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Immunology

Isolation and biochemical characterization of the mammalian reovirus type 3 cell-surface receptor

(anti-idiotype/virus receptor/lymphocytes/neuroblastoma)

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ABSTRACT **A cell-surface receptor for the mammalian reovirus type 3 hemagglutinin was isolated by using anti-idiotypic anti-receptor antibodies. The receptor is a glycoprotein with a molecular mass of 67,000 daltons and a pI of 5.9. Evidence that the isolated structure represents the reovirus receptor was obtained by electrophoretic immunoblot studies, which demonstrated that the 67,000-dalton glycoprotein is the only cell-surface structure recognized by both reovirus type 3 and the anti-receptor immunoglobulin. Comparison of the reovirus receptor on murine thymoma (R1.1) and rat neuroblastoma (B104) cells indicated that similar structures on the cell surface are recognized by the reovirus type 3 and the anti-receptor antibodies as previously suggested from cellular and binding studies. This receptor was found on mouse, rat, monkey, and human cells. Furthermore, diverse tissue types, including lymphoid and neuronal cells, express the receptor structure. The receptor structure is discussed in terms of its role in mediating viral tropism and as an essential cell-surface protein.**

The identification of virus receptors and clarification of their mode of interaction with ligands are important in understanding viral tropism and subsequent disease patterns. The Reoviridae provide a workable and informative model system for studying viral receptor interactions (1–3). The mammalian reoviruses are icosahedral viruses containing segmented, double-stranded RNA genomes. They have been divided by neutralization and hemagglutination patterns into three serotypes—1, 2, and 3. Reovirus type 3 binds to murine lymphoid and neuronal cells and causes a fatal encephalitis when injected into neonatal mice (4). In contrast, reovirus type 1 binds selectively to ependymal cells in the brain (5) and leads to hydrocephalus (6). By using a variety of recombinant reovirus clones in which genetic segments of one serotype have been imposed on a genetic background of another, it has been revealed that the S1 double-stranded RNA segment is responsible for the distinct tropism of type 1 and type 3 viruses (7). The gene for the S1 segment encodes the $\sigma 1$ outer-capsid-protein hemagglutinin (HA), which governs virus attachment to target cell surfaces (8, 9).

In previous studies we have shown that both the cellular and humoral murine immune responses to reovirus type 3 were predominantly directed to the viral HA and that these anti-HA antibodies expressed a restricted set of idiotopes (10). Anti-idiotypic antibodies made in rabbits were shown to mimic HA binding and also to trigger cellular responses in a similar manner to the viral HA (11). These studies demonstrated the presence of a common HA binding conformation on lymphoid and neuronal reovirus receptors that is also shared on anti-HA antibodies and HA-specific T-cell receptors (3). Anti-idiotypic antibodies have similarly been used in studies of the cell-surface receptors for retinol binding protein (12), insulin (13), adrenergic ligands (14), formyl peptide chemotoxins (15) and β_1 H-globulin (16).

More recently we have developed syngeneic anti-HA (idiotype) and anti-anti-HA (anti-idiotype) monoclonal antibodies (17). Monoclonal anti-idiotypic antibody possesses all of the specific binding properties of the polyclonal rabbit anti-idiotype and mimics changes in cellular function induced by HA binding, such as the activation of nonspecific suppressor T cells. Syngeneic monoclonal anti-idiotypic antibodies also have been utilized as a viral vaccine and are capable of inducing potent T cell-mediated cytolytic and delayed-type hypersensitivity responses as well as anti-HA antibody production by B cells (18). These results confirm that both the polyclonal and monoclonal anti-idiotypic antibodies specifically recognize the cell-surface receptor for reovirus type 3. In this report we describe the isolation and biochemical characterization of the reovirus type 3 binding receptor on a panel of mammalian cell lines utilizing anti-idiotypic anti-receptor antibodies. The conservation of receptor structure on a murine thymoma line, a rodent neuroblastoma line, and a human lymphoid line is discussed in relation to potential alternative viral receptor functions.

MATERIALS AND METHODS

Anti-Idiotypic Antibodies. The preparation of rabbit anti-idiotypic antibodies has been described (11). The purified antisera were identified by affinity for the HA-specific Ig 9BG5, a monoclonal antibody recognizing the neutralization domain of type 3 HA (10). The generation and screening of syngeneic monoclonal anti-idiotypic antibodies have been reported (17). Briefly, BALB/c mice were immunized repeatedly with 5×10^6 irradiated idiotype-bearing 9BG5 hybridoma cells. Cells were fused by the method of Köhler and Milstein (19), and the resulting hybridomas were screened for their ability to bind to the 9BG5 monoclonal antibody. One such syngeneic monoclonal antibody, 87.92.6 (IgM; Kappa Scientific, Escondido, CA), was shown to inhibit binding of 9BG5 to purified ^{125}I-labeled reovirus type 3 HA protein. 87.92.6 was purified by sieve and ion-exchange chromatography as described (18).

Virus Purification. Reovirus type 3 was grown in suspension cultures of L cells at 34°C and purified from infected cells as described (20).

Cells. Murine thymoma R1.1, lymphoma YAC, and reovirus type 3 HA-specific hybridoma 9BG5 were maintained in suspension in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% fetal calf serum, 1% glutamine, and 1% penicillin/streptomycin. The rat neuroblastoma B104 cells, murine NIH 3T3 fibroblasts, C3H

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Abbreviation: HA, hemagglutinin.

10T½ cells, and monkey COS7 cells were grown in the same medium on plates. Murine thymoma BW5147, human lymphoid CEM cells, and insulin-specific T-cell hybridoma B3C8 were maintained in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% glutamine, and penicillin/streptomycin. Splenic B and T cells were prepared as described by Wysocki and Sato (21). COS7, B104, and B3C8 were the gifts of G. Freeman (Dana-Farber Cancer Institute, Boston), J. Drebin (Harvard Medical School, Boston), and L. Glimscher (Harvard School of Public Health, Boston), respectively.

Iodination. Cell-surface iodination was performed by using the enzymatic lactoperoxidase method (22). Briefly, 2–5 × 10^7 washed cells were mixed with 1 mCi (1 Ci = 37 GBq) of $Na^{125}I$ (New England Nuclear) in 50 μl of 0.2 M phosphate buffer (pH 7.2) and 25 μl of 1% β-D-glucose, and then 50 μl of hydrated enzymobead reagent (Bio-Rad) was added and the reaction mixture was incubated at room temperature for 30 min. The reaction was stopped by extensive washing in phosphate-buffered saline. Membrane proteins were solubilized following incubation in 500 μl of phosphate-buffered saline containing 0.5% Triton X-100 (Sigma), 0.25% Nonidet P-40 (Bethesda Research Laboratories), and 1 mM phenylmethylsulfonyl fluoride for 30 min on ice. The debris was cleared by centrifugation at 35,000 × *g* for 60 min. The solubilized membrane proteins were used immediately or stored at −20°C.

Purified antibodies (10–100 μg in 10–50 μl) and viral particles (10^{13} per ml) were labeled in an analogous manner. Following incubation with enzymatic reagents, the reaction mixture was passed through a Sephadex G-25 column to remove the free iodine. In each case, >95% of the radioactivity was precipitable in 10% cold CCl_3COOH. Binding analyses were conducted as described by Dower *et al.* (23).

Electrophoretic Immunoblot. Membranes were purified from hypotonically lysed cells by a combination of differential centrifugation and discontinuous sucrose density gradients (24). Membranes from 5 × 10^7 cells were loaded per lane and electrophoresed under reducing conditions on 10% polyacrylamide gels (25). Proteins were transferred to nitrocellulose paper (Bio-Rad) at 250 mA for 18 hr at 4°C by using a Hoefer electrophoresis TE series transfer unit (Hoefer, San Francisco) (26). The nitrocellulose paper was blocked with buffer containing 0.1 M Tris (pH 7.5), 3% bovine serum albumin or 0.25% gelatin, and 0.5% Nonidet P-40 at 37°C for 2 hr and then was hybridized with ^{125}I-labeled probes (2–5 × 10^6 cpm in 10 ml of buffer) for 1 hr at room temperature. The paper was then washed thoroughly in the buffer, dried in air, and exposed to Kodak X-OMAT AR film for 2 days at −70°C. In the hybridization with anti-bovine serum albumin antibodies, 0.25% gelatin (Sigma) was used for blocking.

Immunoprecipitation. Iodinated cell lysates (2–5 × 10^5 cpm) were incubated with 50 μg of antibody (polyclonal rabbit anti-idiotype, monoclonal anti-idiotype, or normal rabbit immunoglobulin) at room temperature for 1 hr. Sepharose protein A (25 μl; Pharmacia) was next added, and the incubation was continued for 2 hr. Beads were collected by centrifugation in a Beckman Microfuge, washed three times in solubilization buffer and then twice in phosphate-buffered saline. Pellets were adjusted to $NaDodSO_4$/PAGE sample buffer containing 2-mercaptoethanol, boiled, and run on a 10% acrylamide slab gel (25). Dried gels were exposed to Kodak X-OMAT AR film at −70°C.

Two-Dimensional Gel Electrophoresis. Immunoprecipitated proteins were adjusted to isoelectric focusing sample buffer (9.5 M urea/2% Nonidet P-40/1.6% Pharmalyte, pH 5–8/0.4% Pharmalyte, pH 3–10/5% 2-mercaptoethanol) (27); Pharmalyte was from Pharmacia. Samples (50 μl) were loaded onto the basic end of 120 × 2.8 mm diameter tube gels containing 9.2 M urea, 4% acrylamide, 2% Nonidet P-40, 1.6% Pharmalyte (pH 5–8), and 0.4% Pharmalyte (pH 3–10). Gels were run at 500 V for 16 hr. The tube gels were then incubated in Laemmli sample buffer for 1 hr and layered on top of 10% $NaDodSO_4$/PAGE slab gels with a 3% stack and run at 30 mA for 5 hr. The gels were fixed, dried, and exposed to a Kodak X-OMAT AR film for 2 days at −70°C.

Neuraminidase Treatment. Immunoprecipitates were incubated with 0.5 unit of neuraminidase (Sigma) for 60 min at 37°C. Following the incubation, samples were adjusted to Laemmli sample buffer and run on a $NaDodSO_4$/PAGE gel as described above.

RESULTS

Specificity of 87.92.6 Binding. The specificity of monoclonal anti-idiotype antibody binding to the reovirus receptor was illustrated by using the murine thymoma cell line R1.1 and the rat neuroblastoma line B104. 87.92.6 antibodies recognized the cell-surface receptor for reovirus as demonstrated by the ability of monoclonal anti-idiotype antibodies to inhibit the binding of reovirus type 3 to both R1.1 and the B104 cell lines. Incubation of R1.1 or B104 cells with monoclonal 87.92.6 antibodies prior to reovirus exposure resulted in a 62% and 71% decrease, respectively, in the mean channel fluorescence as measured on a fluorescence-activated cell sorter (Table 1). Preincubation with either monoclonal anti-H-2^k or anti-Thy-1 antibodies had no effect on the subsequent binding of reovirus.

Binding and Scatchard Analysis of Reovirus Receptors. Iodinated purified 87.92.6 protein was used to determine the number and affinity of reovirus binding sites on target cells (Table 2). To quantitate binding, increasing amounts of ^{125}I-labeled 87.92.6 (1–100 nM) were added to a fixed number (10^6) of cells. The binding of anti-idiotype to reovirus type 3 binding R1.1, B104, and splenic T and B cells displayed linear and saturable kinetics. Reovirus receptor-bearing cells possess between 50,000 and 78,000 reovirus binding sites with an apparent K_d for 87.92.6 of 1 nM. In each instance, the nonspecific component of binding was assessed by measuring the amount of ^{125}I-labeled 87.92.6 bound in the presence of a 100-fold excess of unlabeled anti-idiotype. Nonspecific binding did not exceed 6% of the total bound immunoglobulin. Similar results have been recently reported from binding studies using iodinated reovirus particles (28).

Isolation and Biochemical Characterization of the Reovirus Receptor. Our initial experiments indicated that the most effective reovirus receptor purification was achieved by utilizing polyclonal rabbit anti-receptor antibodies rather

Table 1. Inhibition of reovirus type 3 binding by anti-idiotypic antibody

Cell line	Blocking reagent	Mean channel fluorescence
R1.1	None	117.43
	Reovirus type 3	11.98
	Anti-Id	44.62
B104	None	131.68
	Reovirus type 3	16.78
	Anti-Id	38.19
R1.1	Anti-*H-2*^{k}	116.02
	Anti-*Thy-1.2*	115.38

Cells (10^6) were preincubated with reovirus type 3 (10^{11}) particles or blocking reagents: anti-idiotypic (10 μg), anti-*H-2*^{k} (10 μg), or anti-*Thy-1.2* (10 μg) at 4°C as described. Cells were washed thoroughly and incubated with 10^{11} biotinylated reovirus particles for 30 min on ice, washed, and then stained with avidin-fluorescein. Mean channel fluorescence was determined by using a EPICS V fluorescence-activated cell sorter.

Table 2. Number and affinity of anti-idiotypic antibody receptors on reovirus high-binding and low-binding cells

Reovirus type 3	Number of binding sites	K_d, nM
High-binding cell		
R1.1 thymoma	67,000	1.0
B104 neuroblastoma	78,000	0.9
Splenic T cell	65,000	0.8
Splenic B cell	50,000	0.9
Low-binding cell		
YAC thymoma	<2,000	—

Increasing amounts of purified monoclonal ^{125}I-labeled anti-idiotype were added to 10^6 cells in 50 μl of phosphate-buffered saline containing 1% bovine serum albumin and 0.2% NaN_3. After incubation with labeled immunoglobulins, cells were pelleted through phathlate oils, and cell-bound ^{125}I was determined. The results presented were obtained by Scatchard analysis of binding data, assuming that IgM binds to one cell. Similar results have been reported from Scatchard analysis by using radiolabeled reovirus particles (28).

than monoclonal anti-receptor antibodies. After surface iodination, the solubilized membrane extracts of R1.1 thymoma cells were incubated with anti-receptor antibodies and precipitated with Sepharose-protein A. The precipitate was analyzed by two-dimensional PAGE under reduced conditions. Fig. 1 is an autoradiogram of a two-dimensional map of the polyclonal anti-receptor antibody immunoprecipitate of R1.1 cell extract. The labeled material in the immunoprecipitate has a M_r of 67,000 with a heterogeneous pI around 5.9.

Confirmation that the M_r 67,000 structure is the reovirus receptor was achieved by using immunoblot analysis. R1.1 cell membranes were first purified by using differential and equilibrium gradient centrifugation. Membrane extracts were then run under reduced conditions on 10% NaDodSO$_4$/PAGE and blotted onto nitrocellulose paper. Identification of the reovirus receptor was achieved by independent hybridization with either ^{125}I-labeled reovirus or anti-idiotype. Fig. 2 is an autoradiogram of this hybridization.

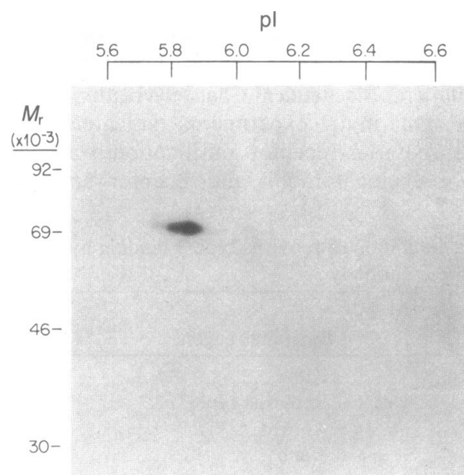

FIG. 1. Two-dimensional gel electrophoresis of cell surface proteins of R1.1 cells immunoprecipitated with polyclonal rabbit anti-receptor antibody. Iodinated cell lysate was incubated with anti-receptor antibody and Sepharose-protein A. The precipitates were reduced with 2-mercaptoethanol and subjected to equilibrium isoelectric focusing in a tube gel, followed by NaDodSO$_4$/PAGE using a 10% acrylamide slab gel. The gel was fixed, dried, and exposed to Kodak X-OMAT AR film at −70°C for 2 days. The pH was determined from blank tube gels run simultaneously with the immunoprecipitates.

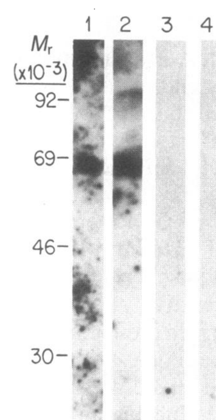

FIG. 2. Electrophoretic immunoblot analysis of R1.1 membrane proteins hybridized with ^{125}I-radiolabeled reovirus or anti-receptor antibodies. Membranes were purified from R1.1 cells, electrophoresed under reducing conditions on 10% NaDodSO$_4$/PAGE and blotted onto nitrocellulose paper. Blotted papers were then blocked with bovine serum albumin or gelatin and independently hybridized with ^{125}I-labeled reovirus (lane 1), monoclonal 87.92.6 antibody (lane 2), polyclonal anti-receptor antibody (lane 3), and anti-bovine serum albumin (lane 4). Washed nitrocellulose paper was exposed to Kodak X-OMAT AR film at −70°C for 2 days. The M_r 67,000 band is apparent in lanes 1 and 2, faint in lane 3, and absent in lane 4.

Lanes 1–4 represent identical samples of R1.1 cell lysates that varied in hybridization treatment. Lane 1 was hybridized with iodinated type 3 reovirus particles. Lanes 2 and 3 were hybridized with iodinated monoclonal 87.92.6 and polyclonal rabbit anti-idiotype antibodies, respectively. Both radiolabeled virus particles and anti-idiotypic antibodies bound to a single band of M_r 67,000. Control hybridization with radiolabeled anti-bovine serum albumin, shown in lane 4, did not detect the same band. Thus, recognition of the M_r 67,000 structure is specific for reovirus type 3 particles and anti-receptor antibodies.

We also attempted immunoprecipitation with monoclonal anti-receptor antibody and whole viral particles, but as mentioned previously, these results have not been as satisfying as with the polyclonal rabbit antibody. Precipitations with monoclonal antibody (Fig. 3, lane 2) or virus (Fig. 3, lane 3) displayed a number of bands in the autoradiogram, including the M_r 67,000 band identified in polyclonal anti-

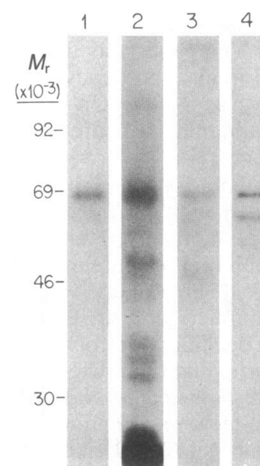

FIG. 3. NaDodSO$_4$/PAGE analysis of the immunoprecipitates of R1.1 cell lysates with anti-receptor antibodies. ^{125}I-labeled R1.1 lysate was precipitated with polyclonal rabbit anti-idiotype (lane 1), monoclonal anti-idiotype 87.92.6 (lane 2), viral particles (lane 3). Lane 4 showed the neuraminidase-treated polyclonal immunoprecipitates.

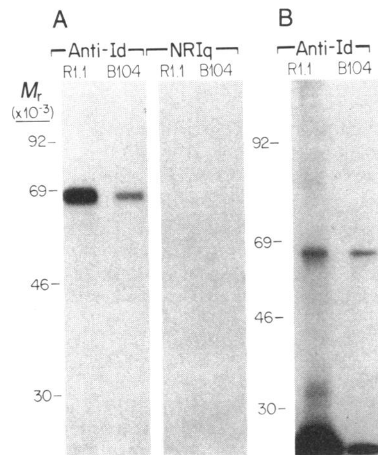

FIG. 4. Comparison of reovirus receptor on murine thymoma R1.1 and rodent neuroblastoma B104 cells. (*A*) $NaDodSO_4$/PAGE analysis under reducing conditions. Lanes: 1 and 2, immunoprecipitates of R1.1 and B104 cell lysates with rabbit anti-idiotypic (anti-Id) anti-receptor immunoglobulin; 3 and 4, parallel precipitates with normal rabbit immunoglobulin (NRIg). (*B*) $NaDodSO_4$/PAGE analysis under nonreducing conditions. Lanes 1 and 2 show the immunoprecipitates of R1.1 and B104 cell lysates with rabbit anti-idiotypic anti-receptor immunoglobulin. The low molecular weight bands do not appear consistently and are probably due to nonspecific bindings of irrelevant molecules.

receptor precipitations (lane 1) and in the immunoblots described above. The results suggest that the M_r 67,000 molecule forms less-efficient precipitation complexes with the monoclonal antibody, possibly a consequence of disassociated IgM molecules. An alternative interpretation is that nonspecific molecules are trapped in the IgM immune complexes. A similar result was obtained when we attempted immunoprecipitation with the monoclonal antibody and a second antibody. Nonspecific components of viral precipitation are most likely related to less effective washing techniques.

Demonstration that the M_r 67,000 receptor on R1.1 is a glycoprotein was achieved by treatment of immunoprecipitates with neuraminidase before analysis on an $NaDodSO_4$/PAGE gel. Neuraminidase catalyzes the release of sialic acid from glycoproteins (29). The autoradiogram (lane 4 in Fig. 3) shows, in addition to the M_r 67,000 band, an additional band of M_r 62,000, the latter band being the partially deglycosylated product. The M_r 67,000 glycoprotein also was observed by using $[^{35}S]$cysteine to label metabolically active cells, showing that the M_r 67,000 glycoprotein was indeed synthesized by the R1.1 line (data not shown).

Comparison of the Reovirus Receptor on Murine Thymoma R1.1 and Rat Neuroblastoma B104 Cells. Reovirus type 3 binds to both murine lymphoid cells and neuronal cells (17), although only neonatal neuronal cells are susceptible to lytic infection. To examine if reovirus type 3 recognizes the same cell-surface structure on lymphoid and neuronal cells, we compared the immunoprecipitates of the murine thymoma R1.1 line with a nitrosoethylurea-induced rodent neuroblastoma line, B104, which is lytically infected by reovirus. Fig. 4*A* is an autoradiogram of the product of $NaDodSO_4$/PAGE analysis under reduced conditions of immunoprecipitations performed with rabbit anti-receptor antibody. Both R1.1 (lane 1) and B104 (lane 2) expressed a major band corresponding to a M_r 67,000 protein. Similar precipitations with normal rabbit immunoglobulin did not detect the M_r 67,000 protein (lanes 3 and 4 in Fig. 4*A*). $NaDodSO_4$/PAGE analysis of the immunoprecipitated protein under nonreduced conditions revealed a similar band corresponding to M_r 67,000 (Fig. 4*B*). These experiments indicate that the protein recognized by the anti-receptor antibody is monomeric. The parallel results on R1.1 and B104 suggest that the reovirus type 3 HA recognizes similar structures on the surface of the murine thymoma and rat neuroblastoma cells.

Other Cell Types that Express the M_r 67,000 Receptor Protein. Earlier studies had identified a panel of cells that bind reovirus type 3 and the anti-receptor antibody (17). Therefore, we have extended our biochemical analysis to include murine splenic T and B lymphocytes; BW5147, a murine lymphoma line; B3C8, a murine T-cell hybridoma specific to insulin; and CEM, a human lymphoma line. These results (data not shown) indicate that, in agreement with fluorescence-activated cell sorter analysis, the M_r 67,000 glycoprotein is present on all reovirus binding lymphoid cells. The presence of the M_r 67,000 structure in the human line CEM indicates that the receptor protein is not species specific.

We also examined three murine fibroblasts—L929, NIH 3T3, and C3H/10T½—and a monkey cell line—COS7 (data not shown). L929 showed an intense band corresponding to the M_r 67,000 glycoprotein, COS7 showed a faint band, whereas NIH 3T3 and C3H/10T½ did not show the band. The absence of the 67,000 dalton band in NIH 3T3 and C3H/10T½ could be a result of absence of the protein, a lower expression of the protein, or the consequence of less efficient iodination. Cytofluorimetry experiments conducted in parallel indicated that the staining of these latter two cells with reovirus and rabbit anti-idiotype is less intense than that seen in R1.1 cells.

DISCUSSION

These studies demonstrate that the cell surface receptor for mammalian reovirus serotype 3 is expressed on normal rodent lymphoid and neuronal cells as well as on several rodent and primate transformed cell lines. Purification of the reovirus receptor was accomplished by immunoprecipitation with an anti-idiotypic anti-receptor antibody. Anti-receptor antibodies provide several distinct advantages for the identification and isolation of cell-surface receptors as compared to the use of reovirus particles. First, antisera and purified monoclonal antibodies can be generated and stored in large quantities, while viruses lose their activity over a short period of time. Secondly, monoclonal antibodies are monospecific, whereas viruses by their mass favor nonspecific binding. A final consideration for the use of antibodies to isolate receptors relates to those viruses that are particularly dangerous to handle.

Unfortunately, the generation of receptor-specific antibodies has been limited by the availability of purified receptors. An alternative approach has been developed using anti-idiotypic antibodies as probes of receptor structure. We previously have discussed the possibility that the anti-idiotype is an "internal image" of the reovirus type 3 HA (1–3, 11, 17). By internal image we mean that the antigen binding site of the anti-idiotype contains conformations that are indistinguishable from those present on the reovirus HA. The evidence supporting this hypothesis is: (*i*) anti-idiotype binding parallels the cellular tropism of reovirus 3 (11); (*ii*) the anti-idiotype and reoviruses have similar biological effects in limiting conconavalin A-induced stimulation of murine lymphocytes (3); (*iii*) anti-idiotype specifically inhibits reovirus 3 binding to target cells (30); (*iv*) anti-idiotype prevents isolated HA protein from binding to monoclonal anti-HA antibodies (11, 17); and (*v*) anti-idiotype specifically stimulates both T- and B-cell immunity to reovirus *in vivo* (18).

Biochemical characterization of the cell surface structure detected by the anti-idiotype shows that it is a glycoprotein

with a M_r of 67,000 and a pI of 5.9. The receptor is monomeric because NaDodSO$_4$/PAGE analysis under reducing and nonreducing conditions revealed a structure of similar molecular weight. The receptor also contains sialic acid residues since neuraminidase treatment created a new band with a lower molecular weight. Immunoblot studies clearly show that the M_r 67,000 glycoprotein is the only cell-surface structure recognized by both reovirus type 3 and the anti-idiotypic immunoglobulin.

Previous studies have shown that murine lymphoid and neuronal cells have receptors for reovirus type 3 HA (17). The murine thymoma cell line R1.1 and the rodent neuroblastoma cell line B104 were used here as models for detailed biochemical study of these cell types. The antibodies detect a M_r 67,000 glycoprotein expressed on the surface of both cell lines. Neuraminidase treatment revealed similarly sized breakdown products of M_r 62,000, confirming that the structures detected by anti-receptor antibodies on these diverse tissues are probably identical. A set of normal murine lymphocytes also express the M_r 67,000 reovirus binding structure. This observation confirms that the M_r 67,000 glycoprotein is not a structure associated only with transformed cell lines. This receptor is present on mouse, rat, monkey, and human cells. Its occurrence on diverse cell types, including lymphoid and neuronal cells, strongly suggests that it is a common cell-surface structure perhaps associated with normal cellular functions.

The isolation of a specific glycoprotein receptor for reovirus type 3 has allowed us to begin studies for the nature and function of the receptor in the normal cell. Over the last few years, others have attempted to identify the nature of the cellular membrane proteins that other viruses have used as receptors. For example, it has been reported that Semliki Forest virus binds to histocompatibility antigens (murine H-2K and H-2D and human HLA-A and HLA-B) (31). However, although binding studies have supported this association, Semliki Forest virus can grow in cells devoid of histocompatibility antigens, raising questions as to the significance of the role of histocompatibility structure as receptors (32). Lactate dehydrogenase virus has been found to interact with mouse Ia antigens, suggesting a role for Ia in targeting virus to a subset of macrophages (33). Rabies virus has been reported to bind in close association with the acetylcholine receptor (34). In each of these instances, it has been difficult to prove definitively whether the association with the virus and cell receptor is the specific, biologically important pathway used for viral binding and entry.

Several membrane proteins with molecular weights similar to the reovirus receptor have been reported. The Fc receptor (35), β-adrenergic receptor (36) and the δ component of the acetylcholine receptor (37) all have molecular weights around 67,000. The Fc receptor is an unlikely candidate because control immunoprecipitation with normal rabbit immunoglobulin did not reveal this band, and the M_r 67,000 structure is also found on tissues that do not express Fc receptors. The β-adrenergic receptor is a reasonable candidate, considering its distribution in reovirus-binding tissues. The binding by reovirus to cell-surface structures such as the β-adrenergic receptor could account for the known tropism of the mammalian reovirus. Preliminary studies have shown some structural similarities between the reovirus type 3 and the mammalian β-adrenergic receptors. Identification of the cell surface receptor for the viral HA should be an important step forward in understanding the pathogenesis of reovirus infection.

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1. Fields, B. N. & Greene, M. I. (1982) *Nature (London)* **300**, 12–23.
2. Noseworthy, J. H. & Greene, M. I. (1984) *Idiotypy in Biology and Medicine*, eds. Cazenue, P. A. & Urbain, J. A. (Academic, New York), pp. 303–328.
3. Nepom, J. T., Tardieu, M., Epstein, R. L., Noseworthy, J. H., Weiner, H. L., Gentsch, J., Fields, B. N. & Greene, M. I. (1982) *Surv. Immunol. Res.* **1**, 255–261.
4. Margolis, G., Kilham, L. & Gonatas, N. (1971) *Lab. Invest.* **24**, 91–100.
5. Tardieu, M. & Weiner, H. L. (1982) *Science* **215**, 419–421.
6. Kilham, L. & Margolis, G. (1970) *Lab. Invest.* **21**, 183–188.
7. Weiner, H. L., Drayna, D., Averill, D. R., Jr., & Fields, B. N. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5744–5748.
8. Weiner, H. L., Ramig, R. F., Mustoe, T. A. & Fields, B. N. (1978) *Virology* **86**, 581–584.
9. Lee, P. W. K., Hayes, E. C. & Joklik, W. K. (1981) *Virology* **108**, 156–163.
10. Burstin, S. J., Spriggs, D. R. & Fields, B. N. (1982) *Virology* **117**, 146–155.
11. Nepom, J. T., Weiner, H. L., Dichter, M. A., Tardieu, M., Spriggs, D. R., Gramm, C. F., Powers, M. L., Fields, B. N. & Greene, M. I. (1982) *J. Exp. Med.* **155**, 155–167.
12. Sege, K. & Peterson, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2443–2447.
13. Shechter, Y., Maron, R., Elias, D. & Cohen, I. R. (1982) *Science* **216**, 542–545.
14. Schreiber, A. B., Couraud, P. O., Andre, C., Uray, B. & Strosberg, A. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7385–7389.
15. Marasco, W. A. & Becker, E. L. (1982) *J. Immunol.* **128**, 963–968.
16. Lambris, J. D. & Pars, G. D. (1982) *J. Exp. Med.* **155**, 1400–1411.
17. Noseworthy, J. H., Fields, B. N., Dichter, M. A., Sobotka, C., Pizer, E., Perry, L. L., Nepom, J. T. & Greene, M. I. (1983) *J. Immunol.* **131**, 2533–2538.
18. Sharpe, A. H., Gaulton, G. N., McDade, K. K., Fields, B. N. & Greene, M. I. (1984) *J. Exp. Med.* **160**, 1195–1205.
19. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–496.
20. Ramig, R. F., Mustoe, T. A., Sharpe, A. H. & Fields, B. N. (1978) *Virology* **85**, 531–544.
21. Wysocki, C. J. & Sato, V. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2844–2848.
22. Tenner, A. J., Lesavre, P. H. & Cooper, N. R. (1981) *J. Immunol.* **127**, 648–653.
23. Dower, S. K., Ozato, K. & Segal, D. M. (1984) *J. Immunol.* **132**, 751–758.
24. Stallcup, K. C., Springer, T. A. & Mescher, M. F. (1981) *J. Immunol.* **127**, 923–930.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Burnett, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
27. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
28. Epstein, R. L., Powers, M. L., Rogart, R. B. & Weiner, H. L. (1984) *Virology* **133**, 45–55.
29. Lauer, W. G. (1974) *Adv. Virus Res.* **18**, 57–104.
30. Kauffman, R. S., Noseworthy, J. H., Nepom, J. T., Finberg, R., Fields, B. N. & Greene, M. I. (1983) *J. Immunol.* **131**, 2539–2941.
31. Helenius, A., Morein, B., Fries, E., Simons, K., Robinson, P., Schirrmacher, V., Terhorst, C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3846–3850.
32. Oldstone, M. B. A., Tishon, A., Dukto, F. J., Kennedy, S. I. T., Holland, J. J. & Lampert, P. W. (1980) *J. Virol.* **34**, 256–265.
33. Inada, T. & Mims, C. A. (1984) *Nature (London)* **309**, 59–61.
34. Lentz, T. L., Burrage, T. G., Smith, A. L., Crick, J. & Tignor, G. H. (1982) *Science* **215**, 182–184.
35. Mellman, I. S. & Unkeless, J. C. (1980) *J. Immunol.* **152**, 1048–1069.
36. Cubero, A. & Malbon, C. C. (1984) *J. Biol. Chem.* **259**, 1344–1350.
37. Raftery, M. A., Hunkapiller, M. W., Strader, C. D. & Hood, L. E. (1980) *Science* **208**, 1454–1457.