The Human Cytomegalovirus Receptor on Fibroblasts Is a 30-Kilodalton Membrane Protein†

HOWARD P. TAYLOR‡ AND NEIL R. COOPER*

*Department of Immunology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road,
La Jolla, California 92037*

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Previous studies have demonstrated that human cytomegalovirus (HCMV) binding to human foreskin fibroblasts (HFF) is mediated by a single type of molecule, likely a glycoprotein, which serves as a specific receptor for the virus. In the present experiments, HCMV was found to bind to an HFF membrane protein with an approximate molecular mass of 30 kilodaltons (kDa); weak binding to 28- and 92-kDa membrane components was also observed. Binding was specific, as it was inhibited by excess unlabeled HCMV. Radiolabeled HCMV also bound selectively to Raji and Daudi lymphoblastoid cell membrane proteins of the same molecular masses. The 30-kDa radiolabeled HFF membrane protein bound to HCMV in solution; this binding was also specific, as it was blocked by an excess of HCMV. These data suggest that a membrane protein with a molecular mass of approximately 30 kDa mediates HCMV binding to several cell types.

Human cytomegalovirus (HCMV) is responsible for significant morbidity and mortality in vivo and in all age groups (18). HCMV infection is also a major problem in immunosuppressed individuals, patients with acquired immunodeficiency syndrome, and other individuals with disease-associated immunosuppression (10, 35, 39). Although HCMV is an important human pathogen, the early events in HCMV infection of human cells have not been defined. Recently, we analyzed the binding of HCMV to human foreskin fibroblasts (HFF), a cell type which HCMV readily infects in vitro (43), and found that HCMV exhibited dose-dependent, high-affinity (K_d , 1.1 nM), and saturable binding to a single type of molecule on the cell surface. Binding was specific, as it was competitively inhibited in a dose-dependent manner by homologous and heterologous HCMV strains but unaffected by herpes simplex virus. Studies with proteases and inhibitors of glycosylation strongly suggested that the viral receptor is a glycoprotein. These data indicate that a single type of molecule, likely a glycoprotein, on the surface of HFF serves as a specific receptor for the virus.

A number of other viruses, including reovirus (9, 47), rabies virus (51), Epstein-Barr virus (11, 13, 34), human immunodeficiency virus (HIV) (5, 23), rhinovirus (1, 15, 41), and poliovirus (28), have been found to bind to specific receptors on the cell surface. In several instances, the normal cell surface molecule used by the virus to initiate infection has been identified; thus, it has been shown that the Epstein-Barr virus (EBV) receptor is CR2 (11, 13, 34), the HIV receptor is CD4 (5, 23), and the rhinovirus receptor is ICAM-1 (15, 41). The HCMV receptor has not been identified, although a series of studies by Grundy, McKeating, and collaborators (16, 17, 27) suggested that HCMV firmly binds β_2 microglobulin, a protein which is normally noncovalently associated with major histocompatibility complex (MHC) class I molecules. They proposed that HCMV coated with β_2 microglobulin binds to MHC class I molecules after displac-

ing β_2 microglobulin from the cell MHC molecules. Another recently published study by Keay et al. (22) used anti-HCMV idiotype antibodies to obtain evidence that a 92.5-kilodalton (kDa) HFF protein is involved in HCMV binding to several cell types, including HFF.

In the experiments presented here, we used a variety of techniques to identify the HCMV receptor. The studies cumulatively indicate that HCMV binds predominantly to a membrane protein with an approximate molecular mass of 30 kDa that is expressed on several cell types.

MATERIALS AND METHODS

Cells. HFF cultures were established and grown at 37°C in 150-cm² plastic flasks or 900-cm² roller bottles (Corning Glass Works, Corning, N.Y.) in Eagle minimal essential medium supplemented with 10% fetal bovine serum, L-glutamine (0.03%), and nonessential amino acids. The cells were maintained for at least eight passages before use. Raji and Daudi lymphoblastoid cells (American Type Culture Collection, Rockville, Md.) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Virus and virus purification. HCMV strain AD169 was obtained from the American Type Culture Collection. The origins and properties of this strain of HCMV have been described elsewhere (37). The titer of infectious HCMV was determined by plaque assay on HFF (49). HCMV was purified by a modification (43) of the method described by Stinski (42). Samples were frozen at -70°C and sonicated briefly before use.

Estimation of number of virus particles. The estimated number of virus particles was based on viral DNA content as described earlier (31). A molecular weight of 1.47×10^8 was used for HCMV DNA (14). The protein concentration of viral preparations solubilized in 0.1% sodium dodecyl sulfate (SDS) was estimated by using the colorimetric assay of Bradford (3).

Radiolabeling of HCMV. The medium of infected HFF cell monolayers at 30% cytopathic effect was changed and replaced with reduced-methionine RPMI 1640 containing [³⁵S]methionine (Amersham Corp., Arlington Heights, Ill.) at 1.11 MBq/ml (30 μ Ci/ml). [³⁵S]methionine-labeled virus was purified as previously described (43). The peak fractions

* Corresponding author.

† Publication 6026-IMM from the Research Institute of Scripps Clinic, La Jolla, CA 92037.

‡ Present address: Duke University Medical Center, Durham, NC 27710.

which contained both HCMV radioactivity and infectivity were pooled and used for these studies. Specific activities ranged from 1.9×10^{-5} to 1.4×10^{-4} cpm per particle.

Biotinylation of HCMV. HCMV was biotinylated by using a modification of a previously described method (20). Purified HCMV was pelleted and suspended in sodium carbonate buffer (pH 8.8). An 11- μ l volume of a 1-mg/ml concentration of *N*-hydroxyl succinimido-2-iminobiotin (Sigma Chemical Co., St. Louis, Mo.) in dimethyl sulfoxide (Sigma) was added to 0.25 mg of virus in 100 μ l of carbonate buffer and incubated for 4 h at 25°C with end-over-end rotation. Unbound biotin was removed by dialysis against phosphate-buffered saline (PBS) in a microdialyzer at 4°C. Biotinylated HCMV was kept at 4°C and used within 4 days.

Cell membrane preparation. Subconfluent HFF monolayers in plastic tissue culture roller bottles were washed three times with PBS and twice with EDTA and incubated for 15 min at room temperature with an EDTA-PBS solution (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], 150 mM NaCl, 4 mM EDTA [pH 7.2] in PBS). The cells were then washed three times in Hanks balanced salt solution (0.14 M NaCl, 5.4 mM KCl, 0.8 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.4 mM KH_2PO_4 , 1.4 mM NaHPO_4 containing 0.5% crystalline, globulin-free bovine serum albumin [Sigma]) (HBSS). The cells were then suspended in 8 ml of disrupting buffer (1 mM CaCl_2 , 0.25 M sucrose, 1 mM MgCl_2 , 10 mM Tris [pH 7.5] containing 200 μ M phenylmethylsulfonyl fluoride [PMSF], 1 μ M leupeptin, 100 μ M EDTA, and 1 μ M pepstatin at a concentration of 2.5×10^7 cells per ml. After cooling to 4°C, the cells were subjected to a pressure of 750 lb/in² for 15 min before nitrogen decompression in a Parr cell disruption bomb. This method yields cell-free homogenates with intact nuclei. The nuclei and cell debris were pelleted at $10,000 \times g$ for 15 min. The supernatant was removed, and the volume was increased to 10 ml with buffer containing 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS; Sigma) and octylglucoside (Sigma) to yield final concentrations of 3.8 mg/ml of CHAPS and 4.0 mg/ml of octylglucoside. These concentrations are below the critical micellar concentrations for these detergents. The membrane preparation was centrifuged at 40,000 rpm in a Beckman 65 Ti rotor for 75 min, and the protein pellet was suspended in either PBS or 0.054 M Tris-0.005 M EDTA and stored at -70°C until use. The same method was used to prepare Daudi and Raji membrane proteins, except for omission of EDTA.

Slot blot assay for virus binding. A relatively rapid slot blot technique was used to screen cell membrane preparations or fractions obtained during purification. One-hundred-microliter volumes of membrane preparations to be tested for virus binding were adsorbed onto nitrocellulose membrane filters (Schleicher & Schuell, Inc., Keene, N.H.) by using a slot blot apparatus from Hoefer Scientific Instruments, San Francisco, Calif. The filters were dried and then incubated for 2 h in 2% nonfat milk protein (Carnation Co., Los Angeles, Calif.) in HBSS to block nonspecific binding sites. The filters were washed three times in 1% $5 \times$ crystallized ovalbumin (Calbiochem-Behring, La Jolla, Calif.) in HBSS (blotting buffer) and then incubated for 2 h with 5×10^9 particles of ³⁵S-labeled HCMV in blotting buffer. Unbound virus was removed by four washings in blotting buffer. The filter was dried, and bound ³⁵S-labeled HCMV was detected by autoradiography. This method was used routinely to screen for HCMV-binding ability.

SDS-PAGE and electroblotting of cell membrane proteins. Cell membrane preparations or other proteins of interest

were run on 10% polyacrylamide gels (24) under native conditions (0.1% SDS, no reduction or boiling) as previously described (4). After SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the gels were electrophoretically transferred to nitrocellulose membranes (44) without denaturing agents. To determine transfer success, the transferred protein bands on the nitrocellulose were stained with AuroDye (Janssen Life Science Products, Piscataway, N.J.). For the virus-binding assay, nonspecific binding sites were blocked by treating the nitrocellulose filters with 2% (wt/vol) nonfat dry milk protein (Carnation) overnight in HBSS at 25°C. After being washed three times with this blotting buffer, the nitrocellulose transfer sheet was cut into strips corresponding to the lanes of the gel. The strips were incubated with 2.5×10^9 to 2.5×10^{10} particles of ³⁵S-labeled HCMV in 0.5 ml of blotting buffer for 3 h at 25°C in the wells of six-well flat-bottom tissue culture trays (Corning Glass Works). During incubation, the culture trays were rotated on a rotary wheel. After incubation, unbound material was aspirated and the strip was washed four times in blotting buffer. The filter was dried, and bound virus was detected by autoradiography.

Radioiodination of proteins in SDS-PAGE gel slices. Proteins were radioiodinated in situ in SDS-PAGE gel slices by using the technique described by Elder et al. (8). Briefly, cell membrane extracts were subjected to SDS-PAGE under conditions designed to minimize protein denaturation as described above. The 30-kDa region of the gel or another area of the gel was excised and radioiodinated with 300 μ Ci of ¹²⁵I as previously described (8). The gel slices were then washed with five changes of 10% methanol, after which cell proteins were electroeluted overnight.

Streptavidin precipitation of biotinylated HCMV-receptor complexes. In situ-radiolabeled and eluted membrane constituents prepared as described above were preabsorbed three times with streptavidin-agarose beads (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The samples were then incubated in 500 μ l of HBSS containing 50 μ g of biotinylated HCMV for 90 min at 25°C. Streptavidin-agarose beads were added to the mixture, and incubation was continued for 30 min. The beads were then washed five times in HBSS, eluted with SDS, and analyzed by SDS-PAGE. Streptavidin precipitation of virus-labeled cell proteins was detected by autoradiography. Competition experiments were performed by using a 15-fold excess of unbiotinylated HCMV.

RESULTS

Binding of HCMV predominantly to an HFF membrane constituent with an approximate molecular mass of 30 kDa. Preliminary studies indicated that HFF membrane preparations generated by nitrogen cavitation bound HCMV. Partitioning of the HCMV-binding moiety into the pellet was obtained with a mixture of 3.8 mg/ml of CHAPS and 4.0 mg/ml of octylglucoside (Fig. 1). These concentrations are below the critical micellar concentration of each detergent. The pellet was solubilized, and increasing amounts were immobilized on nitrocellulose and assessed for the ability to bind 5×10^9 particles of [³⁵S]HCMV in a slot blot apparatus. Binding of [³⁵S]HCMV occurred with 100 μ l of a 3-mg/ml solution of membrane proteins (300 μ g) (Fig. 1), which corresponds to approximately 1.36×10^8 HFF cells.

To further characterize the HCMV-binding moiety, 175 μ g of membrane protein prepared as described above was subjected to SDS-PAGE under various experimental condi-

FIG. 1. Binding of HCMV to HFF membrane extracts. Increasing amounts of HFF membrane proteins were spotted onto nitrocellulose with a slot blot apparatus. After being blocked, the nitrocellulose sheet was incubated with 5×10^9 [^{35}S]HCMV particles for 2 h at 25°C. HCMV binding was assessed by autoradiography.

tions. After being electroblotted onto nitrocellulose without denaturing agents, blocked, and washed, 2.5×10^9 [^{35}S]HCMV particles were incubated with each nitrocellulose strip corresponding to a lane of the gel. The radiolabeled HCMV preparation bound to a band with an approximate molecular mass of 30 kDa in samples subjected to electrophoresis under conditions designed to minimize protein denaturation (0.1% SDS without reduction or boiling of the sample) (Fig. 2, lane a). Minor bands at 28 and 92 kDa were also evident. HCMV binding to a band with a molecular mass of approximately 30 kDa was observed in all such studies performed with many cell membrane preparations and several different [^{35}S]HCMV preparations; in some cases (Fig. 2), the 30-kDa band appeared as a doublet. Binding to the 28- and 92-kDa bands was more variable. A 10-fold excess of unlabeled HCMV inhibited the binding of 2.5×10^9 particles of [^{35}S]HCMV by 55% as determined by scanning of the autoradiogram (data not shown), a level of inhibition similar to that observed in earlier studies of [^{35}S]HCMV binding to intact cells (43). Radiolabeled HCMV preparations failed to bind to any bands in identical detergent extracts subjected to a high (2.5%) SDS concentration with (Fig. 2, lane b) or without (Fig. 2, lane c) reduction; HCMV also did not bind to reduced samples subjected to 0.1% SDS but not boiled (data not shown). Although more than 50 bands were apparent on staining of the electroblotted membrane proteins (Fig. 2, lane d), the 28- to 30-kDa region of the electroblotted gel contained relatively few membrane proteins.

To be sure that HCMV rather than a contaminant in the radiolabeled preparations bound to the electroblotted gels, the 30-kDa region of a membrane lysate which had been subjected to SDS-PAGE, electroblotted onto nitrocellulose, and reacted with [^{35}S]HCMV was excised and the nitrocellulose segment was eluted with 0.1% SDS. The eluted sample was then subjected to SDS-PAGE and analyzed by autoradiography. The bands were identical to those exhibited by [^{35}S]HCMV directly subjected to SDS-PAGE (data not shown), confirming that HCMV binds to the 30-kDa membrane constituent.

Binding of HCMV to a 30-kDa protein in B-lymphoblastoid membrane extracts. Raji and Daudi lymphoblastoid cell membrane preparations were subjected to SDS-PAGE under comparable minimally denaturing conditions, electroblotted, and examined for the ability to bind [^{35}S]HCMV as described earlier. Radiolabeled HCMV bound to a 30-kDa

FIG. 2. HCMV binding to HFF membrane extracts subjected to SDS-PAGE and electroblotting. HFF membrane proteins (175 μg) were subjected to SDS-PAGE under various conditions, electroblotted onto nitrocellulose, and examined for the ability to bind 2.5×10^9 [^{35}S]HCMV particles for 3 h at 25°C. Lanes: a, 0.1% SDS in sample buffer, not reduced and not boiled; b, 2.5% SDS in sample buffer, not reduced; c, 2.5% SDS–5% β -mercaptoethanol; d, AuroDye stain of transferred HFF membrane proteins.

band in HFF, Raji, and Daudi membrane extracts (Fig. 3). HCMV also bound to an identical spectrum of 92-kDa and higher-molecular-mass bands in the three cell types. HCMV bound to proteins with similar molecular masses in murine L-cell membrane extracts (data not shown).

Lack of binding of HCMV to electroblotted β_2 microglobulin or HLA class I antigen. Although neither β_2 microglobulin nor HLA class I antigens have molecular masses in the vicinity of 30, 28, or 92 kDa, the possibility that these proteins migrate aberrantly on minimally denaturing gels and mediate the HCMV-binding moiety was addressed experimentally. However, [^{35}S]HCMV failed to bind to purified β_2 microglobulin or purified HLA class I antigen (45) which had been subjected to SDS-PAGE under minimally denaturing conditions and electrotransferred to nitrocellulose (Fig. 4). Successful transfer of the proteins to nitrocellulose was confirmed by protein staining with AuroDye.

Binding of the 30-kDa HFF membrane constituent to HCMV in solution. The above-described experiments indicated that HCMV bound selectively to a membrane constituent with a molecular mass of approximately 30 kDa immobilized on nitrocellulose. To eliminate the possibility that such binding was an artifact of the test system, the ability of the approximately 30-kDa membrane constituent to bind HCMV in solution was assessed. In these studies, a total of 1.5 mg of an HFF membrane extract was applied to four different lanes and subjected to SDS-PAGE under minimally

FIG. 3. HCMV binding to membrane extracts from several cell types. Membrane proteins from HFF (150 μ g, lane a), Raji lymphoblastoid cells (450 μ g, lane b) or Daudi lymphoblastoid cells (450 μ g, lane c) were subjected to SDS-PAGE under minimally denaturing conditions and electroblotted onto nitrocellulose. Ability to bind 2.5×10^{10} particles of [35 S]HCMV was then assessed.

denaturing conditions. The 30-kDa areas of the gels were excised and radiolabeled with 125 I in situ, and labeled constituents were electroeluted from the gels. The pooled, radiolabeled, eluted sample exhibited an intense 30-kDa band and a weaker 50- to 60-kDa band, possibly representing a dimer, on SDS-PAGE analyses performed under minimally denaturing conditions (Fig. 5, lane a). The radiolabeled, eluted 30-kDa area of the gel was incubated either with 50 μ g of biotinylated HCMV or with 50 μ g of biotinylated HCMV together with 750 μ g of unlabeled HCMV for 90 min at 25°C, after which streptavidin-agarose beads were added to bind biotinylated HCMV and attached labeled membrane proteins. After thorough washing, the beads were eluted with SDS and the eluate was analyzed by SDS-PAGE under minimally denaturing conditions. The 30-kDa and 50- to 60-kDa membrane constituents bound to biotinylated HCMV (Fig. 5, lane b). The binding was specific, as it was markedly inhibited by a 15-fold excess of unlabeled HCMV (Fig. 5, lane c). In similar studies, a minimally denaturing SDS-PAGE gel of an HFF membrane extract was sectioned at 20- to 30-kDa intervals and each segment was separately radiolabeled in situ with 125 I as described above. After electroelution, the labeled fractions were recombined and examined for binding to biotinylated HCMV as described above. Only labeled 28- to 30-kDa membrane constituents bound to biotinylated HCMV (data not shown). These

FIG. 4. Lack of binding of HCMV to electrotransferred β_2 microglobulin or HLA class I antigen. HFF membrane proteins (150 μ g, lane a), purified HLA class I antigen (40 μ g, lane b), and purified human β_2 microglobulin (25 μ g, lane c) were subjected to SDS-PAGE under minimally reducing conditions, electroblotted onto nitrocellulose, and examined for the ability to bind 2.5×10^{10} particles of [35 S]HCMV.

results indicate that the 30-kDa HFF membrane constituent also binds to HCMV in solution, and they further prove the specificity of the reaction.

DISCUSSION

Infection of cells by intracellular pathogens is initiated by attachment of the pathogen to specific proteins, carbohydrate residues, or glycolipids on the cell surface (26, 29). By targeting the pathogen to certain cell types, such cell surface molecules, termed receptors, are largely responsible for the host cell specificity and site of initiation of the infection. Examples for viruses include targeting of HIV to T lymphocytes and monocytes-macrophages as a result of the relatively exclusive presence of CD4, its receptor, on these cell types (5, 23) and the tropism of EBV for B lymphocytes, because of the restriction of CR2 (CD21), its receptor, to this cell type (11, 13, 34). Conversely, the ubiquitous presence on cells of sialic acid residues and the adhesion molecule ICAM-1, the receptors for influenza virus (50) and rhinovirus (15, 41), respectively, explains the ability of these viruses to infect multiple cell types.

The cell surface receptors used by most pathogens have not been identified. This report describes our progress in identifying the HCMV receptor on HFF. The rationale for these studies was provided by a biochemical analysis of the

FIG. 5. Binding of the 30-kDa HCMV-binding moiety to HCMV in solution. Membrane proteins from HFF (1.5 mg) were divided among four lanes and subjected to SDS-PAGE under minimally denaturing conditions. The 30-kDa regions of the gels were excised, radiolabeled in situ with ^{125}I , electroeluted, and pooled. Lane a shows the SDS-PAGE pattern of the electroeluted, labeled 30-kDa region of the gel. The radiolabeled, electroeluted 30-kDa region proteins were examined for the ability to bind 50 μg of biotinylated HCMV alone (lane b) or in the presence of a 15-fold excess of unlabeled HCMV (lane c). Membrane protein binding to biotinylated HCMV was detected by SDS-PAGE following removal of biotinylated HCMV from solution with streptavidin-agarose and elution.

characteristics of HCMV binding to HFF. In these studies (43), we found that attachment is specific, saturable, and of high affinity (K_d , 1.1 nM). These properties identify the binding as receptor mediated. Linear Scatchard plots were obtained at 4°C, indicating that a single type of receptor mediates HCMV binding.

In the present studies, we used several approaches to identify the HCMV receptor. HFF membrane proteins were separated by SDS-PAGE under minimally denaturing conditions (4) and examined for binding to HCMV. Subjecting the samples to high SDS concentrations, reduction, and/or boiling abrogated reactivity with HCMV. Two independent approaches were used to demonstrate HCMV binding to the separated membrane constituents. In the first approach, the gel was electroblotted onto nitrocellulose and the transferred replica was incubated with radiolabeled HCMV. In the second, the separated membrane constituents were radiolabeled with iodine in situ in the gel, electroeluted, and examined for binding to biotinylated HCMV in solution. Both techniques showed that HCMV bound predominantly to an HFF membrane protein of approximately 30 kDa;

some binding to 28- and 92-kDa membrane constituents was also observed on most occasions. Evidence of specificity in each instance was provided by the demonstration that unlabeled HCMV competed for binding to the 30-kDa (and the 28- and 92-kDa) membrane constituent.

Since HCMV binding to the 30-kDa HFF membrane constituent was invariably observed and always represented the major band, we consider it likely that it represents the primary HFF receptor for HCMV rather than a cell surface protein involved in a subsequent step, such as fusion or penetration. The role played by the other observed HCMV-binding proteins is not known. Although the 28-kDa molecule could represent a proteolytic product and the 50- to 60-kDa constituent could represent a dimer, each, as well as the 92-kDa membrane protein, could also represent a subunit of a multimeric receptor. Alternatively, the other proteins could be involved in fusion or another early step in virus entry. It also is not clear whether the 92-kDa HCMV-binding factor observed here is identical to the 92.5-kDa HCMV receptor described recently by Keay et al. (22), who used an anti-idiotypic antibody to the 86-kDa HCMV gH analog (also called gCIII), although this is likely. Immunoblot analyses with the internal-image anti-idiotypic antibodies also showed some binding to higher-molecular-mass (180-kDa) and lower-molecular-mass (45- to 49-kDa and 35-kDa) species (22); these also may be the same as some or all of the other HCMV-binding proteins described in the present studies.

The approximate 30-kDa molecular mass of the apparent HCMV receptor precludes its identity as an MHC class I antigen, as suggested by several studies (16, 17, 27). The human MHC class I molecule consists of a single glycosylated polypeptide chain with a molecular mass of 45 kDa. Binding of HCMV to Daudi lymphoblastoid cells, as shown earlier (43), and to 30-kDa Daudi membrane proteins, as shown here, is also not consistent with the notion that the 30-kDa molecule is an MHC class I antigen, since Daudi cells lack MHC antigens (38). Furthermore, as shown here, HCMV failed to bind to purified MHC class I antigen subjected to the same SDS-PAGE and electroblotting conditions used to show binding to the 30-kDa membrane constituent. The amount of HLA class I antigen used was equivalent to 15 times the amount of HLA class I antigen potentially present on the 150 μg of HFF membrane proteins used in this experiment, if there are 5×10^5 HLA class I molecules per cell. This ratio is probably an overestimation, as it applies to lymphocytes. Thus, our studies do not support a major role for HLA class I antigens in HCMV binding to cells.

HCMV does, however, possess some ability to bind β_2 microglobulin, as shown by Grundy, McKeating, and colleagues (16, 17, 27). While this finding might have been attributed to the presence of an open reading frame in the virus with homology to MHC class I antigens (2), recent electron microscopic studies indicate that β_2 microglobulin binds to the tegument of HCMV (40). Since the tegument is not exposed in intact virions, β_2 microglobulin binding may occur as a result of the presence of disrupted virions in the HCMV preparations. In the present studies, HCMV did not bind to β_2 microglobulin subjected to the same SDS-PAGE and electroeluting conditions as those used to show HCMV binding to the 30-kDa membrane constituent. Thus, our studies do not implicate β_2 microglobulin in HCMV attachment to cells. The presence of a 30-kDa HCMV-binding molecule on HFF, as well as on B-lineage-derived Raji and Daudi lymphoblastoid cells and murine L cells, is of considerable interest and may indicate that the same molecule or a

related molecule is present on multiple cell types from several species. If true, this could explain the ability of HCMV to infect not only HFF but also many other cell types, including human lymphocytes and murine fibroblasts (7, 36).

The study of pathogen ligand-receptor interactions is important for understanding of the pathogenesis of disease. Entirely separate from these considerations, however, is the fact that such knowledge may lead to new therapies for prevention or treatment of disease. For example, it has been demonstrated recently that soluble recombinant CD4 blocks HIV infection *in vitro* and *in vivo* (6, 12, 19, 46, 48). Peptides that duplicate the receptor-binding epitopes of gp120, the HIV ligand protein, also block infection by the virus *in vitro* (21, 25). Similarly, we have recently reported that soluble recombinant CR2 blocks EBV infection *in vitro* (32), as do short peptides that represent the binding epitope of gp350, the EBV ligand protein (30).

Use of extremely sensitive techniques has enabled us to show that the HCMV receptor on HFF is a 30-kDa membrane protein. The 30-kDa molecule is, however, a trace constituent of the HFF membrane, and we were unable to purify it to homogeneity because of its extremely low concentration. Further efforts in this area will require generation of an antibody and isolation of the receptor from extremely large volumes of membrane lysates by affinity chromatography, an approach which enabled us to isolate and characterize the EBV receptor (33). These efforts are under way.

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