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Many Rhinovirus Serotypes Share the Same Cellular Receptor

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Twenty-four human rhinovirus serotypes were grown and purified by centrifugation in metrizamide density gradients. These preparations had a lower buoyant density (1.24 g/cm^3) and higher specific infectivities (1:24 to 1:240) than did rhinoviruses described previously (E. J. Stott and R. J. Killington, Annu. Rev. Microbiol. 26:503–524, 1972). Binding conditions in which the unique cellular receptors for virus attachment were saturated were determined for each serotype. Competition binding assays between pairs of serotypes allowed 20 of the 24 serotypes to be assigned to the same cellular receptor. The remaining four serotypes appeared to attach to a different cellular receptor. Since most serotypes were chosen for study at random, it seems likely that many of the yet unstudied rhinoviruses will share this common cellular receptor.

Picornaviruses attach to animal cells by specific receptors on the cell surface (2). Frequently, the possession of appropriate cellular receptors is sufficient to determine the host range for a particular virus. This can be illustrated by poliovirus, which replicates naturally only in cells of primate origin. Other genera of the *Picornaviridae* family, including the rhinoviruses, appear to bind to different cellular receptors (10, 11, 14, 20). The attachment of human rhinoviruses to cells is generally species specific (7), although some serotypes will replicate in cells of either monkeys (15) or chimpanzees (3). The rates at which human rhinoviruses attach to cells is temperature dependent and may vary by a factor of up to 20 (11). The number of rhinovirus receptor sites per cell of human origin has been estimated to be from 10^4 for serotypes 2 and 14 (11) to greater than 10^6 for serotype 18 (12). Such cellular receptors are sensitive to the action of proteolytic enzymes (18). Since the number of cellular receptors on the cell surface is limited, it is possible to saturate them with virus particles of one serotype and examine the binding of another serotype in competition binding assays. Previous analyses have shown that rhinovirus serotypes 1A and 2 share a common cellular receptor which is different from the cellular receptor utilized by serotypes 3, 14, and 51 (10, 11).

As many as 115 serotypes of human rhinoviruses have been identified (13) on the basis of serological reactions, with very little antigenic cross-reactivity among them (1). This investigation was initiated to establish if this large group of related viruses attaches to susceptible cells by a similarly large variety of cellular receptors.

MATERIALS AND METHODS

Cell and virus growth. HeLa cells were cloned, and a clone (designated HeLa R-19) was selected on the basis of its ability to support the rapid growth of rhinovirus. HeLa R-19 cells were propagated in monolayers in McCoy 5A medium containing 5% fetal calf serum. Mouse L cells were grown similarly.

Rhinovirus serotypes were obtained from the American Type Culture Collection and were grown in HeLa R-19 cell monolayers. As soon as working stock cultures were obtained, the authenticity of each serotype was verified by neutralization with the specific antiserum obtained from the same source. Rhinoviruses were grown by the infection of HeLa R-19 cells in plastic flasks (150 cm^2) at a multiplicity of infection of 1 PFU per cell and incubation at 34°C in McCoy 5A medium plus 2% fetal calf serum for ca. 20 h. Radioactively labeled rhinoviruses were grown similarly, except that Eagle minimal essential medium lacking methionine and serum was used for the incubation. [^{35}S]methionine was added to a concentration of 100 μCi/ml 5 h after infection. Typical virus yields equaled 3×10^8 to 1×10^9 PFU per flask.

Purification of viruses. Infected cell cultures were harvested when cells detached freely from the flask surface (usually after 16 to 24 h). The supernatant fluid was frozen and quickly thawed to release virus particles from the cells. After clarification by centrifugation at slow speed (4,000 × *g* for 5 min), polyethylene glycol 6000 and NaCl were added to 7 and 2.2%, respectively, and the mixture was stirred at 4°C for 4 to 16 h. The precipitated virus was recovered by centrifugation (10,500 × *g* for 15 min) and suspended in R buffer (10 mM Tris-hydrochloride [pH 7.5], 0.2 M NaCl, 50 mM $MgCl_2$, 10% [wt/vol] glycerol). Sodium deoxycholate and Nonidet P-40 were added to 0.3 and 0.6%, respectively, for 30 min at 4°C, and the suspension was clarified by centrifugation (4,000 × *g* for 5 min). The supernatant (7.5 ml) was layered over a 5-ml linear density gradient of 40 to 60% (wt/vol) metrizamide in R buffer lacking glycerol. Isopycnic banding of the virus sample was achieved by centrifugation for 24 h at 150,000 × *g* in a Beckman SW40 rotor. Virus bands were generally the densest of the visible bands in the gradient and were harvested visually. After dilution in 10 volumes of R buffer, the virus was repelleted by centrifugation at 200,000 × *g* for 2 h. Virus pellets were suspended in a small volume of phosphate-buffered saline and stored frozen at −70°C. Infectivity titrations were done by plaque assay in HeLa R-19 cells by standard procedures.

Radioactively labeled virus was concentrated by polyethylene glycol precipitation as described above but was generally layered over an 11-ml linear density gradient of 5 to 20% (wt/vol) sucrose in R buffer. After centrifugation for 2 h at 150,000 × *g* in a Beckman SW40 rotor, the gradients were fractionated (0.4 ml) into tubes containing 0.2 ml of 0.09% bovine serum albumin in R buffer, and portions of each fraction were analyzed for ^{35}S radioactivity. Infectivity profiles across these gradients indicated that infectious particles were associated only with the more rapidly sedimenting peak of radioactivity. Peak fractions of radioactivity were frozen directly, repelleted by centrifugation, or resediment-

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ed in metrizamide gradients. When the last procedure was done, infectivity determinations confirmed that the radioactivity peak coincided with the infectivity peak. Density measurements of fractions from metrizamide gradients were done by accurately weighing 20-μl capillary tubes filled with the test solution. Similar values were obtained by weighing 20-μl volumes of gradient fractions expelled from an automatic pipetter.

Rhinovirus binding assays. Radioactively labeled rhinoviruses, purified either by banding at their buoyant densities in metrizamide or by rate zonal sedimentation in sucrose gradients, were used to study the binding of viruses to host cells. Either method of preparation gave the same results. ^{35}S-labeled virus preparations were diluted in 30 to 50 volumes of Hanks balanced salt solution immediately before binding assays.

HeLa R-19 cells were seeded into the wells of either 24- or 48-well plastic culture plates (Costar or Corning brands) at 25×10^5 or 15×10^5 cells per well and incubated at 37°C for 24 h. Growth medium was removed, and the cells were washed once with 0.5 ml of Hanks solution (GIBCO Laboratories). [^{35}S]methionine-labeled virus (5×10^3 to 10×10^3 cpm) in 60 to 90 μl of Hanks solution was added to each well. Binding was allowed to occur at 34°C with gentle rocking and was stopped by removal of the supernatant inoculum and two gentle washes with 0.2 ml of phosphate-buffered saline. The intact cell monolayer was then dissolved in 1% sodium dodecyl sulfate and transferred to a scintillation vial for direct estimation of ^{35}S radioactivity. Duplicate assays were done when appropriate, and the results showed good agreement.

Blocking of rhinovirus binding by the homologous virus was demonstrated by adding increasing amounts of unlabeled virus to a series of cell monolayers for a 40-min period. Excess virus was removed, and the cells were washed once with Hanks solution. Challenge binding was then done with 60 μl of ^{35}S-labeled virus in Hanks solution, and the radioactivity bound during the 40-min incubation was estimated and expressed as a percentage of the radioactivity bound in a control well to which no blocking virus had been added.

Blocking caused by the prior binding of heterologous viruses was assayed similarly. Concentrations of each virus that caused (in most cases) a greater than 80% blocking of binding of the homologous virus were established from the assays described above. The appropriate concentrations of viruses were then used to saturate the receptor sites before challenge bindings with ^{35}S-labeled heterologous viruses as described above. In such assays, the binding of the labeled virus to control cells was used for calculating the percentages of binding.

Materials. Metrizamide was manufactured by Nyegaard and Co., Oslo, Norway, and L-[^{35}S]methionine (1,200 Ci/mmol) was purchased from Amersham International.

RESULTS

Purification of rhinoviruses. Virus preparations used in studying the interactions of rhinoviruses with HeLa cells were recovered from infected cell lysates by concentration with polyethylene glycol. Virus particles were resolved from contaminating debris by centrifugation overnight in preformed metrizamide density gradients. Rhinoviruses invariably formed visible bands at a higher density than did contaminating particles. Rhinoviruses prepared by centrifugation in metrizamide solutions consisted of uniform, characteristic particles when examined by electron microscopy. Very few empty capsids were present (data not shown). As an additional check on the purity of viruses prepared in this way, samples were radioiodinated by standard procedures, and the labeled proteins were analyzed by sodium dodecyl sulfate-acrylamide gel electrophoresis. The only labeled

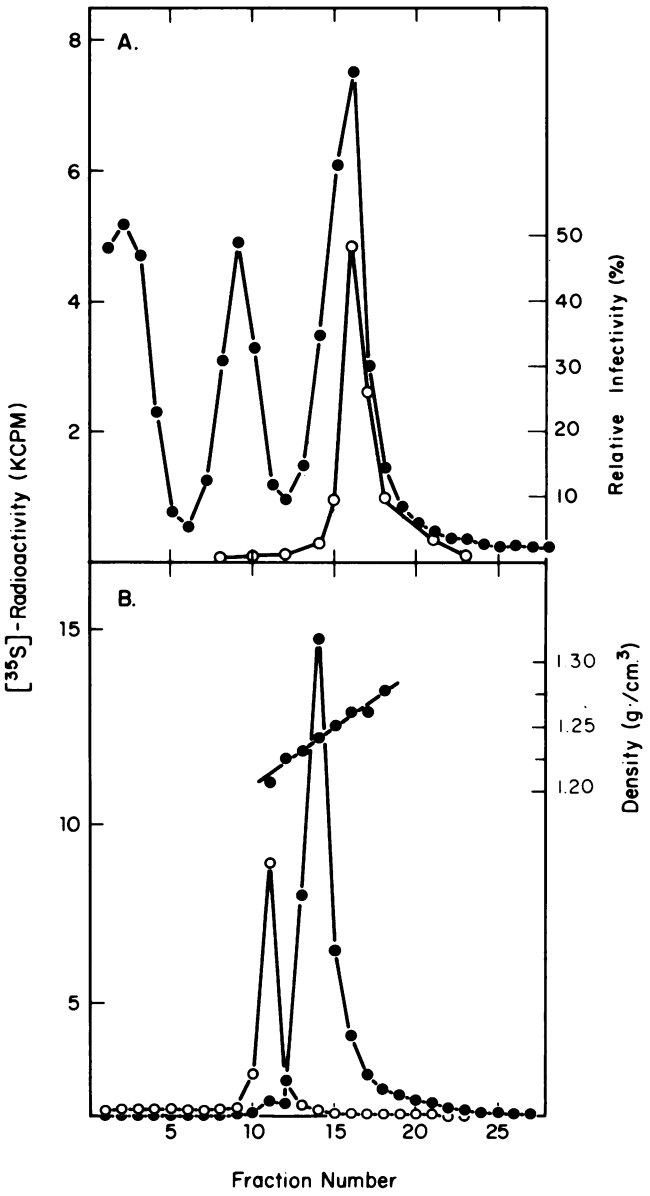

FIG. 1. Density gradient centrifugation of rhinovirus particles. (A) [^{35}S]methionine-labeled rhinovirus type 89 was prepared as described in the text and analyzed by centrifugation for 2 h at 150,000 × *g* through a 5 to 20% (wt/vol) sucrose density gradient. The gradient was fractionated from the top into 0.4-ml fractions, and a portion of each was used for the determination of ^{35}S radioactivity (●) or infectivity (○). (B) Samples from the two peak fractions representing intact virions (●) and empty capsids (○) in (A) were diluted with 7.5 ml of R buffer and layered separately over two 5-ml linear gradients of 40 to 60% (wt/vol) metrizamide. After centrifugation for 48 h at 150,000 × *g* in a Beckman SW40 rotor, the gradients were fractionated into 60 0.2-ml fractions. Total radioactivity was determined, and the central part of each profile was depicted. Density determinations on the gradient fractions containing the virus particles were done as described in the text. Sedimentation in each case was from left to right.

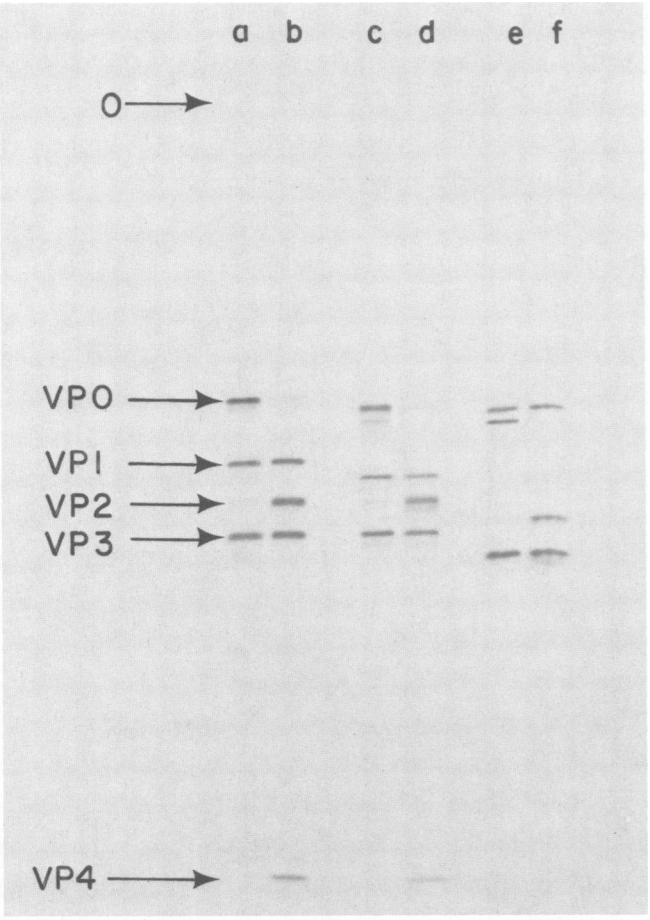

FIG. 2. Polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled rhinovirus proteins. [^{35}S]methionine-labeled serotypes 17, 5, and 15 were grown, and the virus particles were resolved from the empty capsids by rate zonal centrifugation (Fig. 1A). Portions of either the empty capsids (a, c, and e) or the virus particles (b, d, and f) of serotypes 17, 5, and 15 were disrupted with sodium dodecyl sulfate and 2-mercaptoethanol and analyzed on a 12% acrylamide gel by the method of Laemmli (9). Radioactivity in the dried gel was detected by autoradiography. O, Origin.

products detected by autoradiography were the viral structure proteins for the particular serotype (data not shown).

Rhinovirus preparations for serotypes 1A, 11, and 14 were examined by electron microscopy in the presence of a known concentration of standard latex particles. The concentrations of viral particles were determined, and these concentrations, when coupled with the infectivity titers of the preparations, allowed estimates to be made of the infectivity/particle ratios for the three viruses tested. These values of 1:24, 1:65, and 1:240 for serotypes 1A, 11, and 14, respectively, were more than 20 times the previous estimates (12) made with viruses purified by centrifugation in cesium chloride density gradients.

Rhinoviruses grown in the presence of [^{35}S]methionine were usually resolved from empty capsids and other labeled components by rate zonal centrifugation in sucrose density gradients (Fig. 1A). Fractions containing the virus were either frozen directly or diluted with buffer and analyzed further by centrifugation in metrizamide density gradients (Fig. 1B). In Fig. 1, parallel gradients containing either ^{35}S-labeled virus or empty capsids of serotype 89 were analyzed after centrifugation to equilibrium. The measured densities of the fractions containing empty capsids (1.21 g/cm^3) and intact virions (1.24 g/cm^3) are shown. Other rhinovirus serotypes sedimented to the same density value when analyzed similarly (data not shown).

The radiochemical purity of the rhinoviruses purified by sucrose gradients alone was checked by analysis of the labeled proteins after sodium dodecyl sulfate-gel electrophoresis. Profiles for both the empty capsids and the viral particles of serotypes 17, 5, and 15 are shown in Fig. 2. The VP0 protein present in the empty capsids can be readily identified, as it is cleaved to give VP2 and VP4 upon maturation of the virus particle. In the case of serotype 15, the VP0 protein migrated faster than did VP1, and the VP4 protein apparently lacked a methionine residue.

The proteins of all rhinovirus serotypes used in this study were analyzed similarly, and in all cases, negligible radioactivity was found in protein bands other than those of the viral structural proteins. Analysis of the proteins of viruses purified by metrizamide gradient banding revealed profiles indistinguishable from those shown in Fig. 2. Consequently, for many of the analyses shown below, ^{35}S-labeled virus particles purified only by sucrose density centrifugation were used.

Characteristics of binding of rhinoviruses to HeLa cells. [^{35}S]methionine-labeled viruses were adsorbed to HeLa R-19 cell monolayers at 34°C as the basis of the binding assay. Virus particles that failed to attach to the cells within the test period were removed, the monolayer was washed, and the residual radioactivity bound to the cell monolayer was determined. Such assays showed that the binding of viruses to cell monolayers was time dependent and specific for the cell type used in the monolayer (Fig. 3). Binding assays at

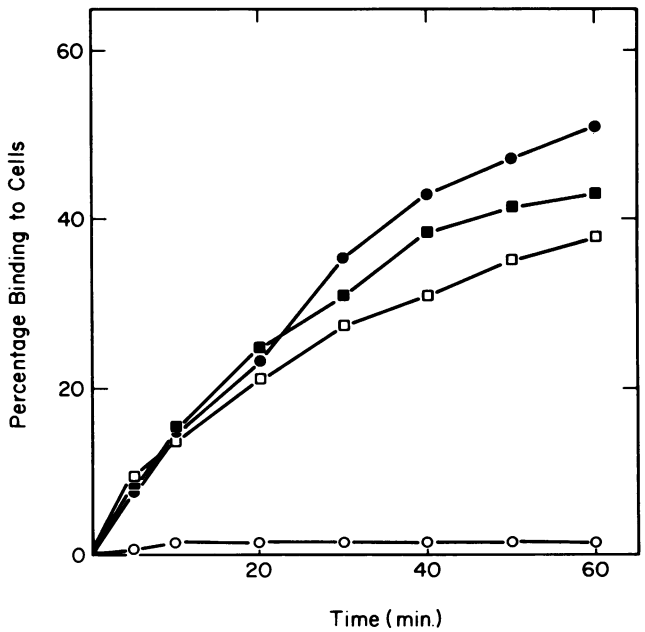

FIG. 3. Binding of radioactively labeled rhinovirus to cell monolayers. Rhinovirus type 1A was bound to parallel cultures of either L cells (●) or HeLa cells (■). The radioactivity bound to the cells at particular times was estimated by scintillation counting and expressed as a percentage of the radioactivity used for each culture. Rhinovirus type 36 was analyzed simlarly on either L cells (○) or HeLa cells (□).

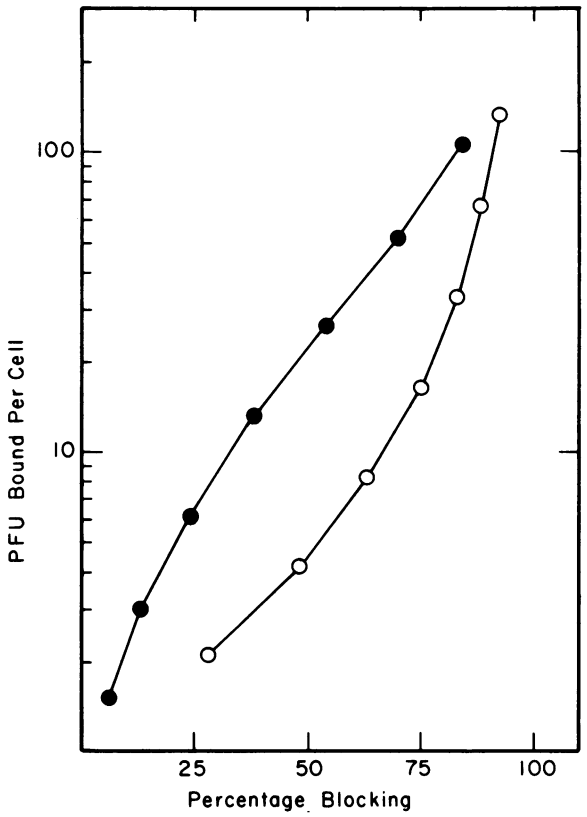

FIG. 4. Saturation kinetics for the binding of rhinoviruses to HeLa cells. Serial doubling dilutions of two preparations of rhinovirus types 9 (○) and 15 (●) were prepared in Hanks solution. A 90-μl volume of the most concentrated dilutions contained either 104 or 130 PFU per cell, respectively, when applied to cultures of 2.5×10^5 HeLa cells. The same volume of the other virus dilutions was added to additional cell cultures and allowed to adsorb at 34°C for 40 min. After removal of the inocula and washing of the cells, the homologous [^{35}S]methionine-labeled serotypes were added as described in the text. The percentage of blocking of binding caused by each dilution was determined.

temperatures lower than 34°C were not considered necessary, as virus binding continued to increase throughout the assay period. The binding of serotype 1A to either HeLa cells or L cells was similar. However, serotype 36 (like most other serotypes tested) bound well to HeLa cells but failed to bind to L cells. This negative result demonstrated that the binding of radioactively labeled virus was receptor specific and not the result of nonspecific adherence to the cells or the culture vessels.

In all assays described, the amount of radioactivity virus added was such that the multiplicity of infectious particles added per cell never exceeded one. Our estimates of the infectivity of our preparations and the published values for the number of receptor sites for rhinoviruses on human cells (11, 12) indicate that the conditions for the binding of radioactive viruses in these assays were well below saturation. Nevertheless, the proportion of any preparation of ^{35}S-labeled virus that bound to HeLa R-19 monolayers within a 1-h period was rarely above 50%.

Blocking of binding by homologous virus. Previous studies by Lonberg-Holm and Korant (11) have shown that saturation of the cell surface receptors can be achieved if the multiplicity of the adsorbed virus is high enough. Such conditions were determined for each of the purified rhinovirus serotypes used in this work. Figure 4 shows the homologous interference provided by the binding of serotypes 9 and 15 to subsequent challenge by the corresponding radioactively labeled viruses. In both cases, greater than 80% inhibition of binding was provided by the prior attachment of 100 to 130 PFU of the appropriate virus per cell. Such values, approaching saturation, were determined for preparations of each serotype before the effects of viruses in heterologous binding assays were studied.

Competition binding assays with heterologous viruses. Ten rhinovirus serotypes were chosen for an initial experiment to determine the number of receptor families that may be involved in the specific binding of rhinoviruses to host cells. Each serotype was allowed to bind to a series of 10 HeLa cell monolayers for 40 min in concentrations approaching saturation (Fig. 4). Excess virus was removed, and the monolayers were washed once. The corresponding [^{35}S]methionine-labeled viruses were added across the 10 rows to produce a complete matrix. After challenge exposure for a further 40 min, excess virus was removed, and the cells were washed twice before estimation of the residual radioactivity. In all cases except that of serotype 11, the viruses produced greater than 80% blocking of their corresponding homologous counterparts when compared with control binding values (Table 1). Heterologous viruses that were blocked at least 90% as effectively as the homologous virus were scored positive (+) to indicate blocking. Cases in which blocking was lower than 20% were scored negative (−), whereas intermediate percentages were scored positive/negative (+/−) to indicate equivocal results. Apart from serotype 14, most of these intermediate values showed greater than 65% blocking.

Serotypes 1A and 2 failed to block the binding of any other

TABLE 1. Competitive inhibition of virus binding by 10 pairs of heterologous rhinoviruses

Blocking serotype	Blocking of [^{35}S]methionine-labeled challenge serotype[a]									
	1A	2	11	3	5	9	14	15	39	41
1A	+	+/−	−	−	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−	−	−
11	−	−	−	+	+	+	+	+	+	+
3	−	−	−	+	+	+	+	+	+	+
5	+/−	−	−	+	+	+	+	+	+	+/−
9	−	−	−	+	+	+	+	+	+/−	+
14	−	−	−	+/−	+/−	+/−	+	+/−	+/−	+/−
15	−	−	−	+	+	+	+	+	+	+
39	−	−	−	+	+	+/−	+/−	+	+	+/−
41	−	−	−	+	+	+	+	+	+/−	+

[a] +, Binding blocked by at least 90%; −, binding blocked by less than 20%; +/−, binding blocked between 20 and 90%.

TABLE 2. Competitive inhibition of virus binding by 12 pairs of heterologous rhinoviruses

Blocking serotype	Blocking of [^{35}S]methionine-labeled challenge serotype[a]											
	1A	2	11	9	14	15	32	36	51	59	67	89
9	−	−	−	+	+	+	+	+/−	+	+	+	+
11	−	−	−	+	+	+	−	+	+	−	−	+
14	−	−	−	+/−	+	+/−	+/−	+/−	+/−	+/−	+/−	+/−
15	−	−	−	+	+	+	+	+	+	+	+	+
32	−	−	−	+	+	+/−	+	+	+	+/−	+	+
36	−	−	−	+	+	+	+	+	+	+	+	+
51	−	−	−	+	+	+	+	+	+	+	+	+
59	−	−	−	+	+	+	+	+	+	+	+	+
67	−	−	−	+/−	+/−	+	+	+/−	+	+/−	+	+
89	−	−	−	+	+	+	+/−	+	+	+	+	+

[a] +, Binding blocked by at least 90%; −, binding blocked by less than 20%; +/−, binding blocked between 20 and 90%.

serotype. Serotype 11 behaved irregularly in that satisfactory blocking of homologous virus was not established even at very high concentrations (data not shown). However, virus concentrations comparable to those used for the other serotypes did produce heterologous blocking for most of the other serotypes. The remaining seven serotypes fell into a group showing very similar binding properties.

A similar analysis (Table 2) included another six rhinovirus serotypes. Several of the serotypes from Table 1 were included to establish a bridge between the two sets of results. The binding patterns of all six of the new serotypes conformed with those of the major group of seven serotypes in the first matrix. Again, serotypes 1A and 2 failed to show any cross-blocking reaction with other serotypes. In all cases except that of serotype 11, the reciprocal binding or blocking assays produced consistent results.

A further eight serotypes were also analyzed (Table 3). Serotypes 44 and 49 resembled serotypes 1A and 2 in their binding interactions with the other serotypes. Serotype 17 resembled serotype 11 in that the binding of radioactive particles could not be prevented by the prior binding of any serotype.

DISCUSSION

Purification of rhinoviruses by banding in metrizamide gradients resulted in virus preparations with specific infectivities at least 20 times higher than those reported for viruses purified similarly in cesium chloride gradients (8). This observation is consistent with various reports (5, 16, 17) that high concentrations of cesium salts alter the structural integrity of rhinovirus particles. Such changes alter the sedimentation characteristics (8), the antigenic neutralization by specific antisera (5), the stability of the viral RNA (4), and the buoyant density of the virus particles (8, 17). When the buoyant densities of rhinoviruses were measured by equilibrium sedimentation in metrizamide solutions, a value of 1.24 g/cm^3 was obtained for serotype 89 (Fig. 1B). This value is considerably lower than the reported values for other rhinoviruses of 1.38 to 1.41 g/cm^3 (see reference 19 for further references) obtained by centrifugation in density gradients made with cesium salts. However, the lower figure reported here is more consistent with density values of 1.26 to 1.27 g/cm^3 reported with potassium citrate (6).

Rhinoviruses purified in metrizamide gradients had high specific infectivities and appeared to be an ideal starting material for the examination of cell-virus interactions. A simple assay for measuring the binding of [^{35}S]methionine-labeled rhinovirus particles to monolayers of HeLa cells was established. The binding characteristics of rhinoviruses were found to be specific and similar to those described previously (10–12). The relatively high specific activity of the radiolabeled virus particles allowed a low multiplicity of infectious particles to be used in the binding assays. Thus, the saturation level of the cell receptors was never approached during the binding of ^{35}S-labeled virus. The fact that less than 50% of [^{35}S]methionine-labeled virus would bind to an excess of cell receptors during a 40- to 60-min period may be explained in terms of the infectivity/particle ratio of virus preparations, where much of the radioactivity was present in noninfectious, nonbinding particles. The lowest level of binding was shown by serotype 14, for which binding values of only 10% were reproducibly obtained. This observation is consistent with a previous report that serotype 14 binds relatively poorly to host cells (10).

Saturating levels of virus particles could be added to cells such that further addition of virus resulted in negligible further binding (Fig. 4). This principle could be extended to examine the interaction of heterologous viruses with cell receptors. Such experiments have been reported in a limited way in previous reports (10, 11). The conclusions of those researchers were that at least two families of cellular receptors were present on human cells; serotypes 1A and 2 shared the same receptor site, whereas serotypes 3, 14, and 51 shared another site (10, 11). Further unpublished observations of these researchers (10) have indicated that serotypes 5, 15, 39, and 41 are also related to serotype 14.

Our work considerably extends these observations and examines the interrelationships among 24 serotypes in an almost reciprocal fashion. Serotypes 1A, 2, 44, and 49 showed virtually no cross-reactivity with any of the other 20 serotypes examined (Table 1, 2, and 3). This result confirmed previous observations that serotypes 1A and 2 were

TABLE 3. Competitive inhibition of virus binding by eight pairs of heterologous rhinoviruses

Blocking serotype	Blocking of [^{35}S]methionine-labeled challenge serotype[a]							
	12	17	22	44	49	58	60	66
1A	−	−	−	+	+	−	−	−
2	−	−	−	+	+	−	−	−
3	+	−	+/−	−	−	+/−	+	+
5	+	−	+/−	−	−	+	+	+
36	+	−	+	−	−	+	+	+
51	+	−	+	−	−	+	+	+
66	+	−	+	−	−	+	+	+

[a] +, Binding blocked by at least 90%; −, binding blocked by less than 20%; +/−, binding blocked between 20 and 90%.

separate from other rhinoviruses examined in terms of their binding reactions with HeLa cells. With the exception of serotypes 11 and 17, the other 18 serotypes appeared to conform to a single pattern with respect to their capacities to block the binding of related serotypes. Within this group, no serious conflict occurred with respect to reciprocal blocking reactions.

There was a very sharp distinction between results that were scored as + or − in Tables 1, 2, and 3. Assays were scored − when the blocking of radioactive virus was less than 20% of the control value. Conversely, when the blocking of binding was at least 90% as effective as that of the homologous virus, then assays were scored +. Most of the intermediate values in Tables 1 and 2 showed blocking effects at least 65% as effective as that of the homologous virus, and so blocking of these serotypes can be considered positive rather than negative. The exceptions to this generalization were serotypes 14 and 67, in which a series of intermediate values were reproducibly obtained. A possible explanation for this result would be that most serotypes bind to receptors with higher affinities than do serotypes 14 and 67 and can displace these weakly binding serotypes upon challenge. The converse of this argument is sustained by the results in Tables 1 and 2, which show that [^{35}S]methionine-labeled serotype 14 was not able to displace any of the previously bound viruses (with the exception of serotypes 1A and 2), and so all assays involving a challenge binding with radioactive serotype 14 were clearly scored as being blocked (+). The results with serotype 67 are similar but less certain, as this serotype did not grow to high titers in HeLa cells or purify well by gradient centrifugation.

Serotype 11 showed irregular behavior in these binding assays. The binding of [^{35}S]methionine-labeled serotype 11 was not blocked by any of the viruses tested, including itself. The same results were obtained when a different preparation of labeled virus was used. In both cases, the gradient profile and the pattern of labeled proteins (data not shown) by gel electrophoresis were consistent with a pure rhinovirus preparation. In a similar way, a fresh preparation of unlabeled serotype 11 again failed to satisfactorily block the cellular receptors for homologous binding. However, a concentration of serotype 11 of 600 PFU per cell—comparable to that used for other serotypes—did effectively block the binding of 10 of the other serotypes (Tables 1 and 2). This observation indicates at least some relationship with the major cell receptor family established in this work. Serotype 17 behaved similarly to serotype 11 in that its cellular receptor site could not be saturated by any virus (Table 3), including itself (data not shown). The results for these two serotypes indicate that they may bind to more than one receptor site on the host cells or that they may bind to cells in a nonspecific fashion.

This study confirms that two families of cell receptors exist for the attachment of human rhinoviruses. However, considerably more perspective is added, as there now appears to be one relatively minor group (serotypes 1A, 2, 44, and 49) and perhaps only one major group comprising a majority of the serotypes. Although only 24 serotypes were included in this analysis, most of them were chosen at random and belonged to the major grouping. It seems possible that an even wider selection of unstudied rhinovirus serotypes may not alter the pattern of relationships already established. If this hypothesis is true, then it seems reasonable to conclude that most of the unstudied rhinovirus serotypes will share the same cellular receptor as the major group described here.

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LITERATURE CITED

1. **Cooney, M. K., J. P. Fox, and G. E. Kenny.** 1982. Antigenic groupings of 90 rhinovirus serotypes. Infect. Immun. **37**:642–647.
2. **Crowell, R. L., and B. J. Landau.** 1983. Receptors in the initiation of picornavirus infections, p. 1–42. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 18. Plenum Publishing Corp., New York.
3. **Dick, E. C.** 1968. Experimental infections of chimpanzees with human rhinovirus types 14 and 43. Proc. Soc. Exp. Biol. Med. **127**:1079–1081.
4. **Gauntt, C. J.** 1974. Fragmentation of RNA in virus particles of rhinovirus type 14. J. Virol. **13**:762–764.
5. **Gauntt, C. J., M. M. Griffith, J. R. Sauck, R. H. Upson, and E. C. Carlson.** 1975. Properties and origins of infectious rhinovirus type 14 particles of different buoyant densities. J. Virol. **16**:1265–1272.
6. **Gerin, J. L., W. R. Richter, J. D. Fenters, and J. C. Holper.** 1968. Use of zonal ultracentrifuge systems for biophysical studies of rhinoviruses. J. Virol. **2**:937–943.
7. **Haff, R. F., B. Wohlsen, E. E. Force, and R. C. Stewart.** 1966. Growth characteristics of two rhinovirus strains in WI-26 and monkey kidney cells. J. Bacteriol. **91**:2339–2342.
8. **Korant, B. D., K. Lonberg-Holm, J. Noble, and J. T. Stasny.** 1972. Naturally occurring and artificially produced components of three rhinoviruses. Virology **48**:71–86.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Lonberg-Holm, K., R. L. Crowell, and L. Philipson.** 1976. Unrelated animal viruses share receptors. Nature (London) **259**:679–681.
11. **Lonberg-Holm, K., and B. D. Korant.** 1972. Early interaction of rhinoviruses with host cells. J. Virol. **9**:29–40.
12. **Medrano, L., and H. Green.** 1973. Picornavirus receptors and picornavirus multiplication in human-mouse hybrid cell lines. Virology **54**:515–524.
13. **Melnick, J. L.** 1980. Taxonomy of viruses. Prog. Med. Virol. **26**:214–232.
14. **Philipson, L., S. Bengtsson, S. Brishammer, L. Svenner-Holm, and O. Zetterquist.** 1964. Purification and chemical analysis of the erythrocyte receptor for haemagglutinating enteroviruses. Virology **22**:580–590.
15. **Pinto, C. A., and R. F. Huff.** 1969. Experimental infection of Gibbons with rhinovirus. Nature (London) **224**:1310–1311.
16. **Rowlands, D. J., D. V. Sangar, and F. Brown.** 1971. Buoyant density of picornaviruses in caesium salts. J. Gen. Virol. **13**:141–152.
17. **Rueckert, R. R.** 1976. On the structure and morphogenesis of picornaviruses, p. 131–213. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 6. Plenum Publishing Corp., New York.
18. **Stott, E. J., and G. F. Heath.** 1970. Factors affecting the growth of rhinovirus 2 in suspension cultures of L132 cells. J. Gen. Virol. **6**:15–24.
19. **Stott, E. J., and R. A. Killington.** 1972. Rhinoviruses. Annu. Rev. Microbiol. **26**:503–524.
20. **Zajac, I., and R. L. Crowell.** 1965. Location and regeneration of enterovirus receptors of HeLa cells. J. Bacteriol. **89**:1097–1100.