to F(ab')$_2$-BSA-Sepharose columns. As controls, equal amounts of extract were also applied to BSA-Sepharose columns and to IgG-BSA-Sepharose columns which had been prepared with either untreated IgG or IgG subjected to the reaction conditions used for pepsin digestion (in the absence of protease). The results shown in Fig. 4 illustrate that markedly less of the IgG-binding protein was retained on the F(ab')$_2$-BSA-Sepharose column compared with retention by the IgG-BSA-Sepharose column. This suggests that the binding is mediated through the Fc region of the IgG molecule.

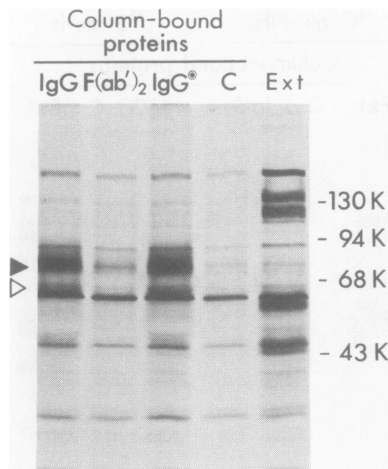

FIG. 4. *Reduction in binding of the HSV-1-induced polypeptides to the affinity columns by removal of the Fc region of IgG. Affinity columns were prepared by the addition to BSA-Sepharose of untreated anti-BSA (IgG) or of anti-BSA which had been digested by pepsin to yield $F(ab')_2$ fragments [$F(ab')_2$] or had been subjected to the same conditions used for proteolysis in the absence of pepsin (IgG©). An extract was prepared from HSV-1(HFEM)syn-infected HEp-2 cells that had been incubated with [^{35}S]methionine from 3 to 18 h after infection, and aliquots were applied to the three affinity columns and to a control BSA-Sepharose (C) column. This autoradiogram of an SDS-acrylamide gel slab shows the polypeptides bound to and eluted from each column along with the polypeptides present in a sample of the unfractionated extract (Ext). The filled and open triangles indicate the positions of two IgG-binding polypeptides, as described in the legend to Fig. 3.*

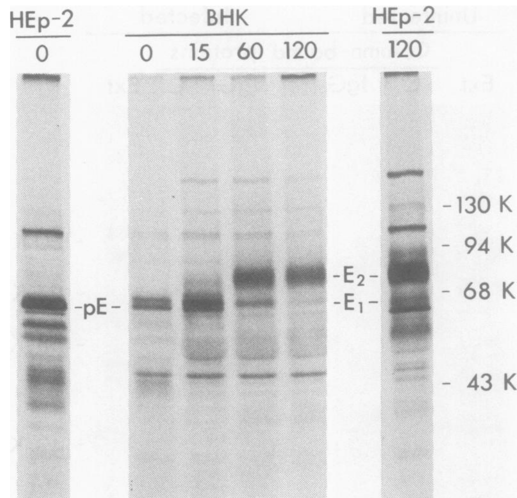

FIG. 5. *Newly synthesized and processed forms of Fc-binding polypeptides produced in HEp-2 cells and BHK cells infected with HSV-1(HFEM)syn. At 6 h after infection the cells were incubated with [^{35}S] methionine for 5 min, and then extracts were prepared immediately (0) or after an additional incubation of the cells in nonradioactive medium (for 15, 60, or 120 min). The extracts were applied to IgG-BSA-Sepharose columns and to control BSA-Sepharose columns, and the bound proteins were eluted for electrophoretic analysis. The designations pE, E_1, and E_2 indicate the positions of polypeptides bound specifically to the IgG-BSA-Sepharose columns but not to the control BSA-Sepharose columns (controls not shown).*

Newly synthesized and stable forms of the Fc-binding glycoprotein. Affinity chromatography of extracts from pulse-labeled HSV-1-infected cells was done to determine whether newly synthesized forms of the Fc-binding polypeptide(s) could bind to the IgG-antigen columns and might perhaps differ in electrophoretic mobility from the processed, stable forms. Infected cells were pulse-labeled for 5 min with [^{35}S]methionine at 6 h after infection and then harvested immediately or after 15, 60, or 120 min of continued incubation in nonradioactive medium. Both HEp-2 and BHK cells were used for this experiment in order to compare the Fc-binding polypeptides produced by different cells. The results shown in Fig. 5 demonstrate that three electrophoretically distinct polypeptides bound specifically to the IgG-BSA-Sepharose columns (control columns without IgG not shown).

Two of these forms, designated pE and gE_1, were detected immediately after the pulse. Within 15 min after the pulse a third form, gE_2, was detectable. By 60 min pE had disappeared, and by 120 min very little of gE_1 remained. These results are consistent with the possibility that all three electrophoretic forms are derived from the same translation product and that post-translational processing occurs with the conversion of pE to gE_1 and gE_1 to gE_2. The results obtained with infected HEp-2 and BHK cells were similar, although pE and gE_1 produced in the BHK cells were more easily recognized as distinct species. Because the apparent conversion of gE_1 to gE_2 is not complete even after 2 h, it seems likely that extracts of infected cells labeled for long periods of time would contain both of these species and that gE_1 is the faster-migrating Fc-binding glycoprotein seen in Fig. 3 and 4 (marked by the open triangles).

To test the possibility that gE_2 has amino acid sequences in common with pE and gE_1, these [^{35}S]methionine-labeled species were isolated by affinity chromatography and preparative electrophoresis and subjected to partial proteolysis by the procedure of Cleveland et al. (5). The

partial proteolytic products obtained with various concentrations of the staphylococcal protease V8 are shown in Fig. 6. It can be seen that, at all concentrations of enzyme tested, the electrophoretic profile of peptides from the pulse-labeled material (pE plus gE_1) is similar to that of peptides from the more slowly migrating stable material (gE_2), except that the profiles are displaced to the extent that the uncleaved polypeptides differ in mobility. This suggested the possibility that all the [^{35}S]methionine-labeled peptides obtained from gE_2 contained the modification responsible for the slower electrophoretic mobility of intact gE_2. If this were true, and if the modification includes glycosylation, then all the methionine-containing peptides from gE_2 should contain carbohydrate and would probably incorporate label from radioactive glucosamine. The results shown in Fig. 7 demonstrate, in fact, that all the [^{35}S]methionine-labeled peptides derived from gE_2 also incorporated label from [^{14}C]glucosamine.

Expression of the Fc-binding glycoprotein on the surfaces of infected cells. Other investigators have quantitated the binding of ^{125}I-labeled IgG to HSV-infected cells to investigate the kinetics of appearance of Fc-binding activity in the infected cell surface (18, 31). Similar experiments were performed in our laboratory (results shown in Fig. 8). Fc-binding activity became detectable by this assay between 3 to 6 h after infection and reached a maximum at 24 h. To determine whether the Fc-binding glycoprotein, detected by affinity chromatography, appeared in the cell surface with similar kinetics, the following experiment was done. Intact infected cells were iodinated with ^{125}I by a lactoperoxidase-catalyzed reaction at 3, 12, or 24 h after infection. Detergent extracts of these iodinated cells were then prepared and applied to both IgG-BSA-Sepharose columns and control BSA-Sepharose columns. Figure 9 shows an electropherogram of the ^{125}I-labeled polypeptides that were bound to and eluted from the IgG-containing columns; the triangles identify two species that bound specifically to the IgG-containing columns but not to the control columns (controls not shown). These two polypeptides, whose electrophoretic mobilities are indistinguishable from those of gE_1 and gE_2 (Fig. 5 and 9), were virtually undetectable in extracts prepared from cells at 3 h after infection, whereas significant quantities were present in the extracts prepared at 12 and 24 h. As is also evident from Fig. 9, samples eluted from the IgG affinity columns sometimes contained heterogeneous labeled material whose electrophoretic mobility was greater than that of the major Fc-binding species. The relationship of this material to gE remains to be determined.

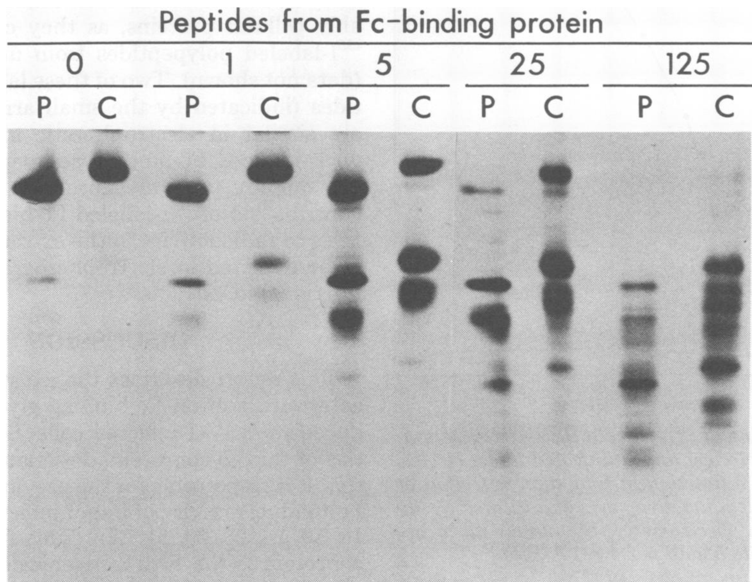

FIG. 6. *Peptides obtained from newly synthesized (P) and processed (C) forms of the Fc-binding glycoprotein after partial proteolysis with various concentrations (0 to 125 μg/sample) of S. aureus protease V8. The pulse-labeled forms of the Fc-binding glycoprotein (P) are the species designated pE and E_1 in the legend to Fig. 5, and the pulse-chased form (C) is E_2. These polypeptides were isolated for this analysis as described in the text.*

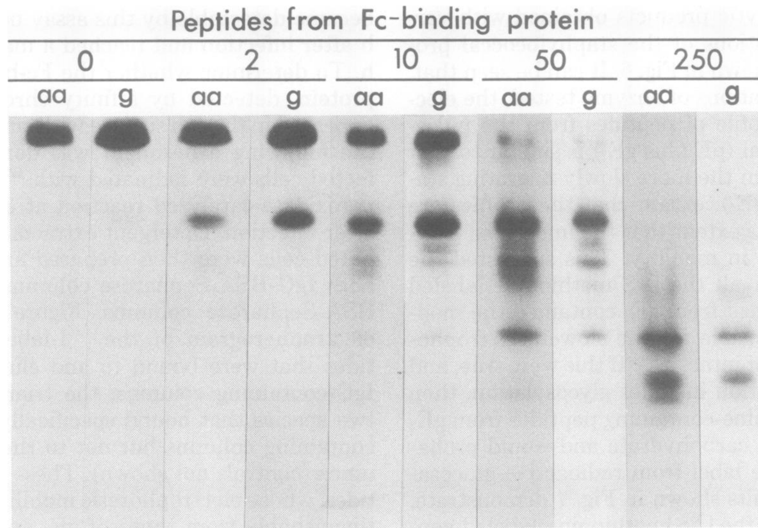

FIG. 7. *Peptides obtained from [^{35}S]methionine-labeled (aa) and [^{14}C]glucosamine-labeled (g) Fc-binding glycoprotein E_2 after partial proteolysis with various concentrations (0 to 250 μg/sample) of S. aureus protease V8.*

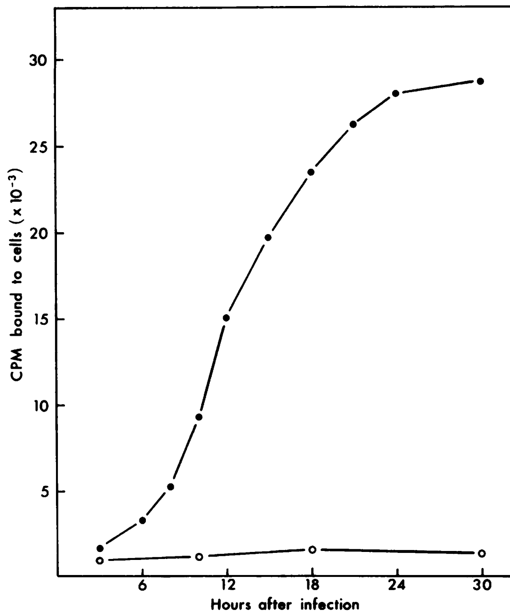

FIG. 8. *Binding of [^{125}I]IgG to HSV-1(HFEM)syn-infected (filled circles) and uninfected (open circles) HEp-2 cells, as a function of time after infection or mock infection. Radioactivity was quantitated by liquid scintillation spectrometry; the values given are counts per minute bound per 4 × 10^6 cells.*

The previously identified HSV-1 glycoproteins gC, gB, and gD were readily detected as ^{125}I-labeled bands in electropherograms of the unfractionated extracts prepared from the iodinated infected cells. The results presented in Fig. 9 indicate that significantly greater quantities of these proteins were present in the surfaces of infected cells at 12 and 24 h after infection than at 3 h, as was also found for the Fc-binding polypeptides. Several other polypeptides detected in the unfractionated extracts were labeled to approximately the same extent at both early and late times after infection and are probably cellular proteins, as they comigrate with ^{125}I-labeled polypeptides from uninfected cells (data not shown). Two of these labeled polypeptides (indicated by the small arrows in Fig. 9) are similar in electrophoretic mobility to the virus-induced Fc-binding polypeptides gE_1 and gE_2 but are probably unrelated to them. The contribution of ^{125}I-labeled Fc-binding polypeptides to radioactivity in the extracts is too small to be detected in electropherograms of the unfractionated extracts.

DISCUSSION

This report describes the isolation and characterization of an Fc-binding glycoprotein produced in HSV-1-infected cells. Several properties of this glycoprotein, designated gE, suggest that it is responsible for the previously described Fc-binding activity of intact infected cells (9, 11, 18, 19, 26, 28, 29, 31, 32). (This Fc-binding glycoprotein is the fifth antigenically or functionally distinct HSV-1-induced glycoprotein to be described. Assignment of the alphabetic designation gE conforms to the nomenclature agreed upon by a group of participants at the Herpesvirus Workshop held in August 1978 in Cambridge, England.) First, gE binds to IgG and this

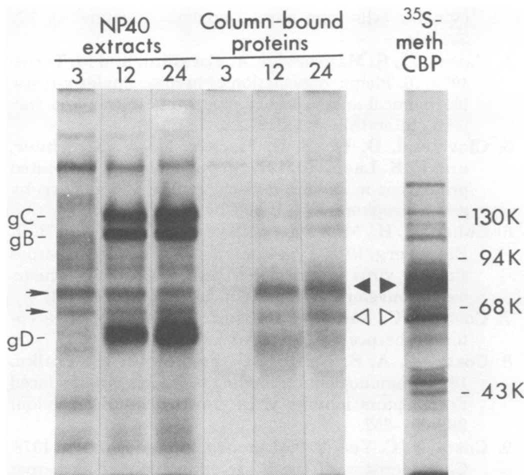

FIG. 9. *Presence and kinetics of appearance of the Fc-binding glycoprotein on the surfaces of infected cells. HEp-2 cells infected with HSV-1(HFEM)syn were labeled with ^{125}I, by a procedure which labels only proteins exposed to the extracellular fluid, at 3, 12, or 24 h after infection. Extracts were then prepared and applied to IgG-BSA-Sepharose columns and to control BSA-Sepharose columns. This autoradiogram of an SDS-acrylamide gel slab shows the ^{125}I-labeled polypeptides bound to and eluted from IgG-BSA-Sepharose, as well as the labeled polypeptides present in small samples of the unfractionated extracts. Also shown is a profile of [^{35}S]methionine-labeled polypeptides which bound to and were eluted from an IgG-BSA-Sepharose column.*

binding depends upon integrity of the Fc region. Second, gE is a constituent of the infected cell surface. Finally, the time of its appearance in the cell surface correlates with the time of appearance of Fc receptors on intact cells. The association of Fc-binding receptor activity with a membrane glycoprotein produced after infection is consistent with previous reports that this receptor is trypsin sensitive and that its expression is prevented by deoxyglucose and by inhibitors of protein synthesis (9, 18, 31).

Although Fc-binding activity was found to be associated with three electrophoretically distinct polypeptides, ranging in apparent molecular weight from 65,000 to 80,000, it appears that these polypeptides share amino acid sequences in common. Both the kinetics of appearance of each Fc-binding polypeptide after a pulse of radioactive amino acid and the results of comparisons by partial proteolysis suggest that a single translation product yields all three forms by sequential stages of posttranslational processing. Previous studies revealed that the HSV-1 glycoproteins gB, gC, and gD are processed similarly to gE, at least with respect to the changes in electrophoretic mobility that accompany posttranslational modifications (6, 27). The finding that newly synthesized as well as stable forms of gE could bind to the affinity columns indicates that Fc-binding activity does not depend on complete processing or complete glycosylation of the protein. It remains to be determined whether all forms of the Fc-binding protein, including the two forms detected on cell surfaces, interact the same way with IgG.

Although the possibility exists that gE is an induced cellular gene product, it seems more likely that the polypeptide moiety of this Fc-binding glycoprotein will prove to be of viral genetic origin. First, gE is apparently produced only after infection of the cell lines analyzed in this study and is synthesized at a time when all or most host protein synthesis is inhibited. Second, the electrophoretic mobilities of the various gE forms produced in HEp-2 cells are similar or identical to those produced in BHK cells. Finally, recent studies (M. Para, R. Baucke, and P. G. Spear, manuscript in preparation) have shown that gE is a constituent of the virion envelope as well as of the infected cell surface.

It can be concluded from the publications cited in the first paragraph of this section that a number of different HSV strains can induce Fc-binding receptors, suggesting that expression of this receptor is not a peculiarity of a few strains. There are differences among HSV strains, however, in the number of receptors detectable by ^{125}I-labeled IgG binding to the surfaces of infected cells. We chose to use HSV-1(HFEM)syn for the studies reported here, on the basis of screening several HSV-1 and HSV-2 strains for maximal expression of cell surface receptors. The results of this screening suggested that syncytial plaque variants of either HSV-1 or HSV-2 expressed larger numbers of Fc-binding receptors than did wild-type strains (R. Baucke and P. G. Spear, unpublished data). It remains to be determined whether the expression of Fc-binding activity is related to virus-induced cell fusion and whether the apparent differences in numbers of receptors are due primarily to variability in the amount of Fc-binding glycoprotein produced, its intracellular distribution, or its binding characteristics. In preliminary studies we have found that the Fc-binding glycoprotein induced by HSV-2(GP) is indistinguishable from the HSV-1(HFEM)syn glycoprotein by two criteria, electrophoretic mobility of intact SDS-solubilized proteins and mobilities of peptides obtained after digestion with V8 protease (Baucke and Spear, unpublished data).

The molecule reported to be responsible for cytomegalovirus-induced Fc-binding activity (23) differs in at least two respects from the HSV-1-induced Fc-binding gE. First, the appar-

ent molecular weight of the cytomegalovirus-induced Fc-binding glycopolypeptide is 43,000 as compared with 65,000 to 80,000 for the various forms of gE. Second, the cytomegalovirus-induced glycoprotein was isolated from cellular extracts prepared without the use of detergents (and therefore may not have been membrane bound), whereas HSV-1 gE could not be solubilized without detergents. It will be of interest to determine whether detergents can solubilize other forms of the cytomegalovirus-induced Fc-binding glycoprotein and to compare the properties of the Fc-binding proteins induced by HSV, cytomegalovirus, and varicella-zoster virus (see reference citations in Introduction).

The function of the herpesvirus-induced Fc-binding glycoprotein and its role in viral replication or pathogenesis, or in both, remain to be determined. The finding that Fc-binding activity can be detected on the surfaces of cells infected by three different human herpesviruses underscores the possible importance of this activity. The fact that detection of the binding activity has so far relied on the use of IgG, however, does not necessarily imply that the purpose of the virus-induced receptor is to interact with IgG. An understanding of the function of this receptor, which in the case of HSV-1 is expressed on the surfaces of both virions (Para et al., in preparation) and infected cells, will depend upon additional experimentation, including exploration of the possibility that the receptor may have affinity for molecules other than IgG.

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ADDENDUM IN PROOF

At the Herpesvirus Workshop held in August 1979 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), a group of the participants agreed upon a convention for the naming of the various processed forms of a single HSV glycoprotein. According to this convention, pE should be designated pgE(64), gE_1 should be pgE(66), and gE_2 should be gE. The numbers within parentheses are apparent molecular weights ($\times 10^{-3}$), used to differentiate among multiple precursor forms.

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