JOURNAL OF VIROLOGY, Sept. 1989, p. 3991–3998
0022-538X/89/093991-08$02.00/0

Vol. 63, No. 9

Human Cytomegalovirus Binding to Fibroblasts Is Receptor Mediated†

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Received 13 February 1989/Accepted 19 May 1989

The binding of radiolabeled human cytomegalovirus (HCMV) strain AD169 to human lymphocytes, lymphoblastoid cell lines, monocytes, and fibroblasts varied over a 20-fold range. Since maximum binding was observed with human foreskin fibroblasts (HFF), interactions of radiolabeled HCMV with this cell type were analyzed quantitatively. Binding of HCMV to HFF at 4°C was specific and saturable; at low viral inputs specific binding averaged 16.4% of input and nonspecific binding was less than 1% of input. Binding curves yielded single-component linear Scatchard plots indicating an average K_d of 1.1 nM and 5,262 available virus-binding sites per cell. A two-component Scatchard curve was obtained at 37°C and reflected viral internalization, since it could be converted to a single-component curve by the use of paraformaldehyde-fixed cells. HCMV strain Towne was found to bind to the receptor used by HCMV strain AD169 with similar affinity. HCMV failed to bind to protease-treated HFF or to HFF grown in the presence of inhibitors of glycosylation. Sialic acid residues, however, were not found to be important in binding. 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.

Human cytomegalovirus (HCMV), a member of the herpesvirus family, is a major pathogen responsible for significant morbidity and disease in utero and in newborns, children, and adults (15). HCMV infection is a serious problem in patients receiving immunosuppressive treatment in preparation for allografts, in acquired immunodeficiency syndrome patients, and in other individuals with disease-induced immunosuppression (4, 9, 11). Primary infection, whether associated with disease or inapparent, is followed by lifelong latent infection. As is the case, however, with many other pathogenic agents, the earliest events in HCMV infection have not been defined. In analogy with other viruses, infection is likely initiated by the specific and firm binding of HCMV, via one or potentially several viral envelope glycoproteins or proteins, to one or more cell membrane structures. Viral endocytosis and/or fusion with the plasma membrane, de-envelopment, movement of the nucleocapsid through the cytoplasm to the nucleus, and passage of the viral genome through the nuclear membrane would follow. The viral and cellular structures and biochemical events responsible for these dynamic processes are largely unknown.

HCMV, with a genome of approximately 240 kilobases, induces in infected cells more than 50 proteins, of which at least 30 are structural proteins of the virus (8, 20, 33, 37). The structure and function of most of these polypeptides are not known. The HCMV glycoproteins that are expressed on the envelope of the viral particle exhibit complex precursor-product relationships and form complexes with each other, making characterization difficult (18, 28). Although antibodies to some of these proteins neutralize viral infectivity, notably those directed to gcIII/p86 (5, 31) and gcII/gp50-52 (18), there is no direct evidence that these molecules serve as the viral attachment protein.

There is little knowledge of the cell surface structure to which HCMV binds except for a recent series of studies by Grundy, McKeating, and collaborators (12, 13, 24, 25) which suggested that HCMV firmly binds β_2-microglobulin, a host protein which is normally noncovalently associated with the major histocompatibility complex class I molecule. They proposed that HCMV coated with β_2-microglobulin binds to human leukocyte antigen class I molecules after displacing β_2-microglobulin from the cell surface major histocompatibility complex molecules. In support, HCMV contains a major histocompatibility complex class I gene (3).

In the present study we examined the interaction of HCMV with human cells. We found that HCMV binds specifically and with moderate affinity to a single receptor on the surface of human foreskin fibroblasts (HFF), which HCMV readily infects. Studies with proteases and inhibitors of glycosylation showed that the receptor is a glycoprotein or closely associated with a glycoprotein. Saturation was achieved with the attachment of approximately 5,000 HCMV particles per cell.

MATERIALS AND METHODS

Cells. HFF cultures were established and grown at 37°C in 75- or 150-cm^2 plastic flasks (Corning Glass Works, Corning, N.Y.) with Eagle minimal essential medium (MEM) supplemented with 10% fetal bovine serum, L-glutamine (0.03%), and nonessential amino acids. HFF were used between passages 8 and 15. Human monocytes and lymphocytes were purified from peripheral blood mononuclear cells by centrifugation over Lymphopaque (Nyegaard and Co., Oslo, Norway) followed by centrifugal elutriation (7). Raji and Daudi lymphoblastoid cell lines (American Type Culture Collection, Rockville, Md.) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. L929 cells (American Type Culture Collection) were cultured in Eagle MEM supplemented with 10% fetal bovine serum, L-glutamine (0.03%), and nonessential amino acids.

Virus and virus purification. HCMV strains AD169 and Towne were obtained from the American Type Culture Collection. The origins and properties of these two strains of

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† Publication 5785-IMM from the Research Institute of Scripps Clinic.

HCMV have been described previously (30, 32). The titer of infectious HCMV was determined by plaque assays (39) on HFF. HCMV was purified by a modification of the method described by Stinski (37). The supernatant was collected from 900-cm^2 roller bottles (Corning) of HCMV-infected HFF monolayers. Cell debris was removed by centrifugation at 4,000 × *g* for 15 min in an IEC CRU-5000 centrifuge. The virus was pelleted from the medium by centrifugation at 19,000 rpm in a Beckman JA20 rotor for 1 h through a 2-ml layer of 20% (wt/vol) D-sorbitol. The pellets were suspended in phosphate-buffered saline (PBS; 0.14 m NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4). The suspension was layered onto a 20 to 70% (wt/vol) linear D-sorbitol gradient and centrifuged at 85,000 × *g* for 1 h. A dense band of viral particles at the 50 to 60% interface was collected and diluted in 4 volumes of PBS. The virus was pelleted by centrifugation at 85,000 × *g* for 1 h. The virus pellets were suspended in PBS containing globulin-free crystalline bovine serum albumin at 0.5% (Sigma Chemical Co., St. Louis, Mo.). Aliquots were frozen at −70°C and sonicated prior to use.

Herpes simplex virus type 1 (HSV-1) was produced in baby hamster kidney cells (BHK-21) and purified on a discontinuous sucrose density gradient (10 to 60%) in TNE buffer (10 mM Tris hydrochloride, 100 mM NaCl, 1 mM disodium EDTA [pH 7.4]) at 120,000 × *g* for 1 h. Influenza virus (PR8) in allantoic fluid harvested from embryonated chicken eggs was a gift from N. R. Klinman (Research Institute of Scripps Clinic, La Jolla, Calif.).

Estimation of the number of viral particles. The number of viral particles was estimated from the viral DNA content (27). Virus was disrupted in 0.3 N NaOH, and the released DNA was detected at 260 nm with a conversion factor for DNA in NaOH of 37 (27). Molecular weights of 1.47×10^8 and 1.1×10^8 were used for HCMV DNA (10) and HSV-1 DNA (17), respectively.

Radiolabeling of HCMV. The medium of infected HFF monolayers at 30% cytopathic effects was replaced with RPMI 1640 containing reduced methionine and [^{35}S]methionine (Amersham Corp., Arlington Heights, Ill.) at 1.11 MBq/ml (30 μCi). [^{35}S]methionine-labeled virus was purified as described above. Specific activities of various preparations ranged from 1.94×10^{-5} to 1.41×10^{-4} cpm per particle.

Virus binding assay. HFF from subconfluent monolayers were removed from plastic tissue culture flasks with Versene (20 mM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid [HEPES], 150 mM NaCl, and 6×10^{-4} M EDTA [pH 7.2] in PBS). The cell monolayers were washed three times with PBS and two times with Versene and left for 15 min at room temperature. The released cells were washed three times with HEPES balanced salt solution (0.14 M NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CaCl_2 \cdot 2H_2O$, 0.4 mM KH_2PO_4, 1.4 mM Na_2HPO_4) containing 0.5% crystalline globulin-free bovine serum albumin (HBSS). The cells were suspended in HBSS to 4×10^5 cells per ml and kept at 4°C until use. In experimental assays ^{35}S-labeled HCMV and unlabeled HCMV in a volume of 250 μl were incubated with 500 μl of the cell suspension containing 2×10^5 cells in duplicate or triplicate. The concentrations of virus, assay conditions, temperatures, and incubation times are specified in Results. After incubation with virus the cells were washed three times with HBSS by centrifugation and suspended in 100 μl of 1 M NaOH prior to scintillation counting in a Beckman LS8000 spectometer to determine the extent of virus binding. Nonspecific binding of ^{35}S-labeled HCMV was routinely ascertained from identical samples containing a 25-fold excess of unlabeled HCMV. Nonspecific binding, which represented less than 15% of the total bound radioactivity, was subtracted. The binding of ^{35}S-labeled HCMV to HFF fixed in 1% paraformaldehyde (Fisher Scientific Co., Fair Lawn, N.J.), purified human monocytes, human lymphocytes, Daudi cells, Raji cells, BHK-21 cells, and L929 cells was also assessed by the same methodology.

Blocking experiments with unlabeled HCMV. Equal numbers of HFF (2×10^5) were incubated with increasing concentrations of unlabeled HCMV at 4°C for 90 min. The concentrations of unlabeled HCMV are specified in Results. After incubation with unlabeled HCMV, a constant concentration of ^{35}S-labeled HCMV was added and incubation was continued for 90 min at 4°C before the ^{35}S-labeled HCMV binding assay described above was done.

Effect of neuraminidase on HCMV binding. HFF removed from monolayers were treated at a concentration of 4×10^5 cells per ml with 0 to 1.0 U of neuraminidase (GIBCO Laboratories, Grand Island, N.Y.) per ml for 30 min at 37°C before the assay for HCMV attachment was done.

Effect of protease treatments on HCMV binding. HFF removed from monolayers were treated at a concentration of 4×10^5/ml at pH 7 with either pronase E (protease type XIV, 4 U/mg; Sigma) at 0 to 300 μg/ml or crystalline trypsin (10,000 benzoylarginine ethyl ester units/mg; Sigma) at 0 to 100 μg/ml for 10 min at 37°C. These levels of protease did not reduce cell viability. The cells were washed three times with PBS containing 5% fetal calf serum; 1 μM pepstatin, 200 μM phenylmethylsulfonyl fluoride, and 1 μM leupeptin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.); and 100 μM EDTA (Sigma) before suspension in HBSS at 2×10^5 cells per ml. The assay for virus binding was then performed as described above.

Kinetics of HCMV receptor regeneration following trypsin treatment. HFF that had been treated with 0.1 mg of crystalline trypsin per ml as described above were washed by centrifugation three times with sterile PBS containing 1% bovine serum albumin, suspended in MEM alone or MEM containing 300 μg of cycloheximide (Sigma) per ml, and incubated at 37°C. Control samples were treated similarly but without trypsin treatment or cycloheximide. Samples of 2×10^5 cells were withdrawn at different times from 0 to 18 h posttrypsin treatment, and the attachment of ^{35}S-labeled HCMV to these cells was determined.

Virus binding to HFF grown in 2-deoxy-D-glucose- or tunicamycin-supplemented media. HFF were treated with trypsin as described above and grown in MEM or MEM supplemented with various concentrations of either 2-deoxy-D-glucose (Sigma) or tunicamycin (Sigma). After 3 days, confluent monolayers were washed extensively with HBSS and cells were removed by Versene treatment. Cells were assayed for the binding of ^{35}S-labeled HCMV as described above.

RESULTS

Analysis of HCMV binding to mammalian cells. In these studies, 4×10^8 particles of ^{35}S-labeled AD169 HCMV were incubated with 2×10^4 cells in suspension for 90 min at 4°C. After washing, bound HCMV radioactivity was determined and the mean of the triplicate samples was ascertained. Specific binding was calculated by subtracting the values obtained from those of identical reaction mixtures containing a 25-fold excess of unlabeled HCMV. Specific binding values were converted to HCMV particles by calculation from the

TABLE 1. Binding of ^{35}S-labeled HCMV to cells in suspension

Cell line or type	No. of viral particles bound/cell[a]
HFF	2,942
Human lymphocytes	161
Human monocytes	187
Raji lymphoblastoid cells	289
Daudi lymphoblastoid cells	311
Murine L cells	2,005
BHK-21	2,177

[a] Specific binding was determined by subtracting nonspecific binding from total binding.

specific radioactivity of the radiolabeled HCMV preparation (3.83×10^{-5} cpm per particle), ascertained by scintillation counting and determination of viral DNA content. HCMV bound to the various cells and cell lines examined (Table 1). Levels of binding ranged from 14.7% of input, or 2,942 virions per cell for low-passage HFF, to 0.8% of input, or 161 virions per cell for human lymphocytes. Since maximum binding was obtained with HFF, which are also readily permissive for HCMV infection, further studies to address the specificity and characteristics of binding were carried out with these cells.

Times course of HCMV binding to HFF. The time course of radiolabeled HCMV binding to HFF at 4°C was assessed. In these studies, ^{35}S-labeled AD169 HCMV was incubated in suspension with 2×10^5 HFF and samples were taken at intervals. Mean values were obtained from three samples at each time point and expressed as viral particles per cell by calculation as described above (Fig. 1). The number of viral particles binding to the cells increased with time up to about 60 min and then began to plateau. Since maximum binding was obtained by 90 min at 4°C, this time was used in subsequent studies.

Specificity of HCMV binding to HFF. To determine whether HCMV binding was specific, we assessed the ability

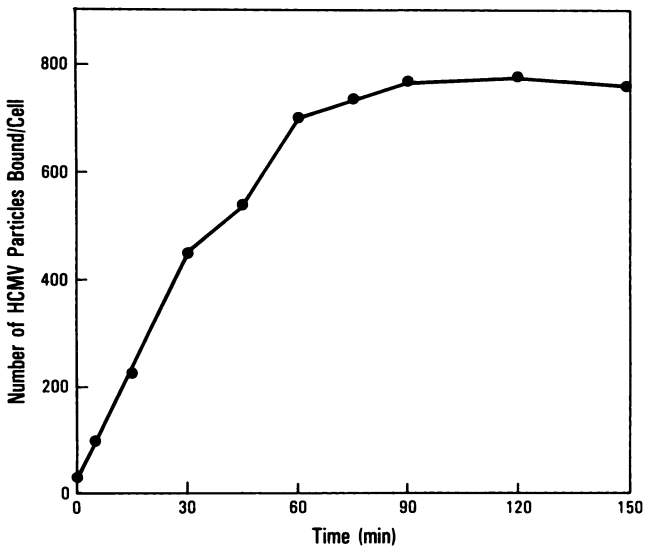


FIG. 1. Kinetics of HCMV attachment to HFF at 4°C. Samples containing 4.93×10^8 particles of ^{35}S-labeled HCMV (2.64×10^{-5} cpm per particle) were incubated with 2×10^5 HFF for increasing periods of time at 4°C. Bound radioactivity was converted to HCMV particles per cell as described in the text. Each point represents the mean of triplicate determinations.

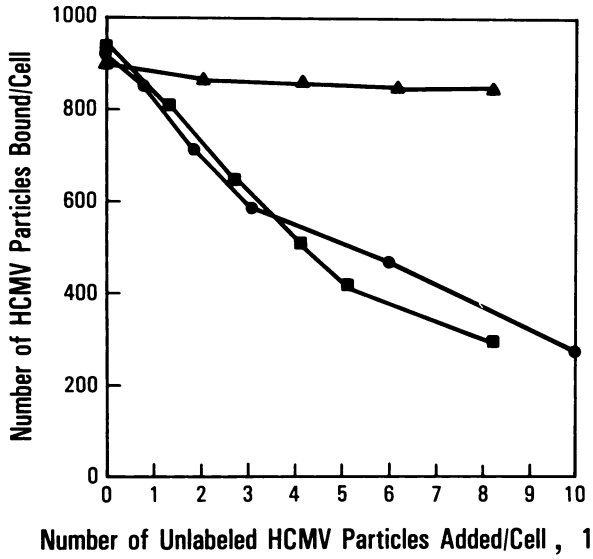


FIG. 2. Specificity of HCMV binding. Samples containing 9.6×10^8 particles of ^{35}S-labeled AD169 HCMV (1.4×10^{-4} cpm per particle) were incubated with 2×10^5 HFF in the presence of increasing amounts of unlabeled AD169 HCMV (●), Towne HCMV (■), or HSV-1 (▲) for 90 min at 4°C. Bound radioactivity was converted to HCMV particles per cell as described in the text. Each concentration was assayed in duplicate.

of unlabeled AD169 HCMV to compete with ^{35}S-labeled AD169 HCMV for attachment to HFF. HFF in suspension were incubated with a constant amount of ^{35}S-labeled AD169 HCMV in the presence of increasing concentrations of unlabeled AD169 HCMV. After 90 min at 4°C, the binding of labeled HCMV was determined. Unlabeled AD169 HCMV competitively inhibited the attachment of labeled AD169 HCMV to HFF (homologous competition) (Fig. 2). In the same study, the potential ability of unlabeled HCMV of the Towne strain to compete with ^{35}S-labeled AD169 HCMV was also investigated. Unlabeled Towne HCMV competitively inhibited the attachment of labeled AD169 HCMV with the same dose-response characteristics as those of AD169 (Fig. 2). Fifty percent inhibition of binding was obtained with 14- and 10-fold excesses of unlabeled AD169 and Towne HCMV, respectively. Unlabeled HSV-1, also examined in this study, did not inhibit the binding of labeled HCMV to HFF (Fig. 2), providing further evidence of specificity.

Saturability of HCMV binding to HFF. To determine whether HCMV bound to HFF in a saturable manner, we incubated increasing amounts of ^{35}S-labeled AD169 HCMV with a constant number of HFF in suspension for 90 min at 4°C. Each concentration was assayed in triplicate. Identical sets of samples contained a 25-fold excess of unlabeled HCMV. Based on the studies described above, the counts that became bound in the presence of this excess of unlabeled HCMV were considered nonspecific and were subtracted from the total binding values to establish the specific binding values. Nonspecific binding was low and accounted for less than 15% of the total binding in this and all other experiments. Three such experiments are presented in Fig. 3. The number of viral particles which became specifically attached increased with viral input. The binding curves were concave relative to the abscissa and showed suggestive evidence of a plateau at higher values, indicative of saturable binding. Specific binding of HCMV, expressed as a percent-

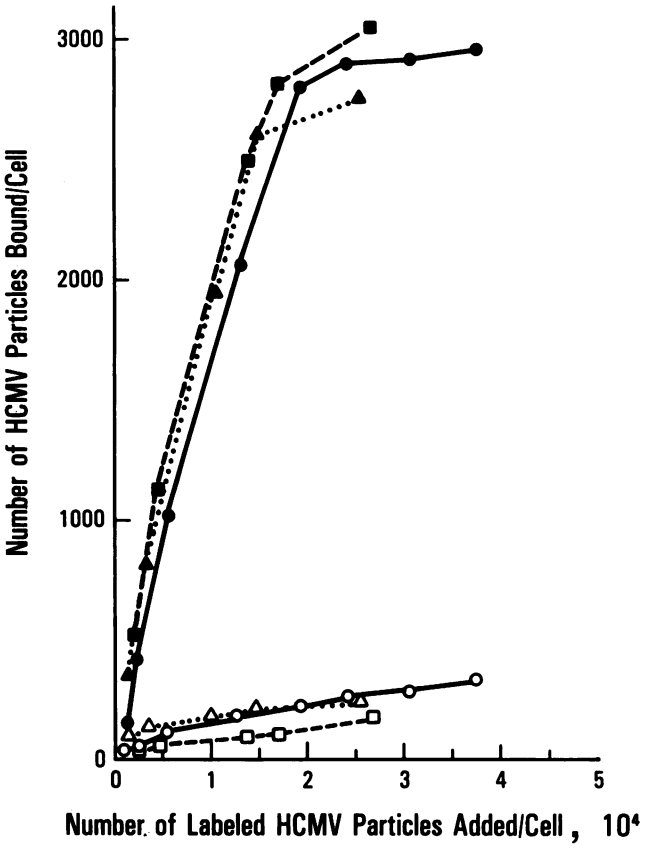


FIG. 3. Saturability of HCMV binding to HFF. Increasing amounts of ^{35}S-labeled HCMV were incubated with 2×10^5 HFF for 90 min at 4°C. Specific binding values (●, ■, and ▲) were obtained by subtracting nonspecific linear binding values (○, □, and △), obtained from identical samples containing a 25-fold excess of unlabeled HCMV, from the total binding values. Each point represents the average of triplicate determinations. Three separate experiments (● and ○, ■ and □, and ▲ and △) done with an HCMV preparation with a specific activity of 2.64×10^{-5} cpm per particle are shown.

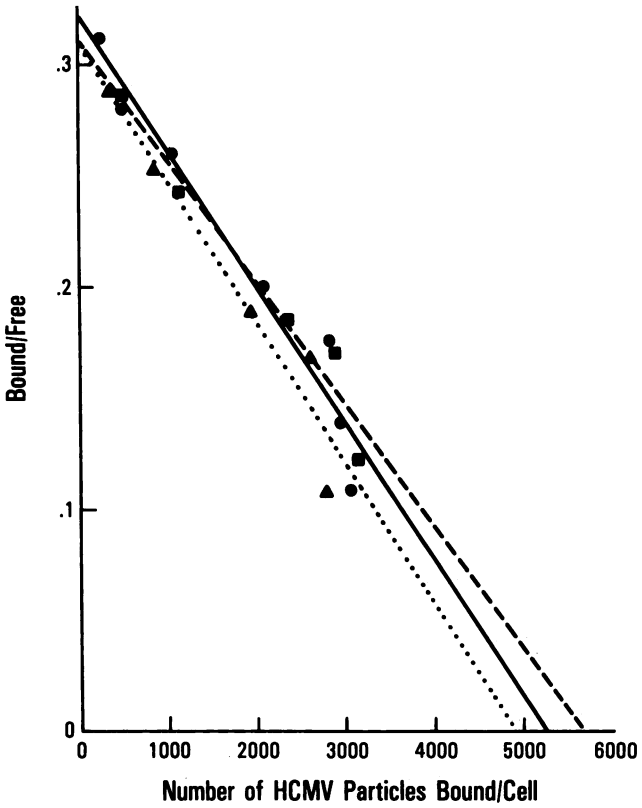


FIG. 4. Scatchard analyses of HCMV binding to HFF. Scatchard analyses (34) of the saturation binding data shown in Fig. 3 are presented. Lines were obtained by linear regression analyses.

age of input, ranged from 16.4% at low inputs to 8.3% at high inputs.

Analyses of binding parameters. Scatchard analyses (34) of the saturation binding curves shown in Fig. 3 yielded linear plots, indicative of the presence of a single binding site on the cells (Fig. 4). The three different binding studies yielded very similar lines. Using molar quantities converted from the slopes of the Scatchard plots, we obtained dissociation constants (K_d values) of 1.06, 1.19, and 1.09 nM in the three experiments. The average K_d determined from these values was 1.11 nM. The theoretical average number of available virus-binding sites per cell predicted by the intercepts on the horizontal axis of the Scatchard plots was 5,262. These data indicate that HFF possess a moderate number of high-affinity binding sites per cell.

Binding analyses carried out at 37°C produced a two-component Scatchard binding curve (Fig. 5). The K_d values of the relatively higher- and lower-affinity components were 0.69 and 9.62 nM, respectively. The two-component curve apparently was a consequence of viral internalization, since a Scatchard analysis of a binding study carried out on paraformaldehyde-fixed cells (which are unable to internalize virus) at 37°C yielded a single-component straight line with a K_d of 1.88 nM, a value similar to that obtained at 4°C with unfixed cells.

Protease sensitivity of HCMV binding to HFF. HFF in suspension at a concentration of 2×10^5 were incubated with various amounts of trypsin or pronase E for 10 min at 37°C and washed extensively in buffer containing protease inhibitors. Cell viability was not impaired by this treatment. ^{35}S-labeled AD169 HCMV was incubated with the protease-treated or untreated HFF cells for 90 min at 4°C. Pretreatment of the HFF with either protease reduced binding in a dose-dependent manner (Fig. 6). Concentrations of 5 μg of trypsin per ml and 30 μg of protease type XIV per ml produced a 50% decrease in HCMV binding. These results indicate that the receptor for HCMV is either a protein or is closely associated with a protein structure.

Role of carbohydrates in HCMV binding to HFF. HFF in suspension at a concentration of 4×10^5/ml were treated with 100 μg of trypsin per ml for 10 min at 37°C, washed extensively, and grown in media or media containing various amounts of tunicamycin or 2-deoxy-D-glucose to confluency, which occurred in 3 days. Growth and viability were not significantly altered by the highest concentrations of tunicamycin (5 μg/ml) or 2-deoxy-D-glucose (30 mM) used. The cells in suspension were examined for ability to bind ^{35}S-labeled AD169 HCMV. Cells treated with either tunicamycin (Fig. 7A) or 2-deoxy-D-glycose (Fig. 7B) exhibited a dose-dependent reduction in the ability to bind ^{35}S-labeled HCMV. Concentrations of 1.9 μg of tunicamycin per ml and 9 mM 2-deoxy-D-glucose produced a 50% decrease in binding. These data, together with the protease sensitivity stud-

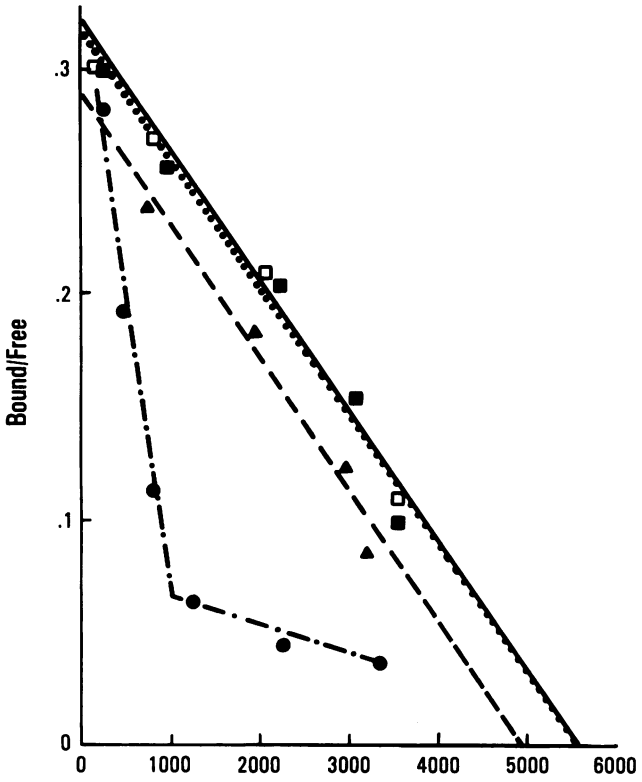


FIG. 5. Scatchard analyses of HCMV binding to untreated and paraformaldehyde-treated HFF at 4 and 37°C. Increasing amounts of ^{35}S-labeled HCMV were incubated with 2×10^5 untreated HFF at 4°C (■) and 37°C (●) and paraformaldehyde-treated HFF at 4°C (▲) and 37°C (□). Data were analyzed by the method of Scatchard (34). Lines were obtained by linear regression analyses.

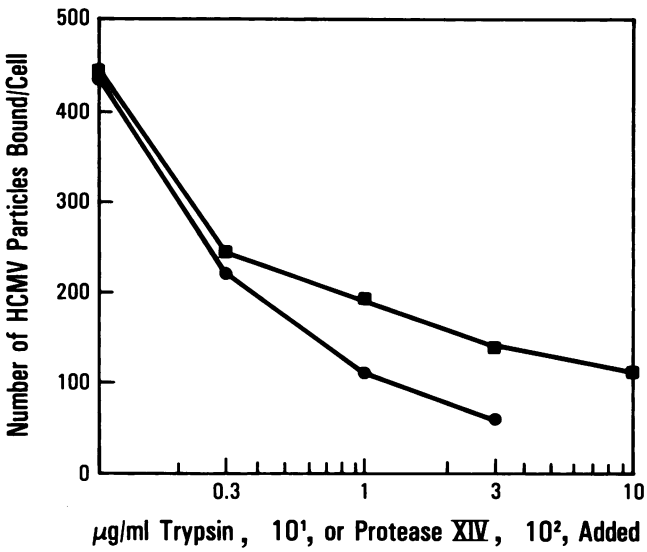


FIG. 6. Protease sensitivity of HCMV binding to HFF. HFF in suspension at a concentration of 2×10^5 were treated with buffer or with various amounts of trypsin (■) or protease type XIV (●) for 10 min at 37°C. After extensive washing with HBSS containing protease inhibitors, 8×10^8 particles of ^{35}S-labeled HCMV (1.94×10^{-5} cpm per particle) were added to 2×10^5 HFF in suspension. Bound radioactivity was converted to HCMV particles per cell as described earlier. Each point denotes the average of duplicate determinations.

ies described above, indicate that a glycoprotein participates in HCMV binding to HFF cells.

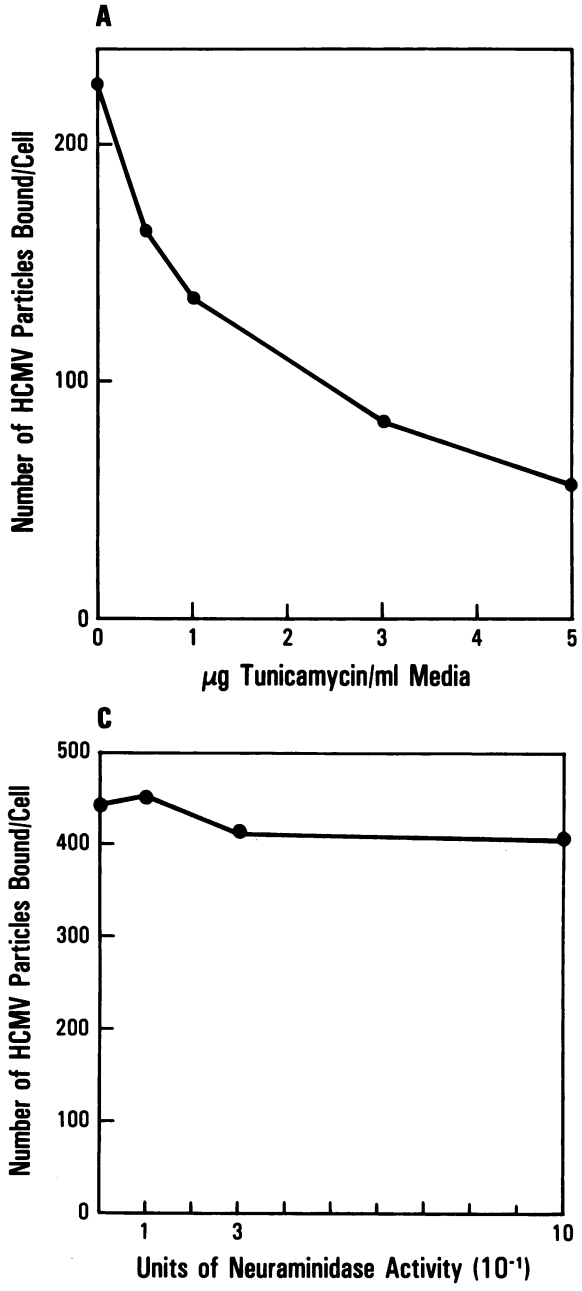
To determine whether sialic acid residues were involved, we treated HFF in suspension at a concentration of 2×10^5/ml with buffer or with various amounts of neuraminidase for 30 min at 37°C. After washing, the cells were examined for ability to bind ^{35}S-labeled AD169 HCMV. Neuraminidase in concentrations as high as 1 U/ml failed to impair HCMV binding (Fig. 7C). In parallel studies, treatment of HFF with 0.1 U of neuraminidase per ml completely blocked the infectivity of influenza virus (PR8) for the cells (data not shown). These data indicate that sialic acid residues are not required for HCMV binding to HFF.

Time course of HCMV receptor synthesis. The ability of trypsin to reduce the binding of HCMV to HFF made it possible to examine the time course of receptor regeneration. HFF were treated with 100 μg of trypsin per ml or buffer for 10 min at 37°C. After washing, the treated and sham-treated cells were divided; half of each was incubated at 37°C in media, and the other half of each was incubated at 37°C in media containing 0.3 μg of cycloheximide per ml. At intervals, samples were removed from the plastic wells and examined for ability to bind ^{35}S-labeled HCMV in suspension. Values were calculated relative to those for the sham-treated samples incubated in media without cycloheximide. Trypsin treatment reduced binding to less than 10% of that of the untreated sample (Fig. 8). Fifty percent recovery of binding activity occurred in 3 h, while full binding required more than 6 h. In the presence of cycloheximide, only partial restoration of binding activity (approximately 38%) was obtained, indicating that the recovery of full binding activity requires protein synthesis.

DISCUSSION

To initiate infection, viral particles must attach to cells and be internalized. In the relatively few systems which have been carefully studied, viral infection is initiated by specific viral binding, via a distinct protein, to a finite number of surface molecules, generally termed viral receptors. Such cell surface receptors play a prominent role in viral pathogenesis. Clearly, they represent the first step in viral infection of cells. Furthermore, they are largely responsible for the host range specificity and tissue tropism exhibited by many viruses. Finally, studies with several viruses have indicated that the receptor "selected" by a virus may facilitate infection and persistence either by triggering intracellular events conducive to infection or by impairing immune recognition and clearance.

Receptor-ligand interactions are generally characterized by the criteria used to analyze interactions between monovalent molecules in free solution. Such reactions exhibit moderate to high affinity binding, reversibility, specificity, and saturability. It is not strictly valid to use the same approaches to analyze the interactions of cell-associated receptors with viral particles, which contain multiple copies of the ligand in close proximity. Viral attachment to cells is likely multivalent, involving many separate interactions of viral ligand proteins with multiple receptor molecules on the cell membrane. Such interactions are undoubtedly influenced by the number, density, and distribution of viral attachment proteins and receptor molecules and, in the case of the cell membrane and possibly viral attachment proteins



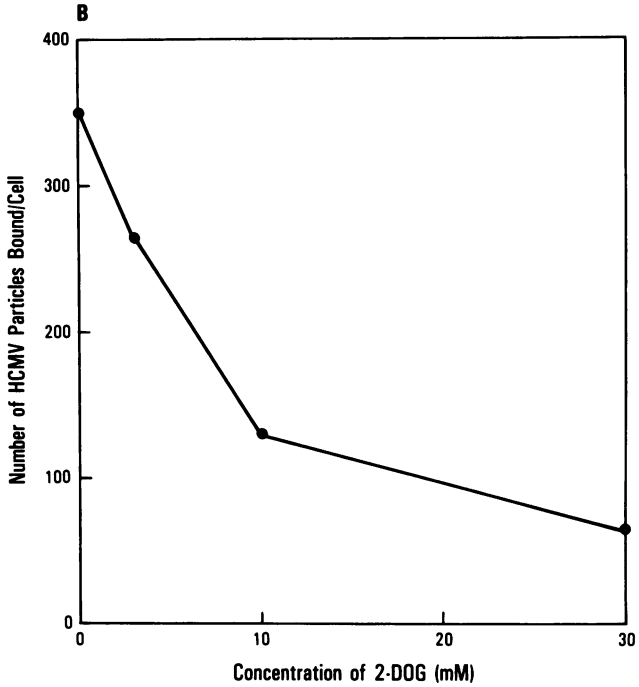


FIG. 7. Role of carbohydrates in HCMV binding to HFF. After treatment with 100 μg of trypsin per ml for 10 min at 37°C, HFF were grown in media containing buffer or various concentrations of tunicamycin (A) or 2-deoxy-D-glucose (2-DOG) (B) for 3 days. The cells were removed and washed, and 2×10^5 untreated or treated HFF in suspension were incubated with 4.5×10^8 particles of ^{35}S-labeled HCMV (5.65×10^{-5} cpm per particle) for 90 min at 4°C. HFF in suspension were treated with various amounts of neuraminidase for 30 min at 37°C (C).

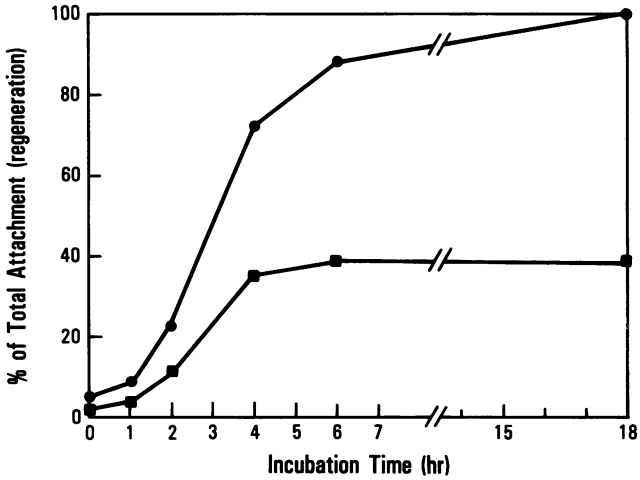


FIG. 8. Time course of HCMV receptor synthesis. After treatment of HFF with buffer or 100 μg of trypsin per ml for 10 min at 37°C, the cells were washed and incubated at 37°C in media (●) or in media containing 0.3 μg of cycloheximide per ml (■). At intervals, samples were examined in suspension for ability to bind ^{35}S-labeled HCMV (3.83×10^{-5} cpm per particle). After 90 min at 4°C, virus binding was determined and values were expressed as a percentage of those observed for the sham-treated samples incubated in media. Each point represents the average of duplicate determinations.

in enveloped viruses, also by the redistribution of receptors and viral ligand proteins and the physical and chemical composition of the lipid bilayer membrane. Regardless of these theoretical limitations, analyses of the parameters of virus-cell interactions have yielded useful information. In addition, such analyses in a number of systems have indicated that viral binding to cells is saturable, specific, and susceptible to quantitative analyses (16). Such studies have been carried out with several viruses, including reovirus (6, 38), Semliki Forest virus (14), rhinovirus (1, 22) rabies virus (40), and foot-and-mouth disease virus (2). Although the determination of binding constants from such plots is not absolute because of the above-noted restraints, such data are useful as minimal estimates and for comparative purposes.

The number of virus-binding sites per cell obtained from such plots and from the curve relating viral input to uptake represents a useful value, although it must be kept in mind that the number of receptor molecules is undoubtedly larger than the number of receptor sites because of the multivalency of binding. Most important, however, a single-component linear Scatchard plot indicates that a given cell type possess a single population of viral receptor sites.

In the present study, we showed that HCMV exhibits specific saturable binding to HFF. Approximately 5,000 virions were bound per cell at saturation. The number of cellular virus-binding sites observed with other viruses has varied widely from about 10^3 for vesicular stomatitis virus, certain enteroviruses, and rhabdoviruses (23, 35) to more than 10^6 for certain retrovirus subtypes (26), although most appear to be in the range of 10^4 to 10^5 (21). HCMV binding to HFF exhibited specificity, a primary criterion of receptor-mediated interactions. The attachment of radiolabeled HCMV was blocked by unlabeled virus in a dose-dependent manner but was unaffected by high concentrations of another human herpesvirus, HSV-1. Studies with other viruses, notably rabies virus (40), rhinoviruses (1), and reovirus (6, 38), have all reported comparable competition by unlabeled homologous virus. In addition to specific binding, HCMV exhibited nonspecific binding to HFF. Such nonspecific binding, assessed by binding studies carried out in the presence of a 25-fold excess of unlabeled HCMV, was small in extent and averaged less than 15% of the total binding in the various studies. Such nonspecific binding, observed also to a comparable extent with other viruses (6, 38, 40), is thought not to lead to infection.

Analyses of the parameters of specific HCMV binding to HFF by Scatchard plots yielded a single-component straight line, indicating that there is a single type of HCMV receptor on the surface of HFF. Single-component linear Scatchard plots have also been observed with reovirus serotypes (6, 38) and Semliki Forest virus (14). The dissociation constant (K_d) for HCMV binding to HFF obtained from the Scatchard plots was 1.1 nM. For comparative purposes reovirus serotype 1 and 3 binding to murine fibroblasts and rat endothelial cells exhibited K_d values of 0.4 and 0.8 nM, respectively (6, 38). Calculations of the K_d from the input uptake curve as the viral concentration necessary to saturate half of the available receptor sites yielded 1.5×10^3 viral particles per cell, corresponding to 0.51 nM, a value similar to that obtained from the Scatchard analyses.

Two-component linear Scatchard plots were obtained when viral binding studies were carried out at 37°C. We considered it likely that the failure to obtain single-component linear Scatchard plots was due to internalization of HCMV and thus interference with association or dissociation of the virus. This was indeed demonstrated to be the case by a study at 37°C with paraformaldehyde-fixed cells, which are unable to internalize virus. In this experiment, a single-component Scatchard plot was obtained and the K_d was similar to that observed at 4°C with unfixed cells.

Approximately 60 min at 37°C was required to achieve maximum binding. These kinetics are similar to those observed for reovirus serotypes 1 and 3 (6), rhinovirus (1), and rabies virus (40). This relatively slow rate, in contrast to the rapidity of interactions between molecules in free solution, likely reflects the time needed to redistribute receptors within the plane of the plasma membrane to form multiple links between viral glycoproteins and individual receptor molecules.

Of considerable interest is the finding that the Towne strain of HCMV possessed the ability to competitively inhibit attachment of the AD169 strain. This result indicates that both HCMV strains bind to the same receptor. Furthermore, the slopes of the binding inhibition curves for the two viral strains were the same, suggesting that both HCMV strains bind to the receptor with approximately equal affinities. HSV-1, however, failed to impair the binding of the AD169 strain, indicating that it binds to a different structure(s) on the fibroblast surface. Grundy et al. (13) had earlier also shown that a urinary tract HCMV isolate but not HSV-1 competed with HCMV AD169 for attachment to cells.

The susceptibility of the receptor to trypsin and pronase treatments indicates that HCMV binding involves a protein component. Consistent with the sensitivity to proteases, cycloheximide prevented full recovery of binding activity; only partial recovery (approximately 38%) was obtained in its presence. It is likely that this partial recovery of binding activity, which is independent of protein synthesis, is due to the migration of previously synthesized receptors from existing intracellular pools to the plasma membrane. If so, the difference between the two curves shown in Fig. 8 reflects the rate of de novo synthesis of receptors. This theoretical curve indicates a half-time of synthesis of 6 h. For comparative purposes, the regeneration of Epstein-Barr virus receptors on Raji cells after cycloheximide treatment was found by Khelifa and Menezes (19) to have a half-time of synthesis of 6 h.

HCMV binding to HFF was impaired by trypsin treatment of HFF followed by 3 days of growth in the presence of tunicamycin or 2-deoxy-D-glucose. Tunicamycin blocks N-linked glycosylation in mammalian cells, while 2-deoxy-D-glucose truncates the high-mannose intermediate form of N-linked carbohydrate residues, interfering with further processing. It cannot be ascertained from the studies reported here whether glycosylation is necessary for processing, transport, and expression of the receptor or associated binding structure on the cell surface, as is often, but not invariably, the case (29, 36). Alternatively, the nonglycosylated molecule which participates in binding could be expressed on the cell surface but require such carbohydrate residues to bind HCMV. Regardless, the data indicate that the HCMV receptor possesses or is associated with N-linked carbohydrate residues.

The studies with proteases and inhibitors of glycosylation indicate that the receptor is a glycoprotein or is closely associated with a glycoprotein. Since single-component Scatchard curves were obtained, the former explanation is more likely. The demonstration in this study that HCMV binds specifically and with high affinity to a single type of molecule, presumably a glycoprotein, on the surface of HFF furnishes a rationale for analyses of the role of this molecule in viral infection as well as for efforts to isolate and characterize the receptor. These studies are under way.

ACKNOWLEDGMENTS

We thank Bonnie Weier for preparation of the manuscript.

This work was supported by Public Health Service grants AI25016, AI17354, and CA14692 from the National Institutes of Health.

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