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Isolation of a Monoclonal Antibody That Blocks Attachment of the Major Group of Human Rhinoviruses

R. J. COLONNO,* P. L. CALLAHAN, AND W. J. LONG

Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486

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Reciprocal competition binding assays have previously demonstrated that 20 of 24 human rhinovirus serotypes tested compete for a single cellular receptor. These studies suggested that the vast majority of rhinovirus serotypes utilize a single cellular receptor. With HeLa cells as an immunogen, a mouse monoclonal antibody was isolated which had the precise specificity predicted by the competition binding study. The receptor antibody was shown to protect HeLa cells from infection by 78 of 88 human rhinovirus serotypes assayed. In addition, the receptor antibody protects HeLa cells from infection by three coxsackievirus A serotypes. The receptor antibody was unable to protect cells from infection by a wide range of other RNA and DNA viruses. Using the receptor antibody and human rhinovirus type 15, we determined that the cellular receptor utilized by the vast number of human rhinovirus serotypes is present only on cells of human origin, with the exception of chimpanzee-derived cells. The receptor antibody has a strong affinity for the cellular receptor as evidenced by its rapid binding kinetics and ability to displace previously bound human rhinovirus virions from receptors. No viral variants were identified which could bypass the receptor blockage.

The human rhinoviruses (HRVs) represent a subgroup of picornaviruses and contain over 100 antigenically distinct serotypes (4, 15). They are of particular medical importance because they have been identified as the major causative agent of the common cold in humans (10). Similar to other viruses, HRVs attach to specific receptor sites located on the surface of cellular membranes as a prerequisite to entry into susceptible cells (2, 5–7, 21). Previous studies have clearly demonstrated that different groups of picornaviruses utilize different cellular receptors for attachment and entry (5, 11–14). Using competition binding assays, Lonberg-Holm et al. suggested that HRVs could be divided into at least two receptor families which did not compete with poliovirus, coxsackievirus B or adenovirus for attachment to cellular receptors (12). They also found that one HRV family would compete with coxsackievirus A21 for a single receptor. Since this study dealt with a limited number of HRV serotypes, we expanded the study to include 24 randomly selected serotypes. Results of reciprocal competition binding studies indicated the existence of only two HRV receptor families (1). However, 20 of the 24 HRV serotypes tested competed for a single receptor referred to as the major HRV receptor group, and only 4 of the 24 fell into a second, minor HRV receptor group. These results implied that a single cellular receptor existed on cell membranes which is utilized by the vast number of HRV serotypes. Recently, monoclonal antibodies have been isolated which specifically block poliovirus and coxsackievirus B attachment (3, 16). We now report the isolation of a mouse monoclonal antibody which is effective in specifically blocking the attachment and subsequent infection of 78 serotypes of HRV.

MATERIALS AND METHODS

Viruses and cells. All numbered serotypes of HRVs and other viruses used were obtained from the American Type Culture Collection. The clinical isolate strain HRV Hanks was a generous gift of Jack Gwaltney, University of Virginia. The isolation, growth, and propagation of HeLa R-19 cells and HRVs have been previously described (1). The cell lines listed in Table 2 were obtained from the American Type Culture Collection or the Merck cell collection and grown as described in the American Type Culture Collection instructions. In most cases, the various virus inocula consisted of infected cell supernatants that had been precleared of cellular debris by low-speed centrifugation and concentrated by polyethylene glycol precipitation. Inoculum titers were determined by plaque assay (HRVs) or by direct infection of cell monolayers to determine the minimal amount of infected cell supernatants needed to destroy monolayers in 1 or 2 days. The preparation of [^{35}S]methionine-labeled HRVs has been described previously (1).

Cell protection assay. HeLa R-19 cells, or other cell lines described below, were plated in 48-well cluster plates (Costar, Cambridge, Mass.) at 1.5×10^5 cells per well and incubated overnight to generate confluent monolayers. Duplicate monolayers were pretreated with hybridoma supernatants or receptor antibody as indicated for 30 min at 37°C before infection with a specified virus. Virus was applied at a multiplicity of infection of 0.1 to 1; in the case of viruses other than HRVs, a minimum amount of virus was added to yield a cytopathic effect within 24 to 48 h. After an overnight incubation, monolayers were checked for cytopathic effect with a light microscope. Treated wells were compared with infected wells of each virus in the absence of added antibody to determine whether the antibody tested had any protective effect. Cultures showing partial protection were repeated with increased amounts of virus and usually resulted in a negative test.

Preparation of membranes. HeLa R-19 cell monolayers were treated with phosphate-buffered saline (PBS) containing 50 mM EDTA for 10 to 15 min at 37°C. Released cells were pooled and washed three times in cold PBS. Cells were suspended in 10 mM phosphate buffer at 6×10^7 cells per ml and allowed to swell on ice before disruption by Dounce homogenization. Cell debris and nuclei were pelleted at $1,000 \times g$ for 5 min. The supernatant was transferred to a new centrifuge tube and pelleted at 45,000 rpm for 1 h in a

* Corresponding author.

Beckman Ti60 rotor at 4°C. The crude membrane pellet was suspended at 8 mg/ml in PBS and stored at −70°C.

Receptor binding assays. Binding assays with tissue culture monolayers have been described previously (1). In in vitro membrane-binding assays, 20 μl of HeLa cell membranes was incubated for 5 min with 10 μl of an antibody solution before the addition of 10 μl (10^4 cpm) of [^{35}S]methionine-labeled HRV or I^{125}-labeled antibody. After the indicated incubation times, usually 1 h at 25°C in a 1.5-ml Eppendorf tube, the membranes were pelleted at 12,000 × *g* for 2 min, and radioactivity in the supernatant was determined. Pellets were suspended in 0.2 ml of 1% sodium dodecyl sulfate and also assayed for radioactivity. Results are expressed as a percentage of total radioactivity found in the pellet fraction. Control binding of ^{35}S-labeled virus ranged from 25 to 40%, whereas I^{125}-labeled antibody ranged from 10 to 20%.

Isolation and purification of receptor monoclonal antibody. Adult BALB/c mice were injected intraperitoneally with a solution containing HeLa R-19 cells, which were recovered from tissue culture monolayers by treatment with PBS containing 50 mM EDTA for 20 min at 37°C. Each mouse received 3 × 10^6 cells suspended in 0.5 ml of PBS containing complete Freund adjuvant. After 38 days, mice were inoculated intraperitoneally with 10^7 HeLa R-19 cells in a volume of 0.5 ml of PBS containing incomplete Freund adjuvant. Three mice that tested positive for antibodies which protected cells against HRV-14 infection (cell protection assay) were primed 4 days before the cell fusion by injection of crude HeLa cell membranes in the tail vein (in aqueous solution, 0.05 ml per mouse).

The spleen cells from immunized animals were washed, pelleted, and fused to SP2/0 myeloma cells as described elsewhere (W. J. Long, W. R. McGuire, A. Palombo, and E. A. Emini, Immun. Methods, in press). Supernatants from growth-positive cultures that had attained at least 40% confluency were assayed in the cell protection assay described above. The hybridoma cells yielding antibody that was positive in the cell protection assay were recloned twice under conditions of limiting dilution to ensure the production of monospecific antibody. To obtain sufficient quantities of the receptor monoclonal antibody, ascites tumors were produced by injecting 10^7 cells per mouse into the peritoneal cavities of mice pretreated with pristane (Aldrich Chemical Co., Milwaukee, Wis.) as described elsewhere (19). Ascites fluid was recovered by syringe at the appropriate time, usually 7 to 10 days after injection. Cells and debris were removed by low-speed centrifugation, and the ascites fluid was filter sterilized. As a first step in purification of the antibody, ascites fluid was passed over a DE-52 (Whatman, Inc., Clifton, N.J.) column in 20 mM sodium phosphate (pH 6.3) to remove any contaminating nucleic acids. Antibody eluting from the DE-52 column was then purified by affinity chromatography on an Affi-Gel protein A column (Bio-Rad Laboratories, Richmond, Calif.) using the MAPS buffer system and directions supplied by Bio-Rad. Eluted antibody was dialyzed overnight against 20 mM Tris hydrochloride (pH 7.7) at 4°C. The receptor antibody was further purified on a high-pressure liquid chromatography Mono Q HR 5/5 column (Pharmacia, Uppsala, Sweden) with a 0 to 1.0 M NaCl gradient in 20 mM Tris hydrochloride (pH 7.7). The purity of the receptor antibody isolated from high-pressure liquid chromatography was demonstrated by analysis on silver-stained polyacrylamide gels and high-pressure liquid chromatography gel filtration and shown to contain less than 1% contaminating protein (data not shown). Iodination and repurification of receptor antibody by using chloramine T have been previously described (W. M. Hurni, W. J. Miller, E. H. Wasmuth, and W. J. McAleer, submitted for publication).

RESULTS

Isolation of a receptor monoclonal antibody. Previous studies (1, 11–13) have clearly indicated that the vast majority of HRV serotypes attach to susceptible cells via a single cellular receptor. We reasoned that a monoclonal antibody specific for this attachment site would block infection by numerous HRV serotypes. BALB/c mice were immunized three times over a period of 154 days with HeLa cells and HeLa cell membranes as described in Materials and Methods. Spleens of three mice were excised and fused to mouse myeloma cells to generate hybridoma cultures. Supernatant fluid from growth-positive cultures was screened in a stringent cell protection assay to identify cultures producing antibody capable of blocking HRV-14 infection. Over 2,000 hybridoma cultures were assayed before a single positive culture was found. Cells in the positive culture were recloned twice to ensure production of monospecific antibody. The receptor antibody was determined to have an immunoglobulin G1 isotype by gel diffusion assays with isotype-specific antisera (data not shown).

Specificity of receptor antibody. As a preliminary test for specificity, HeLa cells were pretreated with receptor antibody for 30 min and then challenged with the 24 serotypes of HRVs previously shown to represent two distinct binding groups (1). Protection of HeLa cells with the receptor antibody coincided precisely with the biologically predicted pattern. The 20 HRV serotypes previously shown by competition binding assays to belong to the major receptor group (HRV-3, -5, -9, -11, -12, -14, -15, -17, -22, -32, -36, -39, -41, -51, -58, -59, -60, -66, -67, and -89) were unable to infect HeLa cells in the presence of receptor antibody, whereas infection by the four minor group serotypes (HRV-1A, -2, -44, and -47) was unaffected.

HRVs utilize unique cellular receptors that are different from those used by other picornaviruses, with the exception of group A coxsackieviruses (11–13). To further demonstrate receptor antibody specificity, cell protection studies were performed by using several picornaviruses and a variety of other RNA and DNA viruses. Only the group A coxsackievirus infections of HeLa cells were blocked by the receptor

TABLE 1. Effect of HRV receptor antibody on infection by other RNA and DNA viruses[a]

Virus[b]	Cell line	Cell protection[c]
Polioviruses 1, 2, and 3	HeLa	−
Coxsackieviruses A13, A18, and A21	HeLa	+
Coxsackieviruses B2 and B3	HeLa	−
Echoviruses 1 and 6	HeLa	−
Vesicular stomatitis virus	HeLa	−
Newcastle disease virus	HeLa	−
Parainfluenza virus 3	HeLa	−
Influenza A virus	HeLa	−
Respiratory syncytial virus	HEP-2	−
Human coronavirus 229E	WI-38	−
Vaccinia virus	HeLa	−
Adenoviruses 1 and 2	HeLa	−

[a] Cell protection assays were as described in Materials and Methods.
[b] Sufficient amounts of virus were used to result in observable cytopathic effect within 48 h.
[c] Cultures were scored positive if a protective difference was observed between antibody-treated and untreated monolayers.

antibody and not polioviruses or group B coxsackieviruses (Table 1). In addition, no protection was observed with any of a wide range of other viruses. Since some viruses of interest do not grow well on HeLa cells, cell production studies were expanded to include echoviruses 3 and 20, hepatitis A virus, and parainfluenza virus 1 and 2 infection of Vero cells. Since Vero cells lack receptors for the receptor antibody (see below), it was not surprising to find that treatment of Vero cells with the receptor antibody showed no effect on the viral infections examined (data not shown). These results again demonstrated that the receptor antibody has the specificity predicted by previous biological studies.

As a third measure of specificity, we have taken advantage of the fact that HRVs are highly restricted to human hosts and show a strong tissue tropism. Parallel binding studies were performed with [^{35}S]methionine-labeled HRV-15 and I^{125}-labeled receptor antibody on a wide variety of cell monolayer cultures. The purpose of the binding study was to ascertain whether there was any cell line that the antibody could bind to that the virus could not. Binding percentages less than 1% were considered negative. The virus and receptor antibody showed identical binding patterns (Table 2). With the exception of chimpanzee liver cells, binding was restricted to cells of human origin. It should be noted that the binding study was designed as a qualitative rather than a quantitative assay. Variations in binding from cell to cell may be of little value, since these experiments were done over an extended period of time, during which different preparations of both virus and antibody were used. The finding that HRV-15 would bind to chimpanzee cells was not surprising, since others (8, 9, 17) have reported virus replication in chimpanzees. In fact, our initial attempts to infect these chimpanzee liver cells with HeLa-grown HRV-14 yielded titers of virus comparable to that obtained in HeLa cells (data not shown).

TABLE 2. Assay for the presence of human rhinovirus receptors[a]

Species	Cell line	Source	% Net binding[b]	
			Virus	Monoclonal antibody
Human	HeLa R-19	Cervical carcinoma	18.4	4.6
	MRC-5	Fetal lung	1.8	1.1
	HFL	Fetal lung	5.7	2.7
	AR 924	Fetal liver	12.4	4.5
	HI 407	Intestine	2.4	6.8
	HV	Vagina	3.8	10.3
	D98S	Sternal marrow	2.8	7.4
	Namalwa	Lymphoblastoid	9.8	28.3
	HL	Epithelial-like	5.9	14.1
	FT	Fetal thymus	15.9	26.6
	KB	Epidermal carcinoma	12.7	21.9
	Chang	Conjunctiva	7.9	16.5
	FS-4	Foreskin fibroblast	4.3	10.5
	WI-38	Lung	6.3	10.1
	RD	Rhabdomyosarcoma	0.1	0
	MIA	Pancreatic carcinoma cells	0.1	0.1
	Hep-2	Laryngeal tumor	0	0.3
	RPMI 2650	Nasal septum (tumor)	0.3	0.1
	RBC	Blood	0.1	0.2
Monkey	CL-1	Chimpanzee liver	3.1	2.0
	Vero	African green kidney	0.2	0.2
	BSC-1	Cercopithecus kidney	0.6	0
	FRHL-1	Rhesus lung	0	0
	COS-7	African green	0	0
Mouse	NIH 3T3	Fibroblast	0.1	0.1
	Lowy EJ	Fibroblast	0.2	0
	C127	Fibroblast	0.2	0.1
	L	Fibroblast	0.1	0.1
Bovine	MDCK	Kidney	0	0.1
Goat	GT	Turbinate	0.2	0

[a] Confluent monolayers in 48-well cluster plates were used to assay the binding of radiolabeled virus and antibody as described in Materials and Methods.

[b] The numbers reflect the percentage of radiolabeled virus or antibody bound to cells after subtraction of the percentage of virus and antibody bound in the presence of 30 μg of unlabeled receptor antibody.

TABLE 3. Characterization of the HRV minor group of viruses

HRV Serotype[a]	% Binding inhibition by HRV-2[b]	% Binding to mouse cells[c]
1A	64	34
1B	74	24
2	62	11
29	75	18
30	79	26
31	54	11
47	71	17
62	57	19
15	0	1

[a] [^{35}S] methionine-labeled virus preparations were prepared and purified as previously described (1).

[b] Competition binding assays on HeLa R-19 cell monolayers (1) involved preadsorption of saturating amounts of purified HRV-2 (10^7 PFU) before binding of the ^{35}S-labeled HRVs shown. The percent binding inhibition was determined by comparison to normal binding on untreated monolayers.

[c] Binding assays were to NIH 3T3 cell monolayers in 48-well cluster plates for 1 h at 34° as described in Materials and Methods.

Convinced that the receptor antibody had the precise specificity desired, we expanded our HRV collection to 88 serotypes and assayed each against the receptor antibody block to confirm our previous prediction that over 80% of HRVs would utilize a single receptor. The results of this extensive binding study illustrated that 78 of the 88 HRV serotypes (HRV-3 through -28, -32 through -43, -45, -46, -48, -50 through -52, -54 through -61, -63 through -81, -83 through -86, -88, and -89; and HRV-Hanks) tested share a single cellular receptor. Six new HRV serotypes (HRV-1B, -29 through -31, -47, and -62) were found which were not inhibited in the presence of receptor antibody. To rule out the possibility of a third HRV receptor group, we used the six newly determined minor HRV serotypes in competition binding assays against HRV-2. These viruses competed with HRV-2 for a single receptor site that was clearly different from that used by the major group of HRVs (Table 3). Previous work had suggested that the minor HRV serotypes were also capable of binding to mouse cells (1, 20). As a further demonstration that the six new serotypes that were not blocked by the receptor antibody belonged to the minor HRV group, binding studies were conducted on mouse 3T3 cells. All six could bind as well to mouse 3T3 cells as HRV-1A and HRV-2 (Table 3). HRV-15, which belongs to the major HRV group, was unable to bind to mouse cells and served as a control.

Characterization of the receptor antibody. To further study the interaction of virus and cellular receptor, a membrane binding assay was developed. The advantages of using membranes instead of cells is that the assay can be standardized, day-to-day variations of tissue culture are eliminated,

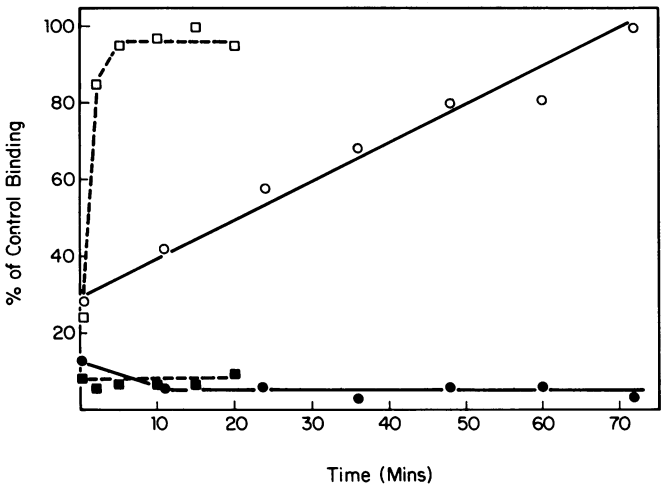

FIG. 1. Binding kinetics of HRV and receptor antibody in a membrane binding assay. Crude membrane preparations were prepared as described in Materials and Methods. Assays (0.22 ml) containing 1.1 mg of membranes and 7×10^4 cpm of either [^{35}S]methionine-labeled HRV-15 (○) or ^{125}I-labeled receptor antibody (□) in PBS were incubated at 25°C. The amount of nonspecific binding of radiolabeled HRV-15 (●) or receptor antibody (■) was determined by setting up parallel assays which contained 20 μg of receptor antibody. At the times indicated, 30-μl samples were removed, and the amount of binding was determined as described in Materials and Methods.

and results are not affected by the eclipse of the virus. To characterize the assay, crude membrane preparations were incubated with ^{35}S-labeled HRV-15 for up to 72 min. Membranes were then pelleted, and the percentage of ^{35}S radioactivity in the pellet fraction was determined as described in Materials and Methods. Similar to binding studies with intact cells (1), the virus required about 1 h to reach maximum binding (Fig. 1). The addition of receptor antibody completely eliminated this binding. Binding kinetics of ^{125}I-labeled receptor antibody differed dramatically from the virus. Receptor antibody attachment occurred within 1 min and was also inhibited by the addition of unlabeled antibody. To show a direct relationship between receptor antibody concentration and inhibition of HRV binding, binding studies were repeated in the presence of increasing amounts of receptor antibody. A direct correlation was observed between inhibition of radiolabeled HRV-14 and HRV-15 binding and the amount of receptor antibody added to the membranes (Fig. 2). The differences observed between HRV-14 and HRV-15 most likely reflect differences in the binding efficiency between the viruses (see below).

Repeated attempts to block receptor antibody attachment with saturating amounts of HRV-15 failed to effectively inhibit antibody attachment (data not shown). We believe that this is due to the greater avidity of the antibody for the receptor. To test this hypothesis, [^{35}S]methionine-labeled HRV-2, HRV-14, and HRV-15 were bound to membranes. Unbound virions were removed by repeated washings of pelleted membrane-virus complex. The membrane-virus complexes were then incubated with increasing quantities of purified receptor antibody, and the release of radiolabeled virus was measured. The antibody was capable of releasing nearly 90% of HRV-14, a weak binding serotype, and 30% of HRV-15, a strong binding serotype (Fig. 3). HRV-2, which served as a control, belongs to the minor group of HRVs and showed no release of virus from the membrane complex.

Can HRVs bypass the receptor block? Since the viral attachment site is highly conserved among the numerous HRV serotypes, the location of this site on the viral capsid is of great importance. Earlier studies by Sherry and Rueckert (18) have demonstrated that natural variants preexist in HRV-14 stock cultures which are resistant to neutralization by neutralizing antibodies. Isolation of a receptor variant would allow us to obtain specific sequence data which would help map the viral attachment site. To screen for the presence of naturally occurring variants, 10^{-7} dilutions of partially purified stock preparations of HRV-2, HRV-14, HRV-15, and HRV-39 were plated on 60-mm plates of HeLa R-19 cells and overlaid with agar. Two additional sets of plates were infected with 0.5 ml of undiluted stock preparation in the presence or absence of receptor antibody. After a 3-day incubation at 34°C, agar overlays were removed, and plates were stained to visualize plaques. Table 4 shows that each of the four viral stock solutions contained approximately 10^9 PFU/ml. Direct infection of plates with undiluted virus results in a multiplicity of infection of greater than 2×10^3 and completely destroyed the cell monolayers. However, in the presence of the receptor antibody, not a single plaque was found in plates infected with the major HRV serotypes 14, 15, and 39. HRV-2 again served as a control and showed no evidence of plaque reduction in the presence of the receptor antibody.

A second plaquing study was initiated in which HRV-15 virions were allowed to attach and enter HeLa cells before the addition of receptor antibody. The addition of 30 μg of receptor antibody at 0.5, 2, 4, or 6 h after viral adsorption also resulted in complete inhibition of plaque formation. Although no direct mutagenesis has been tried, these results clearly show that the virus cannot easily mutate or bypass this receptor blockage. In addition, the latter experiment

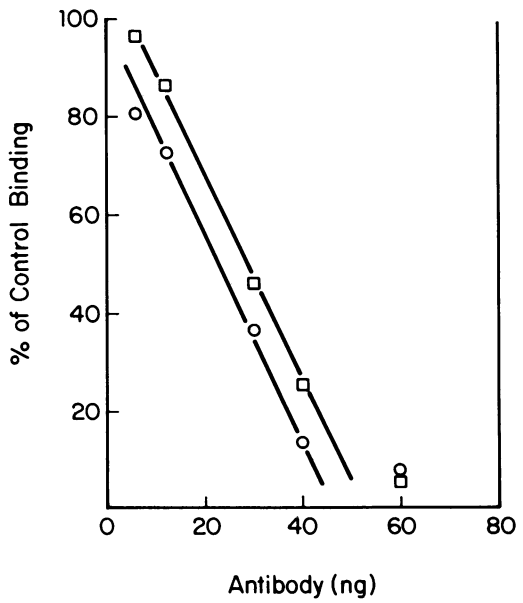

FIG. 2. Inhibition of HRV binding to HeLa membranes by receptor antibody. Membrane binding assays (40 μl) of [^{35}S]methionine-labeled HRV-14 (○) and HRV-15 (□) were performed as described in the Fig. 1 legend. Purified receptor antibody was added as indicated, and the binding inhibition was determined by comparison with control assays.

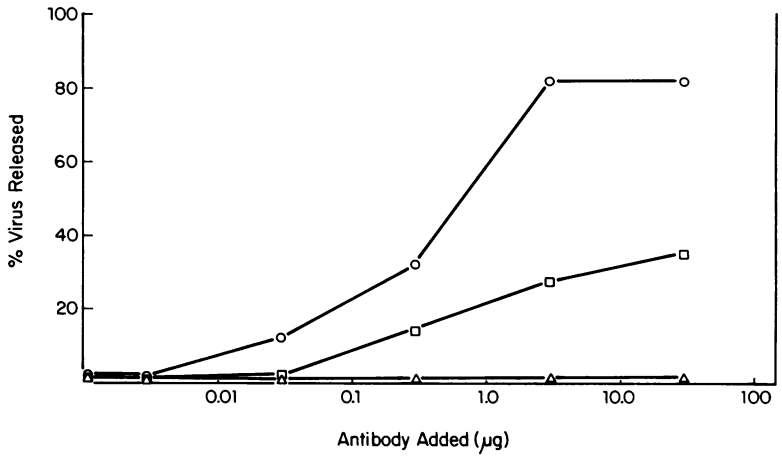

FIG. 3. Receptor antibody-mediated release of bound HRVs. Membrane-virus complexes were prepared by incubation of 960 μg of crude membranes with ^{35}S-labeled HRVs (5×10^4 cpm) for 1 h at 25°C in a total volume of 0.14 ml. The membrane-virus complexes were then separated from unattached virus by pelleting (12,000 × *g* for 2 min) and washing with PBS. The final membrane-receptor pellets were suspended in 130 μl of PBS and distributed into six tubes. Purified receptor antibody was added to each of the tubes as indicated, and the tubes were incubated at 25°C for 1 h. The membranes were then pelleted, and the amount of radiolabeled HRV-14 (○), HRV-15 (□), and HRV-2 (△) that was still associated with the membranes was determined.

demonstrates that HRVs do not infect neighboring cells by any mechanism except receptor-mediated entry.

DISCUSSION

Traditional approaches to controlling HRV infections do not appear to be feasible, since there is a vast number of antigenically distinct serotypes. A greater understanding of the structural and molecular characteristics of these viruses will be necessary for the design of novel anti-HRV agents. Of particular importance will be the discovery of conserved regions among the HRV serotypes which map to important target sites of the virus. The cellular receptor utilized in the attachment of HRVs to susceptible cells represents such a discovery. The data presented clearly demonstrate that the vast majority (89%) of HRV serotypes uses a single cellular receptor for entry into susceptible cells. This was shown by reciprocal competition binding assays (1, 12) and by isolation of a mouse monoclonal antibody capable of blocking infection of HeLa cells by the major group of HRVs.

Three criteria were used to prove that the receptor monoclonal antibody had the precise specificity defined by biological studies. First, the receptor antibody blocked attachment and infection by 20 of the 24 HRV serotypes representing the major group of HRVs and not the 4 HRV serotypes which belong to the minor HRV group and which bind to a different cellular receptor. Second, the receptor antibody failed to protect cells from infection by numerous other viruses including poliovirus, group B coxsackieviruses, and hepatitis A virus. Besides HRVs, the only other virus group to be inhibited by the receptor antibody was the group A coxsackieviruses. This was expected, since previous studies by Lonberg-Holm et al. (12) had shown that coxsackievirus A21 and HRV-14 competed for a single receptor. However, this protection by the receptor antibody has only been observed in HeLa cells. The receptor antibody shows no protective effect against coxsackievirus A infection of mouse cells which lack receptors for receptor antibody attachment but are susceptible to coxsackievirus A infection (data not shown). This result appears to suggest that group A coxsackieviruses can recognize either two different receptors or a unique region on the same receptor that is different than that utilized by HRVs. None of the serotypes in the major group of HRV serotypes has been shown to attach to mouse cells.

TABLE 4. Plaque assay for variants capable of bypassing the receptor antibody block

HRV serotype[a]	Viral plaques[b] at the following virus dilution:		
	10^{-7}	10^0	10^0 + antibody
2	62	TNTC[c]	TNTC
14	40	TNTC	0
15	67	TNTC	0
39	98	TNTC	0

[a] Concentrated stock preparations of HRV-2, HRV-14, HRV-15, and HRV-39 were grown and purified as previously described (1).

[b] Samples containing 0.5 ml of a 10^{-7} dilution or undiluted virus stocks were adsorbed to HeLa cell monolayers in the presence or absence of 30 μg of receptor antibody for 1 h at 34°C. Unadsorbed virus was removed, and the cells were overlaid with 5 ml of McCoy 5A media containing 1% (wt/vol) DEAE dextran, 5% fetal calf serum, and 0.4% agar with or without receptor antibody as indicated. After a 3-day incubation at 34°C, agar overlays were discarded, and cells were stained with 1% (wt/vol) crystal violet in 20% ethanol to visualize plaques.

[c] TNTC, Too numerous to count (complete destruction of cell monolayer).

The third criterion used to show receptor antibody specificity involved parallel binding studies of radiolabeled HRV-15 and receptor antibody. HRVs are highly restricted to human hosts. In fact, no animal models have yet been discovered which can be infected with HRVs and show clinical symptoms of a common cold (20). The binding studies confirmed these findings, since HRV-15 and receptor antibody could attach only to cells of human origin. The only exception to this rule was chimpanzee liver cells, which showed significant binding to both virus and antibody. This result confirmed earlier studies in which HRVs were shown to replicate in chimpanzees (8, 9). Although chimpanzees are capable of shedding HRV, they do not develop clinical symptoms. Infection of chimpanzee liver cells with HRV-14 resulted in complete destruction of the cell monolayer and production of normal titers of progeny virus (data not shown). None of the other monkey, mouse, bovine, or goat

cell lines was able to bind either the HRV-15 or the receptor antibody. In no case could the receptor antibody bind to a cell line that the virus could not. This result clearly shows that the receptor antibody exhibits the same tissue tropism as the virus itself and confirms the specificity of the antibody. The finding of HRV receptors on cell lines derived from virtually all human organs demonstrates that the major HRV receptor is ubiquitous on human cells and not confined to cells located within the nasal cavity. An explanation as to why 5 of the 19 human-derived cell lines lacked receptors for the major group of HRVs is not apparent. Restriction of the virus to the nose and upper throat during an HRV infection is most likely not a result of receptor availability. Instead, other physiological properties of HRVs, such as acid lability and temperature sensitivity, may play an important role.

The receptor antibody showed a high degree of avidity for the receptor site on HeLa cells. This was evident in the instantaneous binding of antibody to the receptor. In addition, the receptor antibody had the capability of physically displacing HRV virions from receptors in which they were previously bound (Fig. 2). Perhaps the greatest evidence of the receptor antibody specificity and affinity for the HRV receptor was demonstrated by experiments where antibody blockage of the receptor was challenged by 10^9 PFU of virus. In these experiments, not a single viral plaque could form. This result strongly suggests that the receptor blockage is not the result of a steric hindrance but is due instead to direct competition binding of the antibody to the viral attachment site on cells.

Subsequent experiments in which the receptor antibody was added at various times after viral eclipse failed to select for viral mutants. Although no direct mutagenesis has been tried, it is apparent that the major group of HRVs cannot easily mutate around the attachment block. The viral attachment site on the viral capsid may involve the spatial arrangement of all four of the viral structural proteins (VP1, VP2, VP3, and VP4). Replacement of one or two amino acids in any one structural gene may not be sufficient to change the binding specificity of the virus. Multiple amino acid changes would probably affect the structural conformation and stability of the capsid and, therefore, result in a lethal mutation. The plaquing assays clearly demonstrate that HRV entry into susceptible cells is completely restricted to receptor-mediated attachment. There is absolutely no indication that HRVs could spread from cell to cell via a secondary route.

The normal functional role of the major HRV receptor remains unknown. In preliminary experiments, exposure of sparsely planted HeLa cell monolayers to a 1,000-fold excess of receptor antibody had no obvious effect on cell morphology or cell number over a 6-day period, which represented over six cell doublings (unpublished data). This appears to indicate that this cellular receptor is of minor importance for cell growth and division in cell culture. However, the finding of the HRV receptor on numerous human cells suggests that it must play some role in vivo. In conclusion, the receptor antibody has illuminated a new target area for control of HRVs and may represent a novel approach in controlling viral infection.

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