MOLECULAR AND CELLULAR BIOLOGY, Aug. 1988, p. 3391–3396
0270-7306/88/083391-06$02.00/0

Vol. 8, No. 8

Entry of Simian Virus 40 Is Restricted to Apical Surfaces of Polarized Epithelial Cells

EDWARD T. CLAYSON AND RICHARD W. COMPANS*

Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Received 1 April 1988/Accepted 9 May 1988

The uptake of simian virus 40 (SV40) by polarized epithelial cells was investigated by growth of cells on permeable supports and inoculation on either the apical or the basolateral surface. Binding of radiolabeled SV40 occurred on the apical but not the basolateral surfaces of permissive polarized Vero C1008 cells and nonpermissive polarized MDCK cells. When similar experiments were performed on nonpolarized Vero or CV-1 cells, virus binding occurred regardless of the direction of virus input. Electron micrographs of Vero C1008 cells infected at high multiplicities revealed virions lining the surfaces of apically infected cells, while the surfaces of basolaterally infected cells were devoid of virus particles. Analysis of the binding data revealed a single class of virus receptors (9×10^4 per cell) with a high affinity for SV40 (K_d = 3.76 pM) on the apical surfaces of Vero C1008 cells. Indirect immunofluorescence studies revealed that synthesis of viral capsid proteins in Vero C1008 cells occurred only when input virions had access to the apical surface. Virus yields from apically infected Vero C1008 cells were 10^5 PFU per cell, while yields obtained from basolaterally infected cells were less than one PFU per cell. These results indicate that a specific receptor for SV40 is expressed exclusively on the apical surfaces of polarized Vero C1008 cells.

Polarized epithelial cells are characterized by the presence of two plasma membrane domains: the apical domain, which lines the epithelial lumen, and the basolateral domain, which faces underlying tissues in vivo. These two domains are separated by a continuous belt of tight junctional complexes which maintain the unique protein and lipid compositions of each domain (30). Certain enveloped viruses have been shown to enter polarized epithelial cells preferentially from the basolateral domain (7, 8). The polarity of viral entry is presumed to be the result of the polarized expression of viral receptors. The entry of a virus exclusively from the apical surface has not been previously reported. We have recently shown that simian virus 40 (SV40), a nonenveloped virus, is released preferentially from the apical surface of polarized epithelial cells (Clayson et al., submitted for publication), and we have investigated the entry of SV40 in the present study.

SV40 adsorbs to the cell surface, where it is internalized either individually or in small groups in endocytic vesicles. These virion-containing vesicles are reported to be transported directly to the nucleus and to fuse with the outer nuclear membrane (11, 20, 23). The virus is then thought to be transported across the inner nuclear membrane (by an unknown mechanism) into the nucleoplasm, where virus uncoating and virus replication occur (2). In order to determine whether the binding of SV40 is polarized in permissive cells grown on permeable supports, an assay for the binding of radiolabeled SV40 virions to cell monolayers was developed. Electron microscopy was also used to demonstrate virus binding to cell surfaces. Productive infection of cells infected from either apical or basolateral surfaces was determined by plaque assay of the progeny virus produced, and viral capsid protein synthesis in cells infected from either apical or basolateral surfaces was analyzed by indirect immunofluorescence.

* Corresponding author.

MATERIALS AND METHODS

Cells and virus. Polarized Vero C1008, CV-1, and primary African green monkey kidney (AGMK) cells were obtained and grown as previously described (Clayson et al., submitted). Nonpolarized Vero and polarized Madin-Darby canine kidney (MDCK) cells were purchased from the American Type Culture Collection and were maintained in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum. Purified canine parvovirus was a gift from Sukla Basak. SV40 stocks were prepared and titers were determined as previously described (Clayson et al., submitted). For preparation of purified ^{3}H-labeled SV40 virus, infected monolayers were incubated in leucine-free Eagle medium supplemented with 2% fetal bovine serum and 10 μCi of L-[4,5-^{3}H]leucine (70 Ci/mmol) per ml. The virus was harvested as described above and then pelleted by centrifugation at 28,000 rpm for 2.5 h in an SW28 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The pellets were suspended in TNE (10 mM Tris hydrochloride, 100 mM NaCl, 1 mM EDTA), layered on solutions of CsCl in TNE (final average density of 1.32 g/ml), and centrifuged for 24 h at 35,000 rpm in a Beckman SW41 rotor. In the gradient, two visible bands were observed. Fractions were collected, and the radioactivity in each fraction was counted by liquid scintillation spectrometry. Two peaks corresponding to the two visible bands were observed. These fractions were separated and subjected to a second equilibrium density gradient. The bands were collected and dialyzed overnight against a 2,000-fold volume of TNE. The identification of "full" and "empty" particles in the two bands was made by electron microscopy. The protein concentration was determined with the Pierce protein reagent (Pierce Chemical Co., Rockford, Ill.). The concentration of viral particles was determined by spectrophotometry as described by Uchida et al. (32) and by calculation from the protein concentration. Finally the two samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fluorography and autoradiography of the gel were performed as described

by Chamberlain (4). Only viral structural proteins were observed in the two samples.

Electron microscopy. Monolayers were prepared for electron microscopy as described elsewhere (Clayson et al., submitted).

Virus binding assay. For the polarized binding experiments, cells were grown to confluency in 12-mm-diameter Millicell-HA filter chambers (Millipore Corp., Bedford, Mass.) in 24-well plates and exposed to ^{3}H-labeled SV40 at 4°C on either the apical or the basolateral surface. At the times indicated in Fig. 1, the inoculum was removed and the cells were washed with phosphate-buffered saline deficient in Ca^{2+} and Mg^{2+} (37°C) for 15 min. The cells were then removed from the filter with trypsin, pelleted by centrifugation, and dissolved in 100 μl of NaOH. The radioactivity in the cell-associated sample was then determined by liquid scintillation spectrometry. Control experiments demonstrated that treating cells with trypsin did not lower the cell-associated radioactivity (data not shown). For the competition and saturation experiments, cells were grown on plastic in 24-well plates instead of permeable supports. Determination of cell-associated counts was performed in the manner described above, except that the trypsinization and centrifugation steps were omitted.

Antibodies and immunofluorescence labeling. Horse anti-SV40 antiserum was purchased from Flow Laboratories, Inc. (McLean, Va.). Fluorescein-conjugated rabbit anti-horse immunoglobulin G was purchased from Miles Laboratories, Inc. (Elkhart, Ind.). Cells were grown to confluency in Millicell-HA filter chambers (Millipore) in six-well plates (Becton Dickinson and Co., Oxnard, Calif.) and infected with stock SV40 virus (multiplicity of infection = 20). At 48 h postinfection, the cells (on filters) were stained for immunofluorescence as previously described (7), except that nuclear staining with Hoechst dye was omitted. The filters were then cut away from their holders, cut into four equal parts, mounted cell side up on a microscope slide in Polymount (Polysciences, Inc., Warrington, Pa.), and covered with a glass cover slip. The slides were viewed by epifluorescence and photographed with a Nikon Optiphot microscope equipped with a modified B2 cube.

RESULTS

Exclusive binding of SV40 virions to apical surfaces. In order to determine whether virus binding occurs in a polarized manner, apical or basolateral surfaces of cells were incubated with purified ^{3}H-labeled virus at 4°C. At selected times postinfection, the cell-associated radioactivity was determined. Virus binding to nonpolarized Vero cells was not dependent on the direction of virus input by 30 min postinoculation (Fig. 1A). The difference in virus binding before 30 min was probably due to the time required for diffusion of the virus through the nitrocellulose filter. By comparison, virus binding to apical surfaces of polarized Vero C1008 cells was 10-fold greater than binding to basolateral surfaces at 4 h postinfection (Fig. 1B). Experiments with empty capsids yielded similar results. These results suggest that a receptor for SV40 is expressed preferentially on the apical surfaces of these cells. Similar binding experiments performed on nonpermissive polarized MDCK cells revealed that virus binding was restricted to the apical surfaces of these cells as well (Fig. 1C). These results suggest that a receptor for SV40 is expressed in a polarized manner in polarized epithelial cell lines.

To further demonstrate that SV40 virions bind exclusively to apical surfaces of polarized epithelial cells, Vero C1008

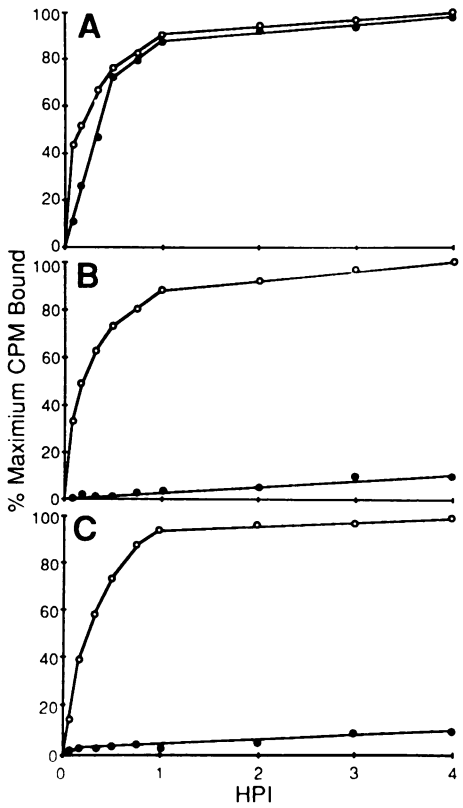

FIG. 1. SV40 binding to plasma membrane surfaces of epithelial cells. Cells were grown on permeable supports and inoculated with purified [^{3}H]leucine-labeled SV40 from either the apical (○) or basal (●) chamber at 4°C. At the indicated number of hours postinfection (HPI), the inoculum was removed and the cell-associated radioactivity was determined by liquid scintillation spectrometry. (A) SV40 binding to nonpolarized CV-1 cells; (B) SV40 binding to polarized Vero C1008 cells; (C) SV40 binding to nonpermissive, polarized MDCK cells.

cells were examined by electron microscopy at 30 min postinfection. Numerous virions bound to apical surfaces of cells infected from the apical chamber were observed; however, no virions were observed to bind to surfaces of cells inoculated from the basal chamber (Fig. 2). It is unlikely that the lack of binding to basolateral surfaces of these cells was due to the inability of virions to pass through the filter, since binding of radiolabeled virus to the surfaces of nonpolarized cells was not restricted by the filter at 30 min postinfection (Fig. 1A).

Competition for binding sites and saturability of the SV40 receptor. The lack of binding of SV40 to basal surfaces of Vero C1008 cells suggests that a receptor for SV40 is expressed only on apical surfaces of epithelial cells. However, the possibility exists that SV40 binds nonspecifically to apical surfaces or that a number of receptor classes with different binding affinities are involved in SV40 binding. If a specific receptor is involved in virus binding, the receptor should exist in a finite number on the cell surface. Therefore, excess unlabeled virus particles should be able to compete with labeled particles for binding sites, and available binding sites should be saturated when cell surfaces are exposed to an excess of virus particles. To determine whether unlabeled particles compete with labeled particles for available binding sites, increasing amounts of unlabeled SV40 were added to Vero C1008 monolayers before addition of a constant

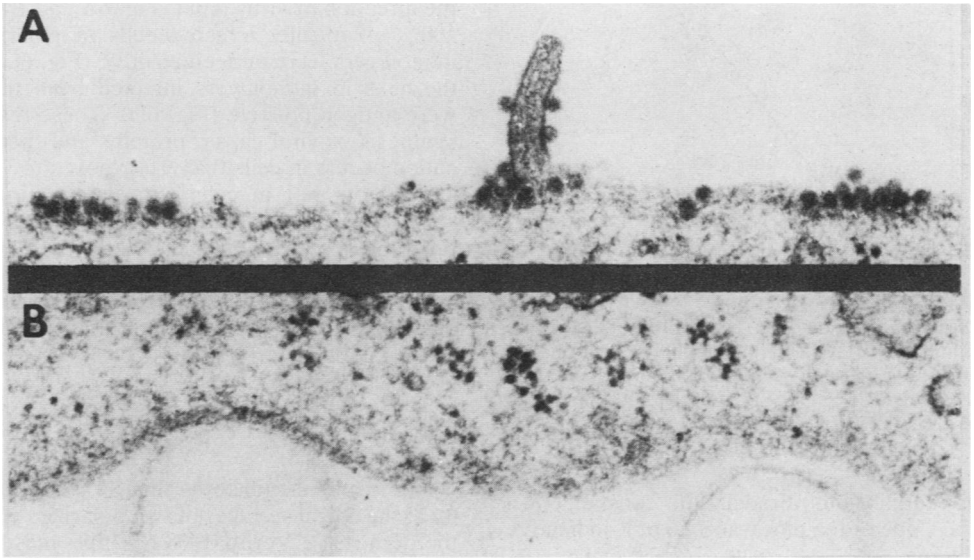

FIG. 2. Electron micrographs of apical and basal surfaces of Vero C1008 cells inoculated with SV40. Cells were grown on permeable supports, inoculated with high multiplicities of SV40 from either the apical (A) or the basolateral (B) chamber for 30 min, and prepared for electron microscopy. Note virions binding to apical surface but not to basal surface. Magnification, ×55,000.

amount of ^{3}H-labeled particles. Unlabeled SV40 virions were able to effectively compete with the labeled particles for binding (Fig. 3), indicating that a finite number of binding sites per cell are present. In contrast, addition of unlabeled canine parvovirus had no effect on SV40 binding.

To determine whether SV40 binding to apical surfaces is saturable, increasing amounts of ^{3}H-labeled SV40 virions were incubated with Vero C1008 monolayers for 2 h. Saturation of available binding sites did occur at high input multiplicities of virus (Fig. 4A). Similar results were obtained when ^{3}H-labeled empty capsids were used (data not shown). The data in Fig. 4A were replotted by the method of Scatchard (28) to estimate the following: the homogeneity of the receptor population, the average number of binding sites per cell, and affinity of the receptor for SV40. The straight line in Fig. 4B is indicative of a single class of receptors. The x intercept corresponds to the average number of receptors per cell, and the results indicate that approximately 9×10^4 receptors for SV40 exist on the apical surfaces of Vero

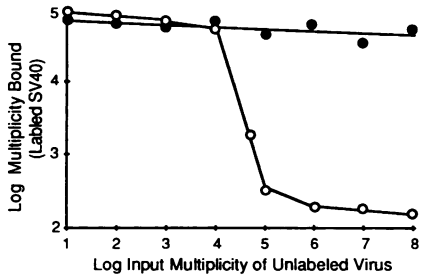

FIG. 3. Competition for SV40 binding sites. Vero C1008 monolayers were incubated with increasing amounts of either unlabeled SV40 (○) or canine parvovirus (●) for 2 h at 4°C before incubation with [^{3}H]leucine-labeled SV40 for 2 h at 4°C. The inoculum was removed, and the cell-associated radioactivity was determined by liquid scintillation spectrometry. Multiplicities of unlabeled SV40 greater than 5×10^4 virions per cell were able to compete for more than 99% of the available binding sites. Canine parvovirus was unable to compete for SV40 binding sites.

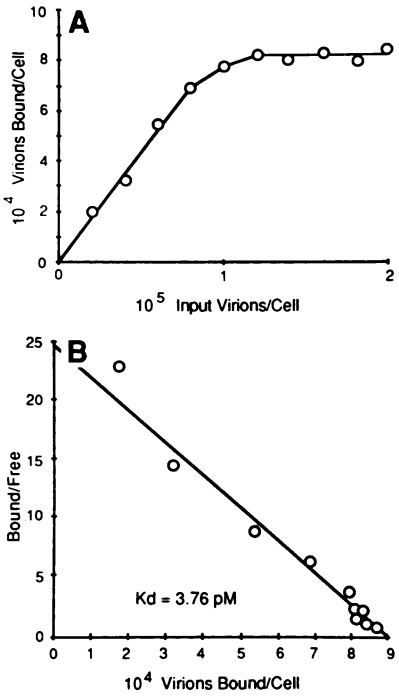

FIG. 4. Analysis of SV40 binding to apical surfaces of Vero C1008 cells. (A) Saturation of SV40 binding sites. Duplicate monolayers of Vero C1008 cells (2.34×10^5 cells) were inoculated with increasing amounts of ^{3}H-labeled SV40 (in 400 μl) at 4°C. At 2 h postinfection, the inoculum was removed and the cell-associated radioactivity was measured by liquid scintillation spectrometry. Saturation of virus binding indicates that binding to cell surfaces is due to the presence of specific receptors. (B) Scatchard analysis of SV40 binding. The data in panel A were replotted according to the method of Scatchard (28). Bound/free, Number of bound virions per well divided by the number of virions remaining free in the media. The straight line indicates a single class of receptors. The x intercept indicates the number of receptor sites per cell (9×10^4). The equilibrium dissociation constant (K_d = 3.76 pM) was calculated from the slope of the line and is indicative of high-affinity binding.

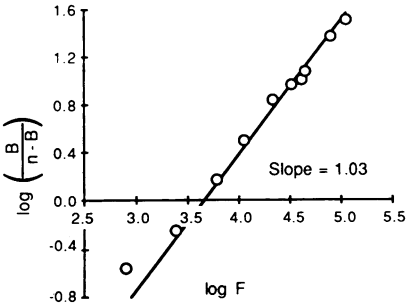

FIG. 5. Hill analysis of SV40 binding. The data in Fig. 4A were replotted by the method of Hill (10). B, Number of bound virions per cell; n, number of receptors per cell as determined in Fig. 4B. The Hill coefficient was calculated from the slope of the line. A value of 1.00 is indicative of no cooperation between receptors.

C1008 cells. The equilibrium dissociation constant (K_d) calculated from the slope of the plot was 3.76 pM, indicative of high-affinity binding.

To examine the possibility that the binding of virus to receptors is influenced by cooperation between receptors, the same data were replotted by the method of Hill (10) (Fig. 5). The Hill coefficient was calculated from the slope of this line. A value of 1.00 is indicative of no cooperation between receptors. The calculated value for the Hill coefficient for SV40 binding to Vero C1008 cells was 1.03, indicating no cooperation between receptors.

Dependence of virus replication on the direction of virus input. To determine whether the production of virus particles is dependent upon the direction of virus input, cells were grown on permeable supports and inoculated with SV40 from either the apical or the basal chamber. Yields of progeny virus were then determined by plaque assay. Approximately equivalent viral yields were obtained from nonpolarized cells (Vero and CV-1), irrespective of the direction of virus input (Table 1). In contrast, the yield obtained from apically infected polarized Vero C1008 cells was 10^5 PFU per cell, compared with less than 1 PFU per cell for basolaterally infected cells. The low yields obtained from basolaterally infected Vero C1008 cells indicate that fewer than 1 of 10^5 cells was productively infected, assuming equivalent yields from each infected cell. To directly examine virus replication, productively infected CV-1 and Vero C1008 cells were identified by indirect intracellular immunofluorescence by using antibodies directed toward viral structural proteins. Between 80 and 100% of the cells in nonpolarized CV-1 monolayers were antigen positive regardless of the direction of virus input (Fig. 6A and B). Although nearly 100% of apically infected cells in polarized Vero C1008 monolayers were antigen positive (Fig. 6C), less than 1% of the cells in monolayers infected from the basal chamber were antigen positive (Fig. 6D). These results indicate that synthesis of viral capsid proteins and therefore virus replication occurs in cells that are exposed to virus on the apical surface but not in cells exposed to virus on their basal surfaces. This restriction was also shown to require intact tight junctional complexes. When Vero C1008 cell monolayers were treated with 30 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′,-tetraacetic acid] for 15 min to open tight junctions just before exposure to SV40 on basal surfaces, the majority of these cells were found to be antigen positive (Fig. 6E).

TABLE 1. Comparison of the yields of progeny SV40 in various cell lines inoculated from either the apical or basal chamber[a]

Cell line	PFU/cell from[b]:		% Cells infected from basal chamber
	A	B	
CV-1	1.0×10^5	0.94×10^5	94.0
Vero	1.0×10^5	0.85×10^5	85.0
AGMK	0.7×10^5	43	0.061
Vero C1008	1.0×10^5	0.85	0.00085

[a] Cells were grown on permeable supports and infected with SV40 (multiplicity of infection = 20). At 60 h postinfection, virus was harvested by two cycles of freeze-thawing and sonification, and titers were determined by plaque assay.

[b] Virus yields obtained from cells inoculated from the apical (A) or basal (B) chamber.

DISCUSSION

Our results demonstrate that SV40 binding occurs primarily to the apical surfaces of two polarized epithelial cell lines, the permissive Vero C1008 cell line and the nonpermissive MDCK cell line. This observation suggests that a receptor for SV40 is expressed on the apical surfaces of several different epithelial cell types and is consistent with the cellular tropism of SV40. Productive infection by SV40 occurs only in cells of monkey origin, but transformation by SV40 has been reported in cells of rabbits, hamsters, mice, rats, cows, guinea pigs, and humans (31). Scatchard analysis of the binding of SV40 to Vero C1008 cells indicates that 9×10^4 binding sites per cell with a high affinity for SV40 (K_d = 3.76 pM) exist on the apical surface of these cells. We could not detect any significant difference in receptor numbers and binding affinities between whole virions and empty capsids, suggesting that both use the same receptors. The observation that fewer than 1 of 10^5 cells within the polarized Vero C1008 monolayers took up virus from the basolateral surface suggests that a small subpopulation of nonpolarized or incompletely polarized cells exists within these monolayers. Similar observations have been reported for the polarized MDCK cell line (7).

Virus entry is thought to occur by one of three alternative mechanisms: endocytosis, fusion of the viral membrane with cellular membranes (enveloped viruses), or possibly direct translocation of virus particles across the plasma membrane. Recent evidence from several laboratories indicates that the endocytic pathway used by SV40 and other papovaviruses is different from that used by other viruses which use endocytosis as a means of entry. Most viruses require transport through acidic compartments (endosomes and lysosomes) for uncoating and activation (5, 13, 17), which can be blocked by neutralizing the low pH of these compartments with lysosomotropic agents (12, 18, 19, 21). Papovaviruses are reported to be uncoated in the nucleus (2, 14), and workers in several laboratories have reported that lysosomotropic agents have no effect on SV40 entry (24, 29, 33). Papovavirus-containing vesicles have been reported to be transported to the nucleus, where they are thought to fuse with the outer nuclear membrane, delivering virus particles into the perinuclear cisternae (9, 11, 20, 23). It has been reported that plasma membrane markers are transported to the outer nuclear membrane in SV40- or polyoma-infected cells but not in control cells; therefore, this vesicular transport pathway may be unique to papovaviruses (9, 23). All the viruses which have been previously reported to enter cells in a polarized manner (vesicular stomatitis virus [7], Semliki Forest virus [8], and reovirus types 1 and 2 [27]) enter

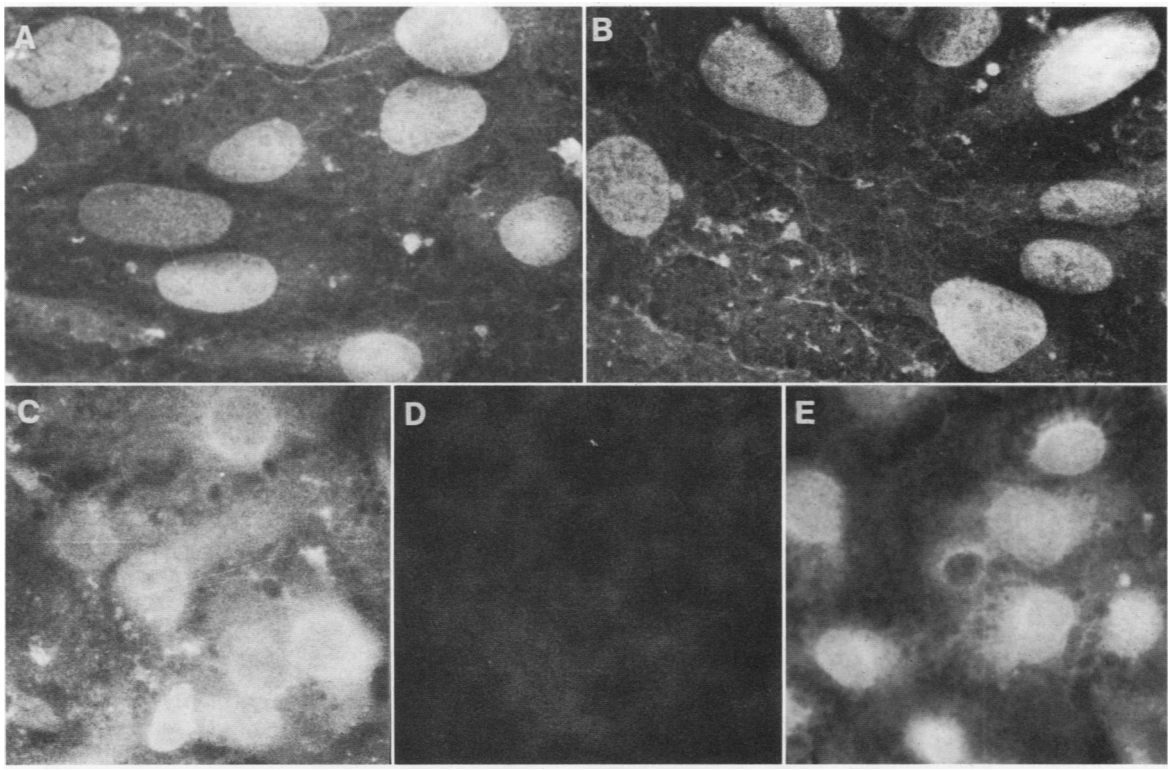

FIG. 6. Indirect intracellular immunofluorescence of cells infected with SV40. Nonpolarized CV-1 and Vero C1008 cells were grown on permeable supports and inoculated with SV40 (multiplicity of infection = 20) from either the apical or the basal chamber for 1 h at 37°C. At 48 h postinfection, the cells were fixed and prepared for immunofluorescence with antiserum directed toward viral structural proteins. Magnification, ×90. (A) CV-1 cells inoculated from the apical chamber; (B) CV-1 cells inoculated from the basal chamber; (C) Vero C1008 cells inoculated from the apical chamber; (D) Vero C1008 cells inoculated from the basal chamber; (E) Vero C1008 cells treated with 30 mM EGTA for 15 min to open tight junctional complexes before inoculation with SV40 from the basal chamber.

exclusively from the basolateral surface. Some viruses, such as influenza A, have been reported to enter from both apical and basolateral surfaces (7). We believe that the present results are the first report of a virus which enters polarized epithelial cells exclusively from the apical surface.

Our results concerning the uptake of SV40 by polarized epithelial cells grown on permeable supports should be relevant to virus infection of epithelia in vivo. We have recently shown that SV40 release occurs also from apical surfaces of infected cells (Clayson et al., submitted). The spread of viral infection in vivo then would be expected to be confined to the epithelial surfaces of intact tissues. Influenza and parainfluenza viruses bud from the apical surfaces of polarized epithelial cells (26), which may play a role in limiting the spread of infection to the epithelial lining of infected tissue. In contrast, vesicular stomatitis virus enters and buds from the basolateral surface of epithelial cells (7, 26). The latter process may facilitate the spread of infection to underlying tissue and blood supply. Little is known about the pathogenesis of SV40 in its natural host, the rhesus monkey. Apparently, virus growth occurs initially in either the respiratory tract or the digestive tract, depending on the route of inoculation, resulting in a viremia. This infection is rapidly cleared in immunocompetent individuals and is followed by a persistent long-term infection in the kidneys, resulting in a lifelong viruria in the absence of a viremia or any other clinical signs of infection (1, 22). These observations suggest that viral infection in the kidney is restricted to the epithelium and are consistent with our results.

We have recently suggested that a receptor-mediated vesicular transport process may be involved in SV40 release (Clayson et al., submitted). In this and other studies, cytoplasmic virions were always enclosed within smooth membrane vesicles and were found to adhere closely to the vesicle membrane (11, 20). It is possible that the mechanism for the polarized entry and release of SV40 depends on the polarized transport and expression of the SV40 receptor. It seems likely that the receptor for SV40 is shuttled between the outer nuclear membrane and the apical domain of the plasma membrane. The normal cellular function and the biochemical nature of the SV40 receptor are currently unknown; however, some information is available about the receptors of other papovaviruses. It has been reported that polyomavirus binds to a receptor complex made up of at least four polypeptides on the surfaces of primary mouse kidney cells. The receptor moieties within this complex were not determined, but it was speculated that carbohydrate and lipid residues also play a role in virus binding (16). Specific sialic acid residues have been reported to be required for polyomavirus infection of mouse 3T6 cells (6); however, others have suggested that binding of polyomavirus to sialic acid residues represents nonspecific binding which fails to result in productive infection (3). BK and JC viruses, other members of the papovavirus family which infect humans, are also believed to bind to sialic acid residues, on the basis of their ability to hemagglutinate erythrocytes in vitro (15, 25). Hemagglutination has not been reported for SV40, and neuraminidase treatment of cells has no effect on SV40 uptake (31); therefore, it seems unlikely that sialic acid residues play a role in SV40 binding. Efforts are currently

directed at determining the biochemical nature of the SV40 receptor.

ACKNOWLEDGMENTS

We thank E. Arms for assistance with electron microscopy and B. Jeffrey for secretarial assistance.

This study was supported by Public Health Service grants AI 12680 from the National Institute of Allergy and Infectious Diseases and CA 13148 from the National Cancer Institute and by Public Health Service Research Service Award AI 07150.

LITERATURE CITED

1. **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.
2. **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.
3. **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.
4. **Chamberlain, J. P.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. Anal. Biochem. **98**:132–135.
5. **Dales, S.** 1973. Early events in cell-animal virus interactions. Bacteriol. Rev. **37**:103–135.
6. **Fried, H., L. D. Cahan, and J. C. Paulson.** 1981. Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells. Virology **109**:188–192.
7. **Fuller, S., C. H. von Bonsdorff, and K. Simons.** 1984. Vesicular stomatitis virus infects and matures only through the basolateral surface of the polarized epithelial cell line, MDCK. Cell **38**:65–77.
8. **Fuller, S. D., C. H. von Bonsdorff, and K. Simons.** 1985. Cell surface influenza haemagglutinin can mediate infection by other animal viruses. EMBO J. **4**:2475–2485.
9. **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.
10. **Hill, A. V.** 1913. The combination of hemoglobin with oxygen and carbon monoxide. Biochem. J. **7**:471–480.
11. **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.
12. **Kielian, M., and A. Helenius.** 1985. pH induced alterations in the lysogenic spike protein of Semliki Forest virus. J. Cell Biol. **101**: 2284–2291.
13. **Lenard, J., and D. K. Miller.** 1982. Uncoating of enveloped viruses. Cell **28**:5–6.
14. **Mackay, R. L., and R. A. Consigli.** 1976. Early events in polyoma virus infection: attachment, penetration, and nuclear entry. J. Virol. **19**:620–636.
15. **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.
16. **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.
17. **Marsh, M.** 1984. The entry of enveloped viruses into cells by endocytosis. Biochem. J. **218**:1–10.
18. **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.
19. **Marsh, M. M., G. Griffiths, G. E. Dean, I. Mellman, and A. Helenius.** 1986. Three-dimensional structure of endosomes in BHK-21 cells. Proc. Natl. Acad. Sci. USA **83**:2899–2903.
20. **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.
21. **Mellman, I., R. Fuchs, and A. Helenius.** 1986. Acidification of the endocytic and exocytic pathways. Annu. Rev. Biochem. **55**: 663–700.
22. **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.
23. **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.
24. **Norkin, L. C., and K. H. Einck.** 1978. Cell killing by simian virus 40: protective effect of chloroquine. Antimicrob. Agents Chemother. **14**:930–932.
25. **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.
26. **Rodriguez-Boulan, E., and D. D. Sabatini.** 1978. Asymmetric budding of viruses in epithelial monolayers: a model system for the study of epithelial polarity. Proc. Natl. Acad. Sci. USA **75**: 5071–5075.
27. **Rubin, D. H.** 1987. Reovirus serotype 1 binds to the basolateral membrane of intestinal epithelial cells. Microbiol. Path. **3**:215–219.
28. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
29. **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.
30. **Simons, K., and S. D. Fuller.** 1985. Cell surface polarity in epithelia. Annu. Rev. Cell Biol. **1**:243–288.
31. **Tooze, J. (ed.).** 1973. The molecular biology of tumor viruses, p. 357–358. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Uchida, S., K. Yoshiike, S. Watanabe, and A. Furuno.** 1968. Antigen-forming defective viruses of simian virus 40. Virology **34**:1–8.
33. **Upcroft, P.** 1987. Simian virus 40 infection is not mediated by lysosomal activation. J. Gen. Virol. **68**:2477–2480.