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Anti-Idiotypic Antibodies to a Polyomavirus Monoclonal Antibody Recognize Cell Surface Components of Mouse Kidney Cells and Prevent Polyomavirus Infection†

SUSAN J. MARRIOTT, DANIEL J. ROEDER, AND RICHARD A. CONSIGLI*

Section of Virology and Oncology, Division of Biology, Kansas State University, Manhattan, Kansas 66506

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Anti-idiotypic antibodies have been successfully used to identify and isolate the receptor for several cell ligands. To prepare an immunologic probe for identification of the polyomavirus receptor on mouse kidney cells, polyclonal antisera against antipolyomavirus antibodies were prepared in rabbits. Fab fragments of the previously characterized monoclonal antibody E7, which neutralizes polyomavirus infection, were used for immunization (S. J. Marriott and R. A. Consigli, J. Virol. 56:365–372, 1985). Sera containing the greatest anti-idiotype activity were identified by enzyme-linked immunosorbent assay (ELISA) and purified by a series of affinity columns. The anti-idiotypic antibodies recognized the E7 idiotope in an ELISA, and anti-idiotype binding could be inhibited by preincubation of E7 monoclonal antibody with polyomavirus virions. When mixed with anti-idiotype immunoglobulin G (IgG), E7 was no longer capable of binding or immunoprecipitating polyomavirus virions or neutralizing polyomavirus infection. Direct immunofluorescence showed anti-idiotype IgG reactivity with a cell surface component of mouse kidney cells. Anti-idiotype $F(ab')_2$ effectively competed with polyomavirus for binding to mouse kidney cells and displayed binding kinetics similar to those of polyomavirus. Virus infection of mouse kidney cells was blocked in a dose-dependent manner following treatment of the cells with anti-idiotype IgG. The anti-idiotype identified several proteins (95, 50, and 24 to 31 kilodaltons) in an immunoblot of mouse kidney cell membrane proteins but reacted predominantly with a single 50-kilodalton protein in a radioimmunoassay. The anti-idiotype failed to react with virus proteins in three assays, including ELISA, immunoprecipitation, and immunoblotting. The implications of this work for future identification and characterization of the polyomavirus receptor on mouse kidney cells are discussed.

Anti-idiotypic antibodies (anti-Id) appear to play an important role in the regulation of the immune response through a succession of auto-anti-idiotypic responses (12, 13, 20) and have been useful as probes of protein function (16, 24). As first described by Sege and Peterson (35) for insulin- and retinol-binding protein receptors, anti-Id have been successfully used to characterize other hormone receptors, including adrenergic receptors (18, 34) and those for thyrotropin (19), acetylcholine (9, 40), and glucose transport protein (13). Several of these anti-Id appear to mimic their respective agonists both kinetically and pharmacologically and therefore have been useful in identification of ligand receptors. Anti-Id also promise to play an important role in identification of viral receptors, as has previously been demonstrated in the reovirus system (10, 22, 31). Production of anti-Id has proven especially useful when purification of the receptor is not feasible by conventional methods, such as ligand affinity chromatography.

Previous studies have suggested that polyomavirus binds to the surface of mouse kidney cells (MKC) via at least two distinct mechanisms: one which results in productive virus infection and utilizes a specific receptor and one facilitated by nonspecific adsorption resulting in lysosomal degradation of virions and capsids (4, 25). The virus interacts with the cell through different isoelectric species of the major capsid protein VP1. VP1 species E functions as the specific virus attachment protein, whereas VP1 species D and F are responsible for nonspecific binding of virions and capsids to MKC and also function as the viral hemagglutinin (1, 3).

Previous work in our laboratory has resulted in production of monoclonal antibodies (MAbs) directed against the major structural protein, VP1, of polyomavirus (26). Two of these antibodies (E7 and G9) recognize VP1 attachment species D, E, and F and have the ability both to neutralize polyomavirus infection of MKC and to inhibit hemagglutination of guinea pig erythrocytes. E7 was chosen as the immunogen for preparation of anti-Id because of its enhanced ability to neutralize polyomavirus infection. This report describes production and characterization of antibodies directed against the idiotope of MAb E7. These anti-Id bind to the surface of MKC and subsequently inhibit polyomavirus binding to and infection of MKC.

MATERIALS AND METHODS

Cell and virus propagation. Primary cultures of MKC were prepared as previously described (36) and maintained in Dulbecco modified Eagle medium containing 10% fetal calf serum. Wild-type small plaque polyomavirus was used to infect cells at a multiplicity of infection of 10. Infected cultures were maintained in serum-free Dulbecco modified Eagle medium (29). Virions were purified from infected cell lysate as described previously (5, 6, 8, 28). Purified virions and capsids were stored in the presence of the protease inhibitors aprotinin, leupeptin, phenylmethylsulfonyl fluoride, *N-p*-tosyl-L-lysine chloromethyl ketone, and L-1-tosylamide-2-phenylmethyl chloromethyl ketone (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 10 μg/ml each (26).

* Corresponding author.

† Contribution no. 86-512-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506.

Radioactive labeling procedures. All labeling reactions were performed in vitro with ^{125}I by the chloramine-T method (15). The reactions were quenched by addition of unlabeled tyrosine to a final concentration of 0.1 mg/ml. Unbound ^{125}I was removed from all chloramine-T-labeled substrates by centrifugation of the labeling mixture through a 2-ml Sephadex G25 column at 2,000 rpm for 10 min in an International PR-2 centrifuge and overnight dialysis against 0.01 M Tris hydrochloride, pH 7.4.

Antigen preparation and antiserum production. Fab fragments of neutralizing MAb E7 were prepared as described previously (7, 26). Freund adjuvant (Colorado Serum Co., Denver, Colo.) was added to the Fab fragments, and the mixture was emulsified in a Sorvall Omnimixer by using the microattachment. Antisera were produced in New Zealand White rabbits by subcutaneous injection of the antigen at multiple sites along the back. The rabbits were given injections at biweekly intervals. The initial injection was with complete Freund adjuvant, and all subsequent injections were with incomplete Freund adjuvant. The animals received 100 μg of purified E7 Fab during each injection. Anti-E7 rabbit serum was collected periodically throughout the immunization schedule.

ELISA. To determine which sera possessed the greatest anti-Id activity, two types of enzyme-linked immunosorbent assay (ELISA) were used: (i) an indirect ELISA to detect total anti-E7 binding to E7 immunoglobulin G (IgG) and (ii) a modified indirect ELISA to detect anti-E7 serum binding to non-idiotypic epitopes on E7 IgG. For the indirect ELISA, MAb E7 IgG or normal mouse IgG was coated on microtiter plates (Costar, Cambridge, Mass.) at a concentration of 5 μg/ml as described previously (26). Anti-E7 serum was diluted 1:50, and 50 μl was added to each well for 4 h at 37°C. The plate was washed, and alkaline phosphatase-labeled goat anti-rabbit IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) was added to each well followed by *para*-nitrophenylphosphate substrate as described previously (26). A_{405} was measured on a Bio-Tek model EL307 enzyme immunoassay reader. All absorbances were standardized to preimmune serum which was designated zero absorbance.

The modified ELISA was performed by coating microtiter wells with E7 IgG or normal rabbit IgG as described above and incubating them with 0.5 μg of purified polyomavirus virions per well for 1 h at 37°C. The plates were then incubated with anti-E7 serum, followed by alkaline phosphatase-labeled goat anti-rabbit IgG and substrate as described above. The amount of specific anti-Id activity was determined by subtracting nonspecific IgG reactivity measured on the modified ELISA from total IgG binding measured on the indirect ELISA.

Antibody purification. The immunological data presented in this report are based on studies performed with a pool of serum samples collected 2 weeks after the initial immunization which represented maximal E7 idiotope-specific reactivity by ELISA. Although secondary and tertiary anti-E7 responses could be detected by allowing the animal to rest for 1 month before initiation of a new immunization schedule, these responses became progressively less intense. IgG was purified from the pooled sera by Na_2SO_4 precipitation (23) and applied to an Affi-Gel blue column (Bio-Rad Laboratories, Richmond, Calif.) previously equilibrated with 20 mM Tris hydrochloride–28 mM NaCl, pH 8.0. Rabbit IgG was eluted from the column with the same buffer, and its purity was demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fab and $F(ab')_2$ fragments were produced as described previously (26).

To remove IgGs reactive with mouse isotypes, the anti-E7 IgG was passed over a column of normal BALB/c mouse IgG coupled to Affi-Gel 10 (Bio-Rad) (column A). The column provided absorption sites for anti-isotypic IgGs, while other IgGs, including anti-Id, passed through the column with 10 mM Tris hydrochloride, pH 7.4.

The IgGs eluted from column A were placed immediately on an E7 affinity column (column B) produced by coupling E7 IgG with Affi-Gel 10. Anti-Id IgGs were eluted with 0.1 M glycine hydrochloride, pH 2.5, and immediately neutralized with 1 M $NaCO_3$, pH 8.0. All rabbit antibodies are referred to as anti-E7 before application to column A and as anti-Id after passage through column B. The purity of antibodies eluted from these columns was demonstrated by SDS-PAGE.

Immunoprecipitation. ^{125}I-labeled E7 Fab, or polyomavirus virions, were incubated with purified anti-Id IgG or normal rabbit IgG (10 μg) overnight at 4°C in RIPA buffer (20 mM morpholinepropanesulfonic acid [MOPS hydrochloride; pH 7.0], 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1% deoxycholate, 0.1% sodium dodecyl sulfate, and the five protease inhibitors indicated previously) after preadsorption with normal rabbit serum (41). A 100-μl portion of a 10% suspension of *Staphylococcus aureus* (Calbiochem Pansorbin) was added and incubated for 30 min at 4°C. The precipitate was collected by centrifugation and washed four times in TNN buffer (50 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 0.05% Nonidet P-40, 1% aprotinin) (26).

Binding of anti-Id IgG to the MKC surface. A direct cell surface immunofluorescence assay was used to demonstrate anti-idiotype reactivity with the cell surface. Primary MKC were grown to confluency on glass cover slips. The unfixed MKC monolayers were placed on ice 30 min before staining, washed three times with cold phosphate-buffered saline (PBS), and reacted with normal rabbit IgG to block nonspecific reactivity. The cover slips were washed three times in cold PBS and 20 μl of fluorescein isothiocyanate-labeled rabbit anti-Id IgG or normal rabbit IgG, or rabbit anti-mouse IgG was added for 30 min at 4°C. The labeled IgG was removed, and the cover slips were washed three times in cold PBS. Cell surface fluorescence was observed immediately with a Leitz Ortholux UV microscope.

Competition of anti-Id $F(ab')_2$ and polyomavirus virions for binding to MKC. Confluent monolayers containing 10^6 MKC were preincubated at 4°C for 1 h and washed twice with cold HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) binding buffer (0.5 M HEPES [Calbiochem-Behring, La Jolla, Calif.], 8 mM glucose, 1.6 mM bovine serum albumin, 0.15 M NaCl, 4.9 mM KCl, 0.6 mM $MgSO_4$, pH 7.3). Cells were reacted for 1 h at 4°C with 0.1 ml of HEPES binding buffer containing 10^5 cpm (30 ng of protein) of ^{125}I-labeled polyomavirus virions alone or in the presence of a 1,000-fold excess of unlabeled virions, anti-Id $F(ab')_2$, or rabbit anti-mouse $F(ab')_2$ as determined by the protein content. To determine the cell-associated counts at a given time, the monolayers were washed five times with cold PBS, pH 7.3. The cells were solubilized in 0.5 N NaOH, and radioactivity was determined with an LKB 1275 Minigamma Counter.

Anti-Id IgG inhibition of MKC infection by polyomavirus. MKC were grown to confluency on glass cover slips. The MKC monolayers were treated with anti-Id IgG, normal rabbit IgG, or rabbit anti-mouse IgG dilutions ranging from 0 to 10 μg per plate for 1 h at 4°C. Antibody dilutions were

prepared in serum-free Dulbecco modified Eagle medium. Following this treatment, virus (1,000 hemagglutination units) was added to the IgG-treated cells and the incubation was continued for an additional hour. The infection solution was removed, plates were washed three times with PBS, and serum-free Dulbecco modified Eagle medium was added. Infection was allowed to proceed for 40 h at 37°C, at which time the cover slips were fixed with ethanol-glacial acetic acid (95:5) followed by acetone-methanol (2:1) and stained for immunofluorescence (29). The degree of polyomavirus infection was determined by calculating the percentage of fluorescent nuclei in the various cultures.

Membrane preparation. Membranes were prepared by the method of Thom et al. (39). Briefly, confluent MKC monolayers (10- by 100-mm^2 dishes) were washed with cold PBS and isotonic borate (0.05 M boric acid, 0.15 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.2). The cells were then scraped in isotonic borate and centrifuged for 10 min at 1,500 × *g*. The pellet was suspended in 2 ml of isotonic borate and added dropwise to 100 ml of cold, stirring hypotonic borate (0.02 M boric acid 0.2 mM EDTA, pH 10.2). After stirring for 10 min, 8 ml of 0.5 M boric acid, pH 10.2, was added, and stirring continued for another 5 min. The solution was filtered through cheesecloth and centrifuged for 10 min at 1,500 × *g* to pellet nuclei and unbroken cells. The supernatant was centrifuged at 15,000 × *g* for 30 min. The resulting membrane-rich pellet was suspended, layered on top of a 35% sucrose cushion made in PBS, and centrifuged for 45 min at 18,000 × *g*. Plasma membranes were collected from the sucrose-PBS interface, diluted 1:1 with PBS, and centrifuged for 1 h at 33,000 rpm in an SW50.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Pellets were suspended in 20 mM HEPES (pH 7.4)–10% glycerol, divided into aliquots, and stored at −70°C.

Immunoblot analyses. MKC membrane proteins separated by 15% SDS-PAGE were transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described by Bittner et al. (2). The residual binding capacity of the nitrocellulose was blocked by incubation overnight with TDN buffer (10 mM Tris hydrochloride [pH 7.5], 0.05 M NaCl, 2 mM disodium EDTA–$2H_2O$) containing 4% bovine serum albumin and 10 μg of normal rabbit IgG per ml. The proteins were probed directly with ^{125}I-labeled anti-Id IgG, normal rabbit IgG, or rabbit anti-mouse IgG in TDN buffer containing 4% bovine serum albumin and 10 μg of normal rabbit IgG per ml. After incubation for 12 to 18 h, the antibody was removed and the nitrocellulose membrane was washed extensively in TDN containing 4% bovine serum albumin and 10 μg of normal rabbit IgG per ml. The blots were exposed to Kodak XAR-5 X-ray film with a Cronex Lightning-Plus intensifier screen.

Receptor radioimmunoassay. Polyomavirus virions, anti-Id IgG, or rabbit anti-mouse IgG were adsorbed to microtiter plates at a concentration of 5 μg/ml as described previously (26). MKC were extracted with octyl-β,D-glucopyranoside as described previously (27). The microtiter plates were washed, and ^{125}I-labeled octyl-β,D-glucopyranoside extract or MKC membranes (100,000 cpm) were added to separate wells. After 4 h of incubation at 37°C, the plates were washed and SDS-PAGE sample buffer was added to solubilize bound proteins (38). The solubilized proteins were analyzed by 15% SDS-PAGE.

RESULTS

This report involves production and characterization of antibodies against a mouse monoclonal antipolyomavirus antibody (E7) and demonstration that these antibodies react with the idiotope of E7. In addition, these anti-Id recognize a cell surface component of MKC and inhibit polyomavirus infection of MKC. Polyclonal anti-Id were produced by immunizing rabbits with Fab fragments of polyomavirus-neutralizing MAb E7. Reactivity of the purified anti-Id IgG with the immunogen MAb E7 was demonstrated by ELISA binding to E7 IgG. Specific binding of anti-Id IgG to the E7 antigen increased following column purification and could be inhibited by preincubation of polyomavirus virions with E7 (data not shown). To further demonstrate that anti-E7 has specificity for the idiotope of E7, several experiments (Table 1) were performed. Binding of ^{125}I-labeled polyomavirus virions to E7 in the presence of anti-Id was only 4% of that observed in the absence of anti-Id. When E7 was mixed with anti-Id, its ability of either to immunoprecipitate or to neutralize polyomavirus infection of MKC was reduced. Taken together, these results demonstrate specificity of the anti-E7 antibody for the idiotope of E7.

TABLE 1. Reactivity of anti-Id IgG with the E7 immunogen

Expt	% of total cpm or infected cells[a]	
	E7	E7 + anti-Id
A	100	4
B	100	7
C	18	100

[a] In experiment A, ^{125}I-labeled polyomavirus virions (10^6 cpm) were incubated in wells which had been coated with E7 IgG (5 μg/ml) alone or E7 followed by anti-Id IgG (50 μg/ml). The percentage of the total counts per minute bound in each well is reported. In experiment B, ^{125}I-labeled polyomavirus virions (10^6 cpm) were mixed with either 10 μg of E7 IgG or a mixture of E7 and 100 μg of anti-Id IgG. The percentage of the total counts per minute immunoprecipitated from each solution is reported. In experiment C, polyomavirus virions (1,000 hemagglutination units) were mixed with either 10 μg of E7 IgG or a mixture of E7 and 100 μg of anti-Id IgG, and the solutions were used to infect MKC. The percentage of infected cells is reported as calculated from the total number of fluorescing nuclei.

To determine whether the anti-Id would be useful for identification of MKC components involved in attachment of polyomavirus, three criteria were established. (i) The anti-Id must bind to a surface component of MKC, (ii) the anti-Id must compete with polyomavirus virions for attachment to the MKC surface, and (iii) pretreatment of MKC surfaces with anti-Id must block subsequent polyomavirus infection. Fluorescent antibody staining of live MKC with fluorescein isothiocyanate-labeled anti-Id IgG demonstrated that the anti-Id was capable of binding to a cell surface component(s) of MKC (Fig. 1A). The punctate fluorescence observed in MKC stained with anti-Id was absent in cells stained with rabbit anti-mouse IgG (Fig. 1B) or normal rabbit IgG (data not shown). Anti-Id F(ab')$_2$ and Fab fragments produced staining patterns similar to that of IgG (data not shown).

A competitive binding assay was performed to investigate the ability of anti-Id F(ab')$_2$ to compete with polyomavirus virions for binding to the MKC surface. Previous work in our laboratory has demonstrated that maximum competition for labeled polyomavirus binding is achieved in the presence of a 1,000-fold excess of polyomavirus (4). Even with a 1,000-fold polyomavirus excess, 80% of the total binding of labeled virus is observed and is thought to result from nonspecific association with the cell. The binding curve observed for ^{125}I-labeled polyomavirus virions, alone or in the presence of either a 1,000-fold excess of unlabeled polyomavirus virions or a 1,000-fold excess of unlabeled anti-Id F(ab'$_2$) as determined by protein content, is shown in Fig. 2. The ability of

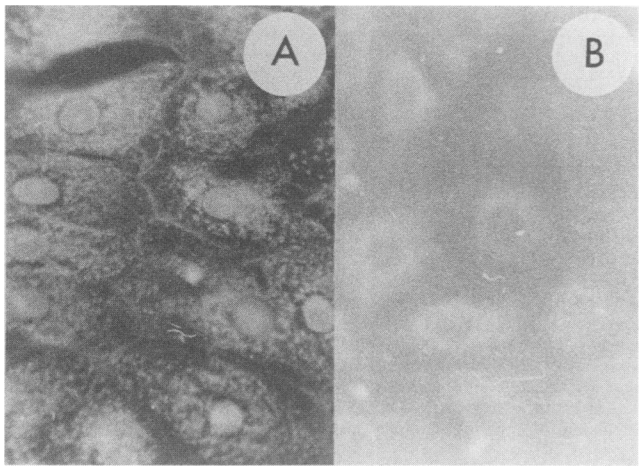

FIG. 1. Anti-Id IgG reactivity with MKC surfaces. Fluorescein isothiocyanate-labeled anti-Id IgG (A) or rabbit anti-mouse IgG (B) was used to stain the surface of MKC at 4°C.

anti-Id to compete for binding of ^{125}I-labeled virions is similar to that observed for unlabeled virions and indicates that anti-Id and polyomavirus bind with similar kinetics to the MKC surface.

The third criterion required that the anti-Id react with an MKC component which is required for proper polyomavirus attachment to and subsequent infection of MKC. Following treatment of MKC monolayers with dilutions of anti-Id IgG, MKC were infected. At 40 h postinfection, the cells were fixed and a fluorescent immunoassay was performed to detect the extent of viral infection (Fig. 3). The amount of infection observed in cells treated with 10 μg of anti-Id IgG before infection was greatly reduced from that observed in cells treated with rabbit anti-mouse IgG before infection and demonstrates the ability of anti-Id to recognize a component of the MKC surface which is required for initial attachment of polyomavirus virions. This effect was dose dependent, as demonstrated by dilution of the anti-Id IgG resulting in a corresponding increase in polyomavirus infection. Anti-Id F(ab')$_2$ treatment of MKC before infection resulted in a similar reduction in polyomavirus infection; however, pretreatment of cells with Fab fragments resulted in no observable decrease in infection, presumably because of the single reactive site of the Fab fragment (data not shown). Figure 3 also illustrates that a 1,000-fold increase in the specific activity of column-purified anti-Id IgG over that of anti-E7 IgG was determined by a 50% reduction in infected cells.

Since we had established that anti-Id IgG could block virus infection, an immunoblot was used to identify the particular MKC membrane components recognized by anti-Id IgG. Recognition of specific MKC membrane proteins implies that these proteins are involved in the initial virus-host interaction. ^{125}I-labeled anti-Id IgG recognized six (Fig. 4, lane 4) of the numerous proteins observed upon Coomassie blue staining (Fig. 4, lane 3) of the purified membranes. The reactive proteins had molecular masses of approximately 24 to 31, 50, and 95 kilodaltons. Proteins of identical electrophoretic mobilities were recognized by ^{125}I-labeled anti-Id F(ab')$_2$ and Fab (data not shown). Anti-Id IgG did not recognize polyomavirus proteins (Fig. 4, lane 2). Membrane proteins showed no reactivity with rabbit anti-mouse IgG (Fig. 4, lane 5) or normal rabbit IgG (data not shown). The results of the immunoblot demonstrated that anti-Id IgG recognized several specific MKC membrane proteins and was unreactive with polyomavirus proteins.

To investigate the possibility that some of the proteins showing reactivity on an immunoblot resulted from the denaturing conditions of SDS-PAGE, we developed an assay in which the anti-Id or polyomavirus virions could react with ^{125}I-labeled MKC proteins in their native conformation. In this receptor radioimmunoassay, anti-Id and polyomavirus virions reacted predominately with a 50-kilodalton protein in both isolated ^{125}I-labeled MKC membranes and ^{125}I-labeled

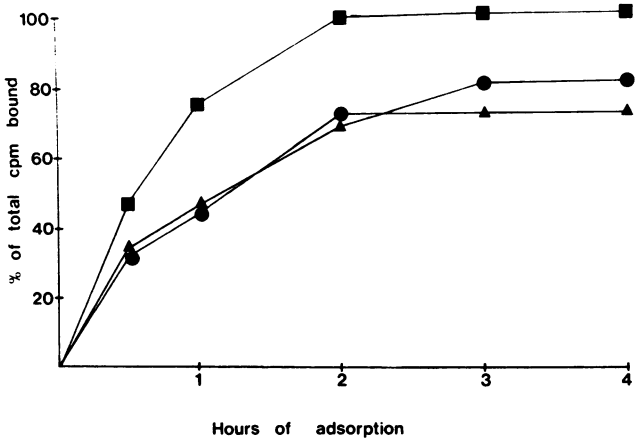

FIG. 2. Competitive inhibition of ^{125}I-labeled polyomavirus virion adsorption to MKC by unlabeled virions and anti-Id F(ab')$_2$. Polyomavirus virions were allowed to adsorb to MKC monolayers alone (■) or in the presence of a 1,000-fold excess of unlabeled virions (▲) or anti-Id F(ab')$_2$ (●). At various times after addition, cells were washed and solubilized, and cell-associated counts were determined. The counts per minute bound were calculated as a percentage of the highest number of cell-associated radioactive counts.

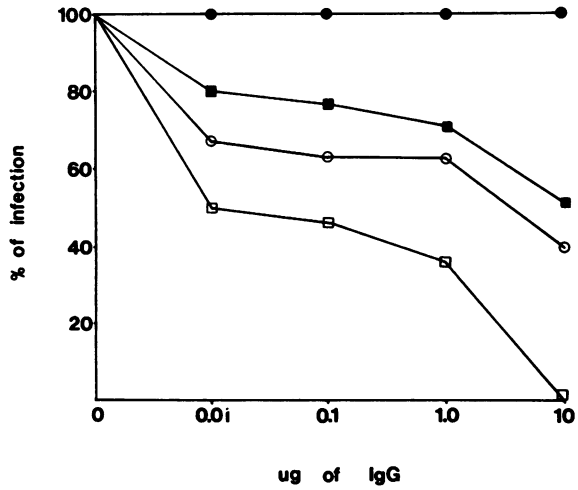

FIG. 3. Anti-Id IgG inhibition of polyomavirus infection. Various protein concentrations of rabbit anti-mouse IgG (●), unpurified anti-E7 IgG (■), anti-E7 IgG after passage through column A (BALB/c IgG) (○), or purified anti-Id IgG after passage through column A and column B (E7 IgG) (□) were applied to MKC monolayers for 1 h at 4°C. The cells were infected as described in Materials and Methods and, at 40 h postinfection, stained by indirect immunofluorescence to determine the extent of viral infection. The number of fluorescent nuclei observed in samples treated with rabbit anti-mouse IgG at each concentration was designated 100%.

octyl-β-D-glucopyranoside-extracted MKC (Fig. 5). Anti-Id and polyomavirus virions also reacted with a 23-kilodalton protein in the MKC membrane preparation. No membrane proteins or octyl-β-D-glucopyranoside-extracted proteins were reactive with rabbit anti-mouse IgG immobilized on microtiter plates (data not shown).

DISCUSSION

Development of a method to purify polyclonal rabbit anti-Id was essential for studies to identify the MKC receptor for polyomavirus. During immunization we noted a cyclic appearance of anti-Id as previously described by Strosberg et al. (37) with anti-Id to β-adrenergic receptors. They described the appearance of a second set of antibodies, anti-anti-Id, which were generated against anti-Id and functioned to clear anti-Id from circulation. Anti-anti-Id bind to anti-Id and may bind to the ligand for the receptor (30). This phenomenon helps to explain the transient nature of the anti-Id response and emphasizes the importance of removing polyomavirus-reactive anti-anti-Id from immune serum. We demonstrated the purity of our anti-Id, including the absence of anti-anti-Id, by the absence of reactivity with polyomavirus virions in ELISAs as well as by immunoprecipitation and immunoblotting.

Ability to produce an anti-Id with the characteristics of the one discussed requires that the actual attachment site of polyomavirus be exposed on the virus surface and that this site provide the epitope recognized by neutralizing antibodies. The neutralization epitope and virus attachment site of one virus system have been examined in great detail and found to be different. In the picornavirus system, Rossman et al. (32) have demonstrated that the actual attachment site for human rhinovirus 14 lies in a cleft on the virus surface which is not accessible to neutralizing antibody. Thus, an internal image or anti-Id antibody made to a neutralizing antirhinovirus antibody will not contain a cell-reactive antibody specific for the rhinovirus receptor. Our ability to generate an anti-Id capable of blocking virus infection implies that the virus attachment protein-reactive epitope is exposed on the surface of polyomavirus and is accessible to direct binding of neutralizing antibodies.

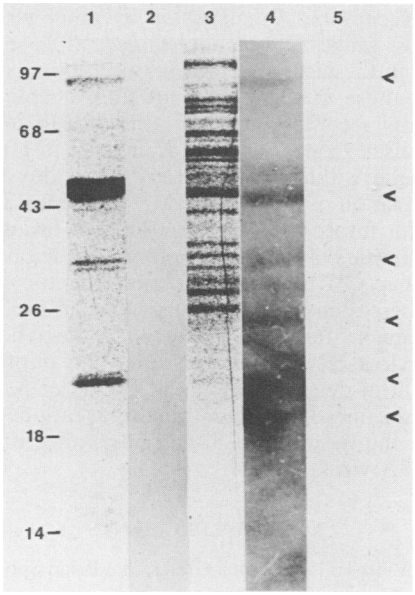

FIG. 4. Immunoreactivity of anti-Id IgG with polyomavirus virions and MKC membrane proteins. Polyomavirus virions (lanes 1 and 2) or MKC membrane proteins (lanes 3 to 5) were separated by SDS-PAGE. The separted proteins were either stained (lanes 1 and 3) with Coomassie blue or transferred to a nitrocellulose membrane and probed with either ^{125}I-labeled anti-Id IgG (lanes 2 and 4) or ^{125}I-labeled rabbit anti-mouse IgG (lane 5). The immunoreactive proteins were detected by autoradiography. The migration of molecular mass markers (in kilodaltons) is shown on the left. The arrowheads on the right indicate the reactive proteins.

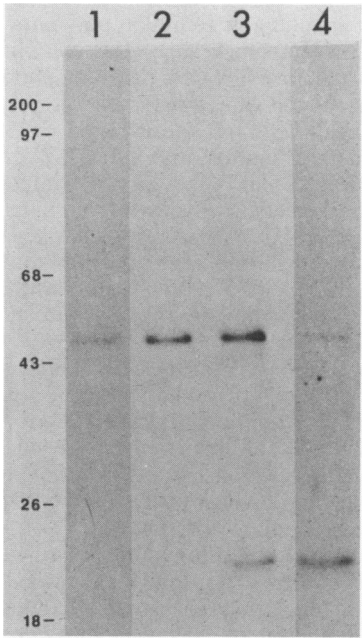

FIG. 5. Receptor radioimmunoassay. Anti-Id IgG (lanes 1 and 3) or polyomavirus virions (lanes 2 and 4) were immobilized on microtiter plates. ^{125}I-labeled octyl-β,D-glucopyranoside extract of MKC (lanes 1 and 2) or ^{125}I-labeled MKC membranes (lanes 3 and 4) were incubated with the immobilized preparations. After washing, the reactive proteins were solubilized from the microtiter plates by incubation with SDS-PAGE sample buffer. Each sample was analyzed by 15% SDS-PAGE, and the reactive proteins were identified by autoradiography. The migration of molecular mass markers (in kilodaltons) is indicated on the left.

Attachment of polyomavirus to MKC can occur via a specific cellular receptor or nonspecific adsorption (4). The immunizing MAb, E7, recognized VP1 species D, E, and F, which comprise both of the specific (species E) and the nonspecific (species D and F) virus attachment proteins. Therefore, the resulting anti-Id activity could be directed against either the specific cellular receptor, the nonspecific cellular adsorption site(s), or both. Attachment to the specific MKC receptor can be competed for by using excess virions and typically accounts for 20% of total virion binding. Anti-Id IgG was capable of inhibiting 20% of virion attachment (Fig. 2), indicating that the anti-Id may compete for virus binding in a manner very similar to that of the virus itself. Since infection appears to result solely from virions which attach to MKC via the specific receptor, it is reasonable to assume that anti-Id binding to specific receptors would inhibit virus infection. In light of the inhibition of infection studies, which demonstrated nearly complete inhibition of polyomavirus infection by pretreatment of MKC with anti-Id IgG (Fig. 3), it appears that the activity of this anti-Id is directed against the specific polyomavirus receptor on MKC.

Anti-Id Fab were unable to inhibit polyomavirus infection of MKC, whereas anti-Id $F(ab')_2$ effectively interfered with polyomavirus infection of MKC (data not shown). We have previously speculated that attachment of polyomavirus to the host cell resulting in infection via the specific route may require a two-step binding process (17). We feel that the ability of anti-Id IgG and $F(ab')_2$ to block infection probably stems from their bivalency, allowing complete interaction with the cellular receptor. Further evidence supporting this idea is the fact that both Fab and $F(ab')_2$ fragments have identical immunoblot reactivities with membrane proteins, yet Fab are unable to compete for virus infection, indicating that differential ability to block infection is not due to differential specificity but must be attributed to the bifunctional activity of the $F(ab')_2$ fragment. Thus, anti-Id IgG and $F(ab')_2$ binding may mimic virus binding to the cell because of their bifunctional activities and may elicit virus-specific host cell alterations which remain to be identified but undoubtedly play an important role in the early events of polyomavirus infection.

We have demonstrated the ability of anti-Id IgG to compete with polyomavirus for binding to MKC (Fig. 2) and subsequently to block polyomavirus infection of MKC (Fig. 3). These antibodies may, therefore, provide a convenient system for studying the intracellular events which occur upon virus binding to a host cell. Whether these anti-Id can mimic the physiological effects of polyomavirus attachment to MKC remains to be determined. If the polyomavirus receptor is found to be homologous to a hormone receptor, as is the case in at least one other virus system (10), these intracellular events may provide important information about normal cell metabolism.

Several membrane proteins were recognized on an immunoblot by using ^{125}I-labeled anti-Id IgG probe (Fig. 4, lane 4). Since these proteins were separated by SDS-PAGE, the presence of multiple reactive bands may reflect the denaturing conditions of the gel in which all amino acid sequences are available for reaction with the anti-Id IgG. Proteins with similar molecular masses were recognized when the anti-Id was used to probe octyl-β-D-glucopyranoside detergent extracts of MKC (27). The receptor immunoassay was used to investigate the reactivity of anti-Id and polyomavirus with either detergent-extracted proteins or MKC membrane proteins which had not been denatured. This assay showed predominately a single reactive 50-kilodalton protein. A 23-kilodalton protein found in MKC membranes was also reactive with both anti-Id and polyomavirus. These two proteins may form a receptor complex in intact membranes of which only the 50-kilodalton protein is extractable with the octyl-β-D-glucopyranoside detergent. This 50,000-molecular-weight protein may represent the virus-binding component of the MKC receptor for polyomavirus.

By several different techniques, our laboratory has repeatedly identified putative receptor proteins with molecular masses of 95, 50, and 24 to 31 kilodaltons (17, 27). We are currently investigating the possibility that the 24- to 31-kilodalton proteins are degradation products of the 50- or 95-kilodalton proteins. Griffith and Consigli (17) identified proteins with molecular masses of 24 to 31 kilodaltons as possible receptor proteins based on their immunoblot reactivity with an anti-120-kilodalton protein antibody. Proteins of molecular masses of 50 and 95 kilodaltons were also visible on this immunoblot but were less reactive. Marriott et al. (27) have reported both 50- and 95-kilodalton proteins which were detergent extracted from MKC surfaces and react with anti-Id antibodies. In this assay, polyomavirus reacted strongly with a group of proteins between 24 and 31 kilodaltons in addition to the 50- and 95-kilodalton proteins. Intensities of the reactive proteins appears to depend on the probe used, for example, anti-120-kilodalton protein (17), anti-Id, or polyomavirus (27). The work reported here substantiates the previously reported molecular masses of polyomavirus receptor proteins on MKC and describes the characterization of a new immunologic probe which will be useful for further characterization of the receptor.

Glycosylation appears to be an integral component of several virus receptors including polyomavirus. In the polyomavirus system, binding to (4) and hemagglutination of (11) guinea pig erythrocytes is abolished by pretreatment of the erythrocytes with neuraminidase. However, neuraminidase treatment of MKC only partially inhibits virus binding (4) and does not completely abolish polyomavirus infection (Griffith and Consigli, unpublished data), indicating that a sialic acid linkage, if it exists on the MKC receptor for polyomavirus, appears to be either protected from neuraminidase treatment or not required for virus binding. A requirement for some type of glycosylation of the polyomavirus receptor on MKC was shown by Marriott et al. (27), who have demonstrated that tunicamycin completely prevents recovery of infectivity after receptor removal in MKC. It is interesting that Fried et al. (14) have reported that a specific sialyloligosaccharide modification of the host receptor is required to obtain polyomavirus infection of 3T6 cells. It therefore appears that a sialic acid linkage is essential for polyomavirus hemagglutination of guinea pig erythrocytes (4, 11) and infection of 3T6 cells (14). Although evidence suggests that glycosylation plays a vital role in polyomavirus receptor function, the exact modification has not been fully characterized in the MKC system. Evidence discussed above suggests that differences may exist between the 3T6 and MKC receptors. As suggested by other virus systems (21, 33), host range may be determined, at least in part, by differences in the virus receptor between host cell types.

Whereas these studies have not fully characterized the polyomavirus receptor on MKC, the anti-Id immunologic probe promises to play an important part in attaining that end. We believe that anti-Id described in this report may facilitate isolation of the specific MKC receptor in sufficient quantities for future biochemical studies. Through isolation of large quantities of receptor protein we hope to define the molecular mass of the polyomavirus receptor clearly. Because of their ability to bind the cell surface in a virus-specific manner, these antibodies may also be useful as probes for virus-receptor interactions. This information will be important in detailing the initial events of polyomavirus infection and may have important implications for understanding of the tissue tropism and host range of this transforming DNA virus.

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