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Differential Adsorption of Polyoma Virions and Capsids to Mouse Kidney Cells and Guinea Pig Erythrocytes†

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Adsorption of ^{125}I-labeled polyoma virions and capsids to the surface of mouse kidney cells (MKC) and guinea pig erythrocytes was examined. Purified polyoma capsids lack the ability to compete with polyoma virions for specific binding sites on the surface of MKC. These same capsids were, however, able to block virion adsorption to guinea pig erythrocytes. UV-inactivated virions blocked cellular receptors on MKC and thus inhibited infectious virions from infecting the cells. Capsids were unable to inhibit virion infection of MKC. Adsorption of polyoma virions to MKC and infection of these cells were found to be independent of the ability of the virions to agglutinate guinea pig erythrocytes.

The initial step of infection of permissive or nonpermissive cells by papovaviruses is the adsorption of the virus to the surface of host cells. Adsorption of polyoma virions containing viral DNA, host-contributed histones, and three structural proteins (VP_1, VP_2, and VP_3) is followed by virion penetration of the cell in monopinocytotic vesicles and the deposition of the virion at the nucleus where uncoating occurs (10). Adsorption of polyoma capsids devoid of DNA and histones and containing only the three structural proteins is followed by phagocytic engulfment and degradation in cell lysosomes (10). These findings led us to speculate that polyoma virions contained a class of receptors missing in polyoma capsids. However, since both polyoma virions and capsids can be found in cellular lysosomes (8, 10), but only virions are found in the nucleus (10), we thought that perhaps the nuclear transport of virions might depend on the adsorption of virions to a class of limited surface receptors to which capsids are unable to bind efficiently.

Virus to be used in these experiments was grown in primary mouse kidney cells (16) in serum-free Dulbecco-modified Eagle medium (11). Purification of the virions (small plaque) has been described previously (3, 4, 11). Purification of capsids was accomplished by a series of CsCl velocity gradient centrifugations followed by in vitro labeling with ^{125}I by the method of Frost and Bourgaux (8, 9) and analysis on sodium dodecyl sulfate-polyacrylamide gels to ensure that the preparations were free of histones. Virions labeled in this manner did not exhibit any loss of biological activity.

Specific polyoma adsorption was taken as the difference between total binding (^{125}I-labeled polyoma alone) and nonspecific binding (^{125}I-labeled polyoma plus a 1,000-fold excess of unlabeled polyoma). Specific and nonspecific adsorption of radioactively labeled polyoma virions reached a maximum in 2 h at 4°C. This optimum was maintained for at least 5 h at that temperature (Fig. 1A). Adsorption of ^{125}I-labeled polyoma to mouse kidney cells (MKC) was linearly related to cell numbers in subconfluent and confluent monolayers (data not shown). In similar adsorption experiments performed with labeled polyoma capsids, the total number of counts bound to cells was virtually identical (Fig. 1B). However, the amount of non-specifically bound counts that could not be competed for by excess unlabeled capsids was increased, compared with the amount of nonspecific adsorption observed in the experiment in which polyoma virions were used. The increased level of nonspecific binding observed for capsids thereby resulted in a fivefold decrease in the amount of specifically adsorbed capsids, compared with the specific adsorption of virions. It was found that 7 to 10% of the input virion population could be adsorbed specifically at 2 to 3 h, whereas only 1 to 2% of the input capsid population was adsorbed specifically. These experiments showed that the majority of the cell-associated virions were bound non-specifically and that this type of adsorption reflected the manner by which capsids primarily associated with the cells. The low level of specifically adsorbed capsids suggested that these particles might lack a receptor(s) which

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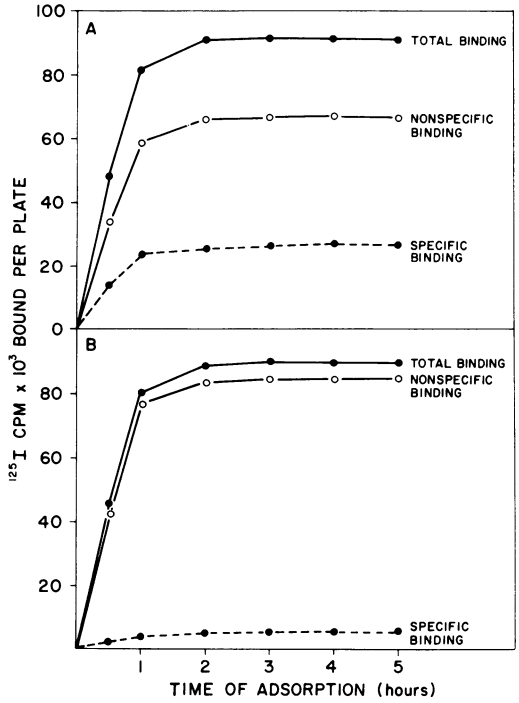

FIG. 1. *Time course of ^{125}I-labeled polyoma virion and polyoma capsid adsorption to MKC. (A) Confluent monolayers containing 3 × 10^6 MKC (4°C) were washed twice with HEPES (N-2-hydroxyethylpiperazene-N'-2-ethanesulfonic acid) binding buffer (0.5 M HEPES [Calbiochem], 8 mM glucose, 1.6 mM bovine serum albumin, 0.15 M NaCl, 4.9 mM KCl, and 0.6 mM $MgSO_4$, pH 7.3). Cells were then reacted with 0.1 ml of HEPES binding buffer containing 3 × 10^5 cpm (50 to 60 ng of protein) of labeled polyoma virions with (○) or without (●) a 1,000-fold excess of unlabeled virions per 35-mm dish. Specific adsorption (●-----●) was taken as the difference between total (●) and nonspecific (○) adsorption at the times indicated after virion addition. To determine the cell-associated counts at a given time, we washed the monolayers five times with 5 ml of cold phosphate-buffered saline, pH 7.3. The cells were dissolved in 1% sodium dodecyl sulfate (percent [weight/volume] in phosphate-buffered saline), and radioactivity was determined by liquid scintillation spectroscopy. (B) Duplicate monolayers of MKC were incubated with 3 × 10^5 cpm (50 to 60 ng of protein) of labeled polyoma capsids with (○) or without (●) a 1,000-fold excess of unlabeled polyoma capsids per culture. Specific adsorption (●-----●) was determined as described above.*

was responsible for the specific adsorption observed with virions.

In most adsorption studies, the hemagglutination model has been used to examine the interaction of polyoma virus with its receptors on host cells (5, 13). Thus, we examined polyoma adsorption to guinea pig erythrocytes (GPRBC) in a manner analogous to the MKC adsorption studies to determine any differences between the binding of the virus to erythrocytes and to cultured MKC. The adsorption of labeled polyoma to GPRBC reached a maximum in 20 to 30 min at 4°C (Fig. 2). Agglutination could be observed in the tubes after about 40 min. Treating the cells with 5 U of neuraminidase decreased virion adsorption by 50%. At concentrations of neuraminidase above 20 U, no virion adsorption above background levels was observed. When the experiments were repeated with capsids, identical results were obtained (data not shown).

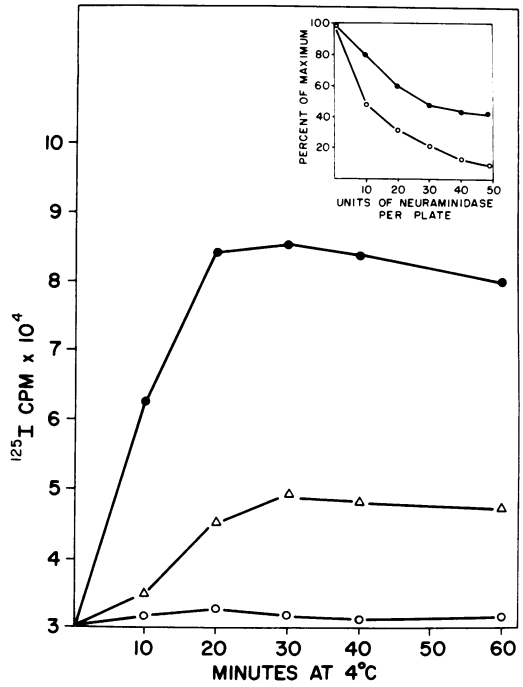

FIG. 2. *Time course of ^{125}I-labeled polyoma adsorption to neuraminidase-treated GPRBC. The washed GPRBC (0.75%) were incubated with 5, 20, 30, 50, 100, or 200 U of neuraminidase (Calbiochem) for 2 h at 37°C. The cells were washed and cooled to 4°C, and 2 × 10^5 cpm of polyoma virus was added per tube. At the times indicated, samples of each tube were withdrawn, and the number of cell-associated counts was determined. Symbols: ●, control, untreated cells; △, 5 U of neuraminidase; ○, 20 to 200 U of neuraminidase. (Insert) Adsorption of ^{125}I-labeled polyoma virions and capsids to neuraminidase-treated MKC. Confluent monolayers were treated with various concentrations of neuraminidase for 2 h at 37°C before the addition of ^{125}I-labeled polyoma virions (●) or capsids (○). The labeled virion and capsid preparations were allowed to adsorb for 2 h at 4°C. The monolayers were then washed, and the number of cell-associated counts was determined.*

During these experiments, the level of nonspecific adsorption of polyoma virions and capsids did not differ significantly from that obtained for total adsorption (data not shown). These results indicated that no specific adsorption of polyoma to GPRBC occurred at 4°C, thereby pointing out a fundamental difference between the adsorption of the virus to the surface of erythrocytes and its adsorption to MKC. Mouse cells have been treated with neuraminidase to free polyoma virions and capsids from cellular debris during purification procedures (17). To determine how pretreating MKC with neuraminidase would affect the adsorption of polyoma, we incubated MKC monolayers with various concentrations of the enzyme. As shown in Fig. 2 (insert), adsorption of capsids was more sensitive to neuraminidase treatment of the cells than was adsorption of virions. At the highest concentration tested (50 U of neuraminidase per plate), virion adsorption was inhibited by 50 to 55%, compared with virus adsorption to untreated MKC. The same concentration of neuraminidase inhibited capsid adsorption by as much as 95%. These results suggested that the level of nonspecific adsorption, which is characteristic of capsid binding, depended on neuraminidase-sensitive (presumably sialic acid) residues on the cell surface. In similar experiments, at concentrations of virions that produced a 27 to 30% infection in untreated MKC when assessed by positive nuclear immunofluorescence at 48 h postinfection, neuraminidase-treated cells (50 to 100 U) yielded a 10 to 16% infection level.

Since the experiments described above (Fig. 1 and 2) suggested that the adsorption of capsids to MKC might be different from the adsorption of virions, experiments were designed to determine whether polyoma adsorption to MKC could be inhibited by capsids or virions. The binding of ^{125}I-labeled polyoma was competitively inhibited by unlabeled virions, but not by unlabeled capsids (Fig. 3). When the unlabeled virions or capsids were allowed to adsorb to the MKC before radioactive polyoma was added, binding of the labeled virus could be 97% inhibited by unlabeled virions and 40% inhibited by unlabeled capsids. These adsorption experiments indicated that, indeed, capsids could not efficiently block virion binding and that the capsids might be deficient in receptors responsible for the specific adsorption of virions to cellular receptors. In similar experiments conducted with GPRBC, capsids were found to completely block virion adsorption (data not shown). Such results are consistent with the concept that virions and capsids share the same surface receptors on GPRBC.

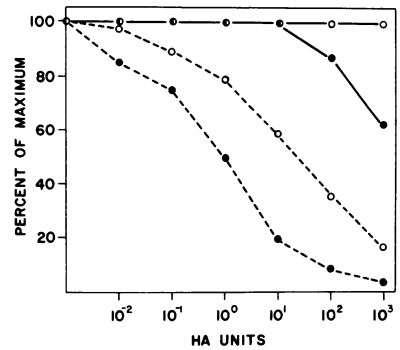

FIG. 3. *Adsorption inhibition and competitive inhibition of ^{125}I-labeled polyoma virions to MKC by unlabeled virions and capsids. Adsorption inhibition assays were performed as described in the legend to Fig. 1 except that before the 2-h adsorption period for 1 hemagglutinating (HA) unit of ^{125}I-labeled virions, the MKC monolayers were incubated with various concentrations of unlabeled virions (●-----●) or capsids (●——●) for 2 h and washed five times with cold phosphate-buffered saline to remove the unbound, unlabeled virions and capsids. Competitive adsorption assays were performed by adding 1 HA unit of ^{125}I-labeled virions to various concentrations of unlabeled virions (○-----○) or capsids (○——○) and allowing them to mix for 15 min before the addition of the mixture to the MKC monolayers. Adsorption was allowed to proceed for 2 h; the cells were washed with phosphate-buffered saline, and the number of cell-associated counts was determined.*

To ensure that the adsorption inhibition experiments were reflecting the biological situation, experiments were designed to assay the biological nature of the specific binding experiments and to determine whether such binding related to the ability of polyoma to infect cells. During these experiments, instead of determining the amount of virus adsorbed to the cells after the adsorption period, we added medium to the cells and allowed the infection to proceed for 48 h. The success of polyoma infection was then determined by an immunofluorescent assay, which was quantitated by counting multiple fields of cells and determining the number of positive fluorescent nuclei. Because the presence of virions was required to conduct these experiments and because such virions would also result in the production of fluorescent nuclei, we tried to eliminate the infective ability of the virions by exposing them to UV light. When polyoma virions (1 ml) were placed 12 in. (ca. 30.48 cm) from the UV source as described by Defendi et al. (6), 4 min of irradiation resulted in producing

a population of polyoma virions which would not yield positive nuclear fluorescence, but would nevertheless inhibit non-irradiated polyoma virions from infecting the cells. These UV-irradiated virions were adjusted to the same concentration as purified capsids, and various dilutions of the irradiated virions or capsids were allowed to adsorb to MKC at 4°C for 1 h. The monolayers were washed, and untreated polyoma virions were then added and allowed to adsorb for 1 h at 4°C. The cells were again washed, medium was added, and infection was allowed to proceed for 48 h at 37°C. The indirect fluorescent-antibody assay was then performed, and the number of positive fluorescent nuclei was determined and used as an index of successful infection. Figure 4 shows that the addition of excess capsids (1,000-fold excess over the untreated polyoma) inhibited about 30% the number of positive fluorescent nuclei as observed in control polyoma-infected cultures. A 10-fold dilution of capsids resulted in only a 5% reduction in the number of infected cells. Further capsid dilutions were incapable of preventing polyoma infection of the cells even though the added capsids were more concentrated than the virions. Polyoma infection, however, could be efficiently inhibited by the virions UV irradiated for 4 min. At the point (10^1 hemagglutinating units) at which capsids could not inhibit polyoma infection, the same concentration of UV-irradiated virions inhibited greater than 90% the extent of polyoma infectivity. These experiments, which demonstrate that capsids do not block specific virion receptors on MKC membranes which are responsible for successful polyoma infection, substantiate the adsorption inhibition and competitive-binding data shown in Fig. 3.

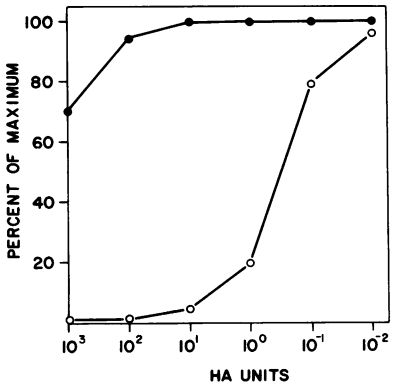

FIG. 4. *Effect of capsid and UV-irradiated virion adsorption on polyoma virus infection of MKC. Capsids (●) and virions UV irradiated for 4 min (○) were adjusted to the same concentration, and dilutions of each were adsorbed to MKC for 1 h at 4°C. The cultures were washed, and 1 hemagglutinating (HA) unit of non-irradiated polyoma virus was added and allowed to adsorb for 1 h. The dishes were washed, and infection was allowed to proceed for 48 h. The number of infected cells was then determined by indirect immunofluorescence as described previously (12).*

The results presented here suggest that polyoma virus is bound to the surface of mouse cells in at least two different manners. The first and most abundant manner is the nonspecific (noninfective) attachment of virus particles to the numerous sialic acid residues on the cell surface. The nonspecific type of adsorption reflects primarily the binding properties associated with capsids and may relate directly to the ability of capsids and virions to adsorb to and agglutinate certain types of erythrocytes. This model predicts that capsids might lack a protein responsible for the specific binding of the virus to the cell. Since sodium dodecyl sulfate-polyacrylamide gels of the pure capsids revealed a total lack of histones, it could be hypothesized that this group of basic proteins might play an important role in the specific binding of the virus to its cellular receptor. Similar concepts have been reported previously (9, 10, 12). However, it is possible that empty capsids lack a protein modification(s) which is present on virions and which acts as a receptor. Such a protein could be a variety of subspecies of major capsid proteins that cannot be resolved by conventional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7, 14, 15).

The second type of adsorption by which polyoma can be bound to cells is the specific attachment of the virus to its cell receptor. This type of binding represents about 10% of the input virus. Specific (infective) adsorption of polyoma to the cell surface is independent of the ability of the virions to agglutinate GPRBC. Additional evidence for this concept has been obtained in our laboratory by using a polyoma mutant (Py 235) which has lost its ability to agglutinate GPRBC (4°C, pH 7.3) (2). Under these same conditions, Py 235 virions were found to specifically adsorb to MKC at levels similar to that of wild-type polyoma, whereas the levels of nonspecific adsorption were reduced by 50% (unpublished data). The specific binding of polyoma to its cell receptor(s) could thus result in the formation of specific monopinocytotic vesicles which surround the virus and transport it to the nuclear membrane. Because both polyoma and simian virus 40 can be found in the perinuclear region as soon as 10 min after adsorption (1, 10), such a nuclear transport system must be viewed as highly efficient. Furthermore, the

amount of virus found associated with the nucleus soon after infection parallels closely the level of specific binding observed. Such results support the concept of an efficient cell surface-to-nucleus transport of virions that have attached to their specific cell receptors which themselves function as the nuclear transport factors.

The information obtained from the adsorption experiments presented here provides the basis for a more detailed understanding of the biophysical and biochemical nature of the polyoma virus-associated receptor protein(s) and the cell-associated receptor components which are responsible for successful polyoma infection. Such studies are currently in progress.

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