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Characterization of Simian Virus 40 Receptor Moieties on the Surfaces of Vero C1008 Cells

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The nature of the simian virus 40 (SV40) receptor on the surfaces of Vero C1008 cells was investigated by a virus binding assay. The optimum pH for SV40 binding to cell surfaces was found to be at 6.5; however, there was little difference in SV40 binding in the range between pH 4.5 and 7.3. The treatment of cell surfaces with several proteases or with an enzyme specific for O-linked carbohydrates significantly reduced virus binding, suggesting that the receptor for SV40 contains protein and O-linked carbohydrates. Treatment of cell monolayers with octyl glucoside removed virus-binding activity from cell surfaces. Recovery of virus-binding activity by octyl glucoside-treated cells took 2.5 h and was inhibited by cycloheximide or tunicamycin. Four polypeptides with molecular weights of 90,000, 58,000, 54,000, and 30,000 were immunoprecipitated from virus-protein complexes derived from octyl glucoside extract solutions and therefore may be components of the SV40 receptor. Competition experiments between SV40 and polyomavirus revealed that these two viruses do not share the same receptor on Vero C1008 cells.

The early events of papovavirus infection have been studied in several laboratories. Virions adsorb to cell surface receptors and are internalized either individually or in small groups in endocytic vesicles (12, 18, 20). Virion-containing vesicles are reported to be transported to the nucleus where they fuse with the outer nuclear membrane (10, 12, 18, 25, 28). Virions are then thought to be transported across the inner nuclear membrane by an unknown mechanism into the nucleoplasm where virus uncoating and replication occur (3, 18). Recent evidence from several laboratories indicates that the endocytic pathway used by papovaviruses is different from that used by other viruses that use endocytosis as a means of virus entry. Most viruses require transport through endosomes and lysosomes for uncoating and activation (8, 17, 23; M. Marsh and A. Helenius, Adv. Virus Res., in press). Entry of these viruses can be blocked by neutralizing the pH within acidic organelles with lysosomotropic agents (14, 23, 24, 26). In contrast, papovaviruses are thought to be uncoated in the nucleus, and several laboratories have reported that lysosomotropic agents have no effect on simian virus 40 (SV40) entry (29, 32, 34). Therefore, it is likely that the entry of papovaviruses does not require an acidification process.

We have previously reported that SV40 entry into polarized Vero C1008 cells occurs exclusively at the apical surface (7). Binding sites for SV40 were found to be localized primarily on the apical surfaces of these cells. It was proposed that the polarized binding and entry of SV40 was due to the exclusive expression of the SV40 receptor on apical cell surfaces (7). Scatchard analysis of SV40 binding indicated that there is a single specific class of receptors on the apical surfaces of Vero C1008 cells with a very high affinity for SV40 (7). It is possible that the signal for the transport of SV40 virions to the nucleus lies within the SV40 receptor.

We have also observed that the release of SV40 occurs exclusively from apical surfaces of Vero C1008 cells (E. T. Clayson, L. V. Brando, and R. W. Compans, submitted for publication). Release of virions from these cells was found to occur by a process other than cell lysis. During the time frame of virus release, virions were observed enclosed in cytoplasmic vesicles and lining cell surfaces. The release of SV40, as well as the appearance of virions in vesicles and on the cell surface, was inhibited by monensin at concentrations that had no effect on viral protein synthesis or on assembly of progeny virions. The results of these studies suggested that the release of SV40 was by a novel exocytic pathway. The sorting signal involved in directing the transport of SV40 virions exclusively to apical cell surfaces is not known; however, it is possible that it lies with the receptor for SV40.

Owing to the results of these earlier studies, we have become interested in studying the transport pathways used by the SV40 receptor. Nothing is known about the biochemical nature or the cellular function of the SV40 receptor. However, some information is available about the receptors used by polyomavirus. Sialic acid (NeuAc) protein modifications in the form NeuAc-α(2,3)Gal-β(1,3)GalNAc, where Gal is galactose and GalNAc is *N*-acetylgalactosamine, either O-linked or possibly as components of N-linked carbohydrates have been reported to be required for polyomavirus infection of mouse 3T6 cells (5, 9); however, others (4) have reported that polyomavirus infection of other cells (primary mouse kidney cells) is not inhibited by neuraminidase treatment of cell surfaces. These investigators have suggested that binding of polyomavirus to NeuAc residues represents nonspecific binding and fails to result in productive infection of mouse kidney cells (4). Evidence has been obtained that the polyomavirus receptor complex on primary mouse kidney cells is made up of at least four proteins with molecular weights of 95,000, 50,000, 31,000, and 24,000 (21, 22). It was suggested that carbohydrates are important components of this receptor complex, but specific carbohydrate linkages were not determined.

In the present study, we used two approaches to characterize the SV40 receptor. In the first approach, cell surfaces were treated with a variety of digestive enzymes and the effects of such treatment on the binding of radiolabeled SV40 were determined. In the second approach, SV40 receptor moieties extracted from cell surfaces by a noncytolytic extraction procedure previously used in the characterization

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of polyomavirus receptor moieties (21) were immunoprecipated and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Virus and cells. Vero C1008 and CV-1 cells were obtained and grown as previously described (Clayson et al., submitted). SV40 stocks were prepared and their titers were determined in CV-1 cells as previously described (Clayson et al., submitted). Preparation of purified ^{3}H-labeled SV40 virus was previously described (7). Mouse 3T3 cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in Dulbecco modified Eagle medium containing 10% newborn calf serum. Cell culture fluid containing mouse polyomavirus was obtained from Richard A. Consigli. Stocks of mouse polyomavirus were prepared in 3T3 cells in a manner similar to that described for SV40 in CV-1 cells (7). Purification of polyomavirus by CsCl gradient centrifugation was as described for the purification of SV40 (7).

Virus binding assay. The virus binding assay was previously described (7). Briefly, ^{3}H-labeled SV40 was incubated with Vero C1008 cells at 4°C for 2 h. Cells were then solubilized in 100 μl of 1 N NaOH, and the cell-associated radioactivity was determined by liquid scintillation spectrometry.

Enzyme treatment of cell monolayers. Confluent monolayers of Vero C1008 cells grown in 24-well plates were washed twice with phosphate-buffered saline (PBS) and were incubated in Eagle minimum essiential medium (EMEM) at the indicated pH for 15 min. Various concentrations of the indicated enzyme in EMEM were added and incubated with cells under the conditions specified in Table 1. The monolayers were then washed with cold PBS and used in the virus binding assay. Treatment of cells with high concentrations of proteases resulted in the cells becoming detached from the plate. Under these circumstances, treated cells were pelleted by centrifugation at 600 × *g* for 10 min and used in the virus binding assay. Untreated cells were detached from the plate (with 50 mM EGTA and stirring), pelleted, and used as controls in the virus binding assay.

Extraction of Vero C1008 monolayers with OG. Confluent Vero C1008 monolayers in 100-mm-diameter plates were extracted with various concentrations of octyl glucoside (OG) as described previously (21) except that multiple extractions were made at 24-h rather than 12-h intervals.

Immunoprecipitation. Vero C1008 monolayers grown in 100-mm dishes were extracted with 0.2% OG containing various protease inhibitors at 10 μg/ml each, as described above. Following extraction, the cells were labeled in vitro for 12 h with a ^{14}C-amino acid mixture or a ^{3}H-amino acid mixture (10 μCi/ml) in EMEM containing 10% calf serum and 10% amino acids. Alternatively, the cells were labeled with L-[^{35}S]methionine (20 μCi/ml) in methionine-deficient EMEM containing 10% newborn bovine serum. Cell monolayers were extracted at an average of four times each at 24-h intervals, and the labeling media were replaced after each extraction. Extract solutions were centrifuged at 1,800 × *g* for 30 min in an IEC centrifuge to remove cell debris. The supernatants were concentrated to 1/100th of the original volume and dialyzed overnight against water. The concentrated extracts were stored at 4°C. To form SV40-receptor complexes, we added unlabeled SV40 virions to 50 μl (100,000 cpm) of labeled extract and incubated them for 1 h at 37°C. The complexes or labeled extracts alone were incubated with antiserum directed toward SV40 structural proteins for 1 h at 37°C and immunoprecipitated with protein A-Sepharose as previously described (Clayson et al., submitted). The precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fluorography to detect labeled proteins was performed as described previously (6). Autoradiography was performed with Kodak X-AR 5 film and an intensifying screen.

Competition between SV40 and polyomavirus. Vero C1008 cell monolayers were grown in 24-well plates. Increasing amounts of purified unlabeled polyomavirus or purified unlabeled SV40 virus were incubated with Vero C1008 monolayers for 2 h at 4°C to minimize internalization. Monolayers were then washed extensively with PBS (4°C) before addition of saturating amounts of ^{3}H-labeled SV40 virus for 2 h at 4°C. The cell-associated radioactivity was then determined as described above.

Enzymes, chemicals, and isotopes. Bromelain was purchased from Sigma Chemical Co. (St. Louis, Mo.), and neuraminidase (*Vibrio cholerae*) was purchased from Calbiochem-Behring (La Jolla, Calif.). The following enzymes were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.): phospholipase C, phospholipase D, chymotrypsin A_4, trypsin, pronase, subtilisin, papain, endoglycosidase F, glycopeptidase F, α-mannosidase, β-fructosidase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, and α-fucosidase. *O*-Glycanase was purchased from Genzyme Corp. (Boston, Mass.). OG was purchased from Sigma. The following protease inhibitors were purchased from United States Biochemical Corp. (Cleveland, Ohio): aprotinin, leupeptin, phenylmethylsulfonyl fluoride, and pepstatin A. The mixture of ^{14}C-labeled amino acids was purchased from Amersham Corp. (Arlington Heights, Ill.), and the mixture of ^{3}H-labeled amino acids was purchased from Schwarz/Mann (Orangeburg, N.Y.). L-^{35}S-labeled methionine was purchased from ICN Biomedicals (Cleveland, Ohio).

RESULTS

SV40 binding is inhibited at high pH. The effect of pH on the binding of SV40 to Vero C1008 cell surfaces was determined for a series of pH values between pH 4.5 and 10.0. The optimum pH for SV40 binding to cell surfaces was found to be pH 6.5; however, there was little difference in binding between the pH range of 4.5 and 7.3, while virus binding decreased sharply at pH values above 7.3 (Fig. 1). For subsequent virus binding experiments, the pH was maintained between pH 6.5 and 7.0 during virus adsorption.

SV40 binding is inhibited by treatment with proteases or *O*-Glycanase. To characterize the biochemical nature of the receptor for SV40, Vero C1008 cells were treated with a variety of enzymes and the receptor activity remaining on cell surfaces was measured with ^{3}H-labeled virus. Treatment of Vero C1008 cell surfaces with phospholipase C, D (Table 1), or both (data not shown) had little or no effect on virus binding. Among the proteases tested, trypsin and chymotrypsin A enhanced receptor activity by about 20%. However, receptor activity was sensitive to treatment with all other proteases tested, suggesting the involvement of a protein species in the interaction between receptor and virus. Treatment of cell surfaces with neuraminidase (*V. cholera*) had little or no effect on receptor activity. To determine whether SV40 recognizes N-linked carbohydrates, we treated cell surfaces with either endoglycosidase

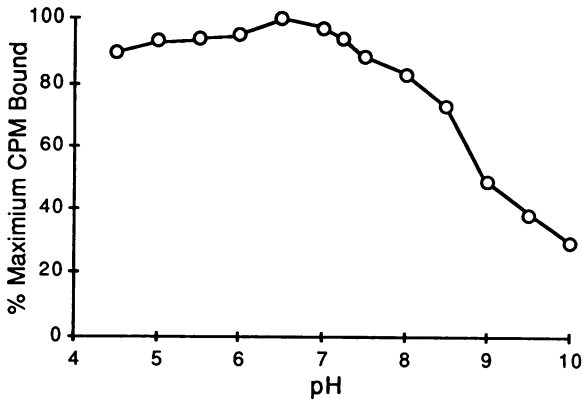

FIG. 1. Effect of pH on SV40 binding. Duplicate cell monolayers in 24-well plates were preincubated with EMEM at the indicated pH for 15 min at 4°C and then were incubated with saturating amounts of ^{3}H-labeled SV40 for 2 h at 4°C to minimize uptake. The cells were then rinsed, and the cell-associated radioactivity was determined by liquid scintillation spectrometry. Values indicated are averages of three or more duplicate monolayers.

F or glycopeptidase F. These enzymes had little or no effect on receptor activity.

O-Glycanase specifically hydrolyzes the Gal-β(1,3) GalNAc core disaccharide from either serine or threonine residues of glycoproteins. Other O-linked carbohydrates are not substrates of this enzyme. Substitution on either the galactosyl or *N*-acetylgalactosaminyl residue interferes with enzyme activity. NeuAc is a common substituent of O-linked carbohydrates and therefore interferes with *O*-Glycanase activity unless enzymatically removed. When cells were treated with neuraminidase followed by treatment with *O*-Glycanase, virus binding was reduced fourfold, suggesting that specific O-linked carbohydrates are components of the SV40 receptor which may be recognized by the virus. Treatment of cells with *O*-Glycanase alone (data not shown) or neuraminidase alone (Table 1) had no effect on SV40 binding, suggesting that the Gal-β(1,3)GalNAc residues involved in SV40 binding to receptors contain one or more terminal NeuAc residues which are not necessary for SV40 infection.

SV40 receptor activity can be extracted from cell surfaces with OG. To determine the effect of OG on SV40 binding, cells were treated with concentrations of OG between 0.01 and 1% followed by a virus binding experiment. Treating cell surfaces with concentrations of OG between 0.1 and 0.3% resulted in a 50% reduction in virus binding (Fig. 2). At concentrations above 0.3%, lysis of cells became apparent; therefore, for further studies with OG, a concentration of 0.2% was used.

Recovery of receptor activity in OG-treated cells is inhibited by cycloheximide or tunicamycin. To determine the length of time required for recovery of receptor activity on cell surfaces, we treated cells with 0.2% OG followed by incubation in growth medium. After incubation periods between 30 min and 4 h, virus binding experiments were performed. A 2.5-h period was required to restore receptor activity to control levels (Fig. 3). This is similar to the 2-h recovery period required to restore polyomavirus receptor activity in OG-treated MKC cells (21). The recovery of receptor activity in OG-treated cells could be due to synthesis of new receptors or to replacement from intracellular pools of receptors. To determine whether the recovery of receptor activity requires protein synthesis or glycosylation or both,

TABLE 1. Effect of enzymes on SV40 binding to Vero C1008 cells

Enzyme	Concn	% Binding compared with control
None		100
Phospholipases		
Phospholipase C[a]	1 μg/ml	94
	10 μg/ml	94
	100 μg/ml	92
	200 μg/ml	—[b]
Phospholipase D[a]	1 μg/ml	96
	10 μg/ml	96
	100 μg/ml	96
	200 μg/ml	—[b]
Proteases		
Chymotrypsin A[c]	10 μg/ml	109
	100 μg/ml	113
	200 μg/ml	118
	1 mg/ml	122
Trypsin[c]	10 μg/ml	104
	100 μg/ml	110
	200 μg/ml	117
	1 mg/ml	123
Pronase[a]	1 μg/ml	93
	10 μg/ml	83
	100 μg/ml	42
	1 mg/ml	9
Subtilisin[d]	1 μg/ml	84
	10 μg/ml	74
	100 μg/ml	64
	1 mg/ml	21
Bromelain[d]	1 μg/ml	74
	10 μg/ml	71
	100 μg/ml	60
	1 mg/ml	15
Papain[e]	1 μg/ml	59
	10 μg/ml	47
	100 μg/ml	36
	1 mg/ml	24
Glycosidases		
Neuraminidase[f]	1 mU/ml	94
	10 mU/ml	100
	100 mU/ml	96
	200 mU/ml	91
Endoglycosidase F[f]	1 mU/ml	104
	10 mU/ml	111
	100 mU/ml	90
	200 mU/ml	96
Glycopeptidase F[g]	100 mU/ml	93
	1 U/ml	93
	10 U/ml	104
O-Glycanase[h]	100 mU/ml	42
	1 U/ml	34
	10 U/ml	26
Mixed glycosidases[i]	100 μg/ml	96

[a] Reaction conditions: pH 7.3, 37°C, 30 min.
[b] —, Significant cell loss occurred at these conditions.
[c] Reaction conditions: pH 7.3, 25°C, 30 min.
[d] Reaction conditions: pH 6.0, 37°C, 30 min.
[e] Reaction conditions: pH 4.5, 25°C, 30 min.
[f] Reaction conditions: pH 5.0, 37°C, overnight.
[g] Reaction conditions: pH 7.3, 37°C, overnight.
[h] Cells were treated with 200 mU of neuraminidase per ml at pH 5.0, 25°C for 60 min prior to treatment with *O*-Glycanase at pH 7.3, 37°C, overnight.
[i] The mixture of glycosidases contained α-mannosidase, β-fructosidase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, and α-fucosidase, each at 100 μg/ml. Reaction conditions: pH 7.3, 37°C, overnight.

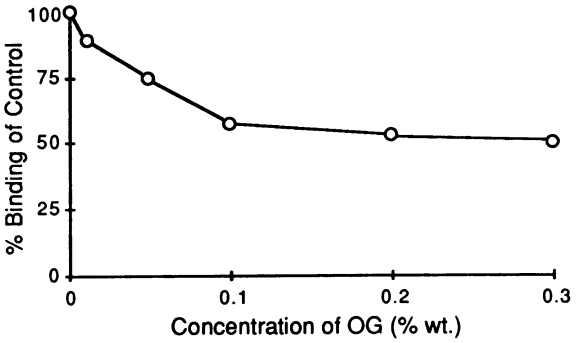

FIG. 2. Extraction of SV40 receptors from Vero C1008 cells. Duplicate cell monolayers in 24-well plates were washed with PBS and incubated with various concentrations of OG in PBS for 30 min at room temperature with periodic rotation. The monolayers were then washed with PBS and used in the virus binding assay. Concentrations of OG at 0.4% or above resulted in cell death. Values indicated are averages from three or more duplicate monolayers.

we added cycloheximide or tunicamycin to the recovery medium of OG-extracted cells. After a 3-h recovery period, a virus binding experiment was performed. Both cycloheximide and tunicamycin inhibited the recovery of receptor activity even at low concentrations (Fig. 4), indicating that protein synthesis and N-linked glycosylation were required to recover receptor activity on cell surfaces.

Immunoprecipitation of SV40-protein complexes. To determine whether OG extract solutions derived from Vero C1008 cell surfaces contained protein moieties which bind to SV40 virions, we incubated radiolabeled OG extract solutions with unlabeled SV40 to form virus-protein complexes. These complexes or OG extract alone were immunoprecipitated with antiserum directed against SV40 structural proteins and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography. Proteins with molecular weights of 90,000, 58,000, 54,000, and 40,000 were

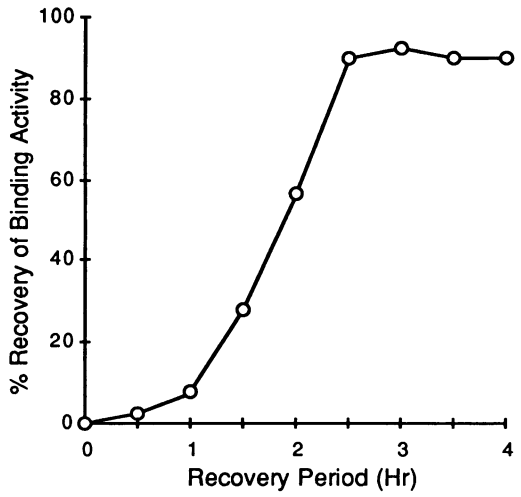

FIG. 3. Recovery of SV40 receptor activity after OG extraction. Duplicate Vero C1008 cell monolayers in 24-well plates were extracted with a 0.2% OG solution and allowed to recover in growth medium at 37°C. At the times indicated, the monolayers were washed with PBS and used in the virus binding assay. Values indicated are averages from three or more duplicate monolayers. Recovery was essentially complete by 2.5 h postextraction.

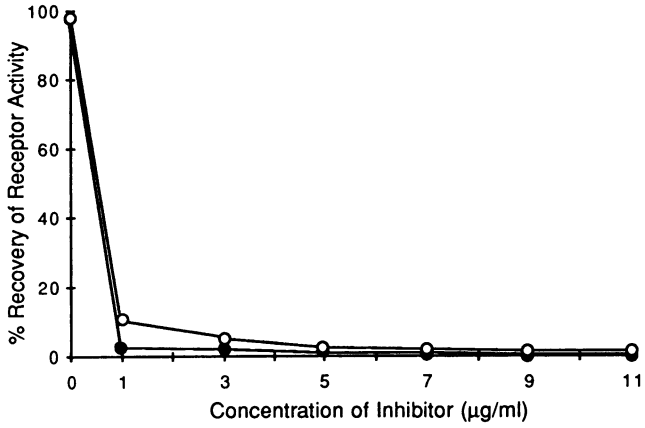

FIG. 4. Effect of tunicamycin or cycloheximide on the recovery of SV40 receptor activity. Duplicate cell monolayers in 24-well plates were extracted with a 0.2% OG solution and allowed to recover in growth medium containing various concentrations of cycloheximide (●) or tunicamycin (○). After a 3-h recovery period, the cells were washed with PBS and used in the virus binding assay. Values indicated are averages from three or more duplicate monolayers.

precipitated from virus-extract mixtures but not from OG extracts alone (Fig. 5), suggesting that these proteins, or a complex made up of these proteins, bind to SV40 virions.

Competition between SV40 and polyomavirus for SV40-binding sites. The results of the immunoprecipitation experiments described above are similar to the results reported for similar experiments involving polyomavirus and its receptor (21). Also, polyomavirus has been reported to bind to specific O-linked carbohydrates (5, 9). Therefore, the possibility exists that SV40 and polyomavirus share the same or similar receptors. To determine whether SV40 and polyomavirus compete for binding to the same receptor, we added increasing amounts of purified unlabeled polyomavirus or purified unlabeled SV40 to Vero C1008 monolayers before addition of a saturating amount of labeled SV40. Unlabeled polyomavirus virions were unable to compete with SV40 virions for available binding sites under conditions in which unlabeled SV40 virions did compete (Fig. 6), indicating that SV40 and polyomavirus do not recognize the same receptor moiety.

DISCUSSION

In a previous study, we have reported that SV40 binds to Vero C1008 cells via a single class of receptors with a high binding affinity for SV40 (7). In this study, we sought to gain information on the biochemical nature of this receptor. A commonly used method for determining whether a viral receptor is a protein, lipid, or carbohydrate moiety is to treat cells or cell membranes with various enzymes and determine the effect of such treatment on virus binding (1, 13, 15, 31). SV40 binding experiments on cells treated with a variety of enzymes revealed that protein moieties on the cell surface were important in the interaction between SV40 and its receptor, suggesting that protein is an integral part of the receptor. Even though treatment of cells with endoglycosidase F or glycopeptidase F had no effect on SV40 binding, tunicamycin was found to inhibit the recovery of receptor activity from OG-treated cells, suggesting that an N-linked carbohydrate is a component of the receptor that is not required for virus binding. Alternatively, the synthesis of

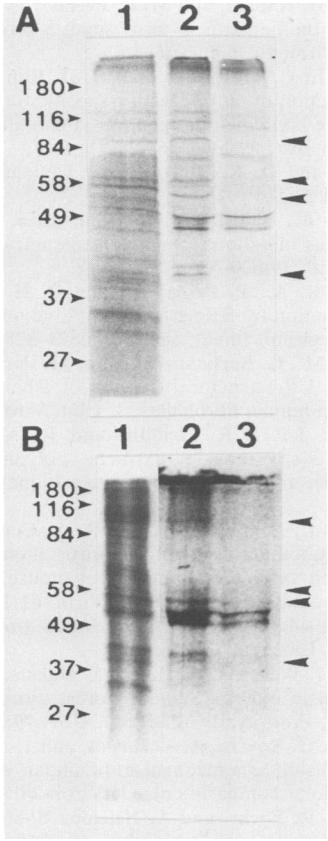

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of immunoprecipitated SV40-protein complexes from OG extract solutions. OG extract prepared from Vero C1008 cells metabolically labeled with either a mixture of ^{14}C-amino acids (A) or with L-[^{35}S]methionine (B) was mixed with unlabeled purified SV40 virions. The OG extract solutions alone (lanes 3) or SV40-protein complexes (lanes 2) were immunoprecipitated with antiserum directed toward SV40 structural proteins. Total labeled OG extract solutions are shown in lanes 1. Numbers represent molecular weights of marker proteins ($\times 10^3$). Arrowheads at the right indicate positions of proteins reactive to SV40 virions.

proteins with N-linked carbohydrates may be necessary in either the synthesis of SV40 receptors or the transport of receptors to the cell surface.

It is interesting that specific carbohydrate chains in the form NeuAc-α(2,3)Gal-β(1,3)GalNAc O-linked, or possibly as components of N-linked carbohydrates, have been reported to be required for polyomavirus infection of mouse 3T6 cells (5, 9). In these studies, neither asialo forms of the receptor nor alternate NeuAc linkages to the receptor permitted polyomavirus infection of these cells. Other workers have suggested that the binding of polyomavirus to NeuAc residues on the surfaces of another cell type (mouse kidney cells) represents nonspecific binding which fails to result in productive infection (4). BK and JC viruses, other members of the papovavirus family which infect humans, are also believed to bind to NeuAc residues on the basis of their ability to hemagglutinate erthrocytes in vitro (19, 30). Hemagglutination has not been reported for SV40, and neuraminidase treatment of cells had no effect on SV40 binding (Table 1); therefore, it seems unlikely that NeuAc residues play a role in the recognition of the receptor by SV40. However, O-linked carbohydrates in the form Gal-β(1,3)GalNAc con-

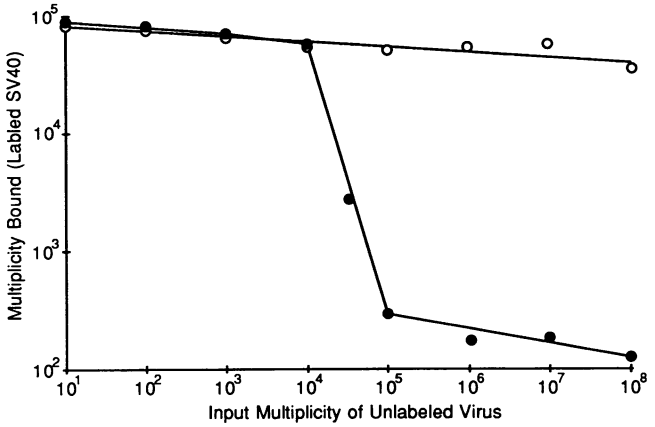

FIG. 6. Competition for SV40-binding sites. Vero C1008 monolayers were incubated with increasing amounts of either unlabeled polyomavirus (○) or unlabeled SV40 (●) for 2 h at 4°C before incubation with [^{3}H]leucine-labeled SV40 for 2 h at 4°C. The inoculum was removed, and the cell-associated radioactivity was determined by liquid scintillation spectrometry. Polyomavirus was unable to compete for SV40-binding sites under conditions in which unlabeled SV40 did compete, indicating that these two viruses use different binding sites.

taining one or more terminal NeuAc residues were found to be necessary for SV40 binding to Vero C1008 cells.

The nonionic detergent OG has been used to extract the receptors for Semliki Forest virus, vesicular stomatitis virus, polyoma virus, and rabies virus from intact cell monolayers (11, 21, 31, 35). At low concentrations, this detergent has been used for the noncytolytic extraction of surface proteins. This treatment leaves intact cells which have been used to study the synthesis of new receptors (16, 21). Extract solutions have been examined for the presence of virus-binding proteins (21). We used these approaches to examine SV40 receptor moieties from Vero C1008 cell surfaces. OG was found to reduce SV40 binding to Vero C1008 cell surfaces. Binding experiments with OG-treated cells revealed that recovery of receptor activity took 2.5 h and was inhibited by cycloheximide or by tunicamycin. These results are similar to those reported for the polyomavirus receptor (21). Immunoprecipitation of SV40-protein complexes from OG extracts revealed four proteins that were not present in precipitates from OG extract solutions alone, indicating that one or more of these proteins bind to SV40 virions. Owing to the denaturing conditions used during gel electrophoretic analysis, it is possible that these proteins are subunits of a larger receptor complex. The presence of protease inhibitors in the extract preparation reduces the possibility that these proteins are proteolytic degradation products of larger receptor proteins and increases the probability that these proteins are subunits of a single protein complex. We have previously obtained evidence that a single class of receptor was involved in SV40 binding to Vero C1008 cells based on Scatchard analysis of virus binding (7), which would also suggest that these proteins make up a single receptor complex. However, the possibility exists that there are a number of receptors for SV40 all having the same affinity for SV40 virus particles. The proteins immunoprecipitated had apparent molecular weights of 90,000, 58,000, 54,000, and 40,000, which are similar to those of proteins immunoprecipitated from polyomavirus-protein complexes from OG extracts of mouse kidney cell monolayers (21). In that study, it was also proposed that these proteins are subunits of a single receptor

complex. Therefore, it is possible that there is a family of related protein complexes found on kidney epithelial cells that are used as receptors by this family of viruses. Both viruses will bind to mouse kidney and monkey kidney cells (33); however, competition experiments between SV40 and polyomavirus (Fig. 6) indicate that these two viruses do not bind to the same epitope, suggesting that these viruses use different receptors.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Armstrong, G. D., R. W. Paul, and P. W. K. Lee.** 1984. Studies on reovirus receptors of L cells: virus binding characteristics and comparison with reovirus receptors of erthrocytes. Virology **138**:37–48.
2. **Ashkenazi, A., and J. L. Melnick.** 1962. Induced latent infection of monkeys with vacuolating SV40 papovavirus. Virus in kidneys and urine. Proc. Soc. Exp. Biol. Med. **111**:367–372.
3. **Barbanti-Brodano, G., P. Swetly, and H. Koprowski.** 1970. Early events in the infection of permissive cells with simian virus 40: adsorption, penetration, and uncoating. J. Virol. **6**:78–86.
4. **Bolen, J. B., and R. A. Consigli.** 1979. Differential adsorption of polyoma virions and capsids to mouse kidney cells and guinea pig erythrocytes. J. Virol. **32**:679–683.
5. **Cahan, L. D., R. Singh, and J. C. Paulson.** 1983. Sialyloligosaccharide receptors of binding variants of polyomavirus. Virology **130**:281–289.
6. **Chamberlain, J. P.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. Anal. Biochem. **98**:132–135.
7. **Clayson, E. T., and R. W. Compans.** 1988. Entry of SV40 is restricted to apical surfaces of polarized epithelial cells. Mol. Cell. Biol. **8**:3391–3396.
8. **Dales, S.** 1973. Early events in cell-animal virus interactions. Bacteriol. Rev. **37**:103–135.
9. **Fried, H., L. D. Cahan, and J. C. Paulson.** 1981. Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells. Virology **109**:188–192.
10. **Griffith, G. R., S. J. Marriott, D. A. Rintoul, and R. A. Consigli.** 1988. Early events in polyomavirus infection: fusion of monopinocytotic vesicles containing virions with mouse kidney cell nuclei. Virus Res. **10**:41–52.
11. **Helenius, A., B. Morien, E. Fries, K. Simons, P. Robinson, V. Schirrmacher, C. Terhorst, and J. Strominger.** 1978. Human (HLA-A and HLA-B) and murine (H-2k and H-2d) histocompatibility antigens are cell surface receptors for Semliki Forest virus. Proc. Natl. Acad. Sci. USA **75**:3846–3850.
12. **Hummeler, K., N. Tomassini, and F. Sokol.** 1970. Morphological aspects of the uptake of simian virus 40 by permissive cells. J. Virol. **6**:87–93.
13. **Kalyanaraman, J. S., M. G. Sarngadharan, and R. C. Gallo.** 1978. Characterization of Rauscher murine leukemia virus envelope glycoprotein receptor in membranes from murine fibroblasts. J. Virol. **28**:686–696.
14. **Kielian, M., and A. Helenius.** 1985. pH induced alterations in the lysogenic spike protein of Semliki Forest virus. J. Cell Biol. **101**:2284–2291.
15. **Komai, K., M. Kaplan, and M. E. Peeples.** 1988. The Vero cell receptor for the hepatitis B virus small S protein is a sialoglycoprotein. Virology **163**:629–634.
16. **LeGrue, S. J., C. M. Macek, and B. D. Kahan.** 1982. Noncytolytic extraction of murine tumor-specific transplantation antigens with the nonionic detergent octyl-β-D-glucopyranoside. J. Natl. Cancer Inst. **69**:131–136.
17. **Lenard, J., and D. K. Miller.** 1982. Uncoating of enveloped viruses. Cell **28**:5–6.
18. **Mackey, R. L., and R. A. Consigli.** 1976. Early events in polyoma virus infection: attachment, penetration, and nuclear entry. J. Virol. **19**:620–636.
19. **Mäntyjärvi, R. A., P. P. Arstila, and O. H. Meurman.** 1972. Hemagglutination by BK virus, a tentative new member of the papovavirus group. Infect. Immun. **6**:824–828.
20. **Maraldi, N. M., G. Barbanti-Brodano, M. Portolani, and M. La Placa.** 1975. Ultrastructural aspects of BK virus uptake and replication in human fibroblasts. J. Gen. Virol. **27**:71–80.
21. **Marriott, S. J., G. R. Griffith, and R. A. Consigli.** 1987. Octyl-β-D-glucopyranoside extracts polyomavirus receptor moieties from the surfaces of mouse kidney cells. J. Virol. **61**:375–382.
22. **Marriott, S. J., D. J. Roeder, and R. A. Consigli.** 1987. Anti-idiotypic antibodies to a polyomavirus monoclonal antibody recognize cell surface components of mouse kidney cells and prevent polyomavirus infection. J. Virol. **61**:2747–2753.
23. **Marsh, M.** 1984. The entry of enveloped viruses into cells by endocytosis. Biochem. J. **218**:1–10.
24. **Marsh, M., J. Wellsteed, H. Kern, E. Harms, and A. Helenius.** 1982. Monensin inhibits Semliki Forest virus penetration into culture cells. Proc. Natl. Acad. Sci. USA **79**:5297–5301.
25. **Maul, G. G., G. Rovera, A. Vorbrodt, and J. Abramczuk.** 1978. Membrane fusion as a mechanism of simian virus 40 entry into different cellular compartments. J. Virol. **28**:936–944.
26. **Mellman, I., R. Fuchs, and A. Helenius.** 1986. Acidification of the endocytic and exocytic pathways. Annu. Rev. Biochem. **55**:663–700.
27. **Meyer, H. M., H. E. Happs, N. G. Rogers, B. E. Brooks, B. C. Bernheim, W. P. Jones, A. Nisalak, and R. D. Douglas.** 1962. Studies on simian virus 40. J. Immunol. **88**:796–806.
28. **Nishimura, T., N. Kawai, M. Kawai, K. Notske, and I. Ichihara.** 1986. Fusion of SV40-induced endocytotic vacuoles with the nuclear membrane. Cell Struct. Funct. **11**:135–141.
29. **Norkin, L. C., and K. H. Einck.** 1978. Cell killing by simian virus 40: protective effect of chloroquine. Antimicrob. Agents Chemother. **14**:930–932.
30. **Padgett, B. L., C. M. Rogers, and D. L. Walker.** 1977. JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: additional biological characteristics and antigenic relationships. Infect. Immun. **15**:656–662.
31. **Schlegel, R., T. S. Tralka, M. C. Willingham, and I. Pastan.** 1983. Inhibition of VSV binding and infectivity by phosphatidylserine: is phosphatidylserine a VSV-binding site? Cell **32**: 639–646.
32. **Shimura, H., Y. Umeno, and G. Kimura.** 1987. Effects of inhibitors of the cytoplasmic structures and functions on the early phase of infection of cultured cells with simian virus 40. Virology **158**:34–43.
33. **Tooze, J. (ed.)** 1973. The molecular biology of tumor viruses, p. 355–359. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Upcroft, P.** 1987. Simian virus 40 infection is not mediated by lysosomal activation. J. Gen. Virol. **68**:2477–2480.
35. **Wunner, W. H., and K. J. Reagan.** 1985. Nature of the rabies virus cellular receptor, p. 152–159. In R. L. Crowell and K. Lonberg-Holm (ed.), Virus attachment and entry into cells. American Society for Microbiology, Washington, D.C.