Characterization of a YAC-1 Mouse Cell Receptor for Group B Coxsackieviruses

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A receptor on YAC-1 cells, a mouse T-lymphoma cell line, bound all six serotypes of the group B coxsackieviruses (CVB). In addition, the cells produced infectious virus. Each of the CVB competed for the same receptor on YAC-1 cells. CVB3 bound relatively slowly to YAC-1 cells ($k = 4 \times 10^{-11} \text{ min}^{-1} \text{ cell}^{-1}$), and there were only 500 attachment sites per cell. A rabbit antiserum prepared against the HeLa cell receptor protein Rp-a specifically inhibited the binding of CVB1 and CVB3. A virus-receptor complex with CVB3 could be isolated from detergent (0.5% sodium deoxycholate, 1% Triton X-100)-solubilized YAC-1 plasma membranes. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the iodinated virus-receptor complex revealed a band with the same mobility as Rp-a. The results suggested that the YAC-1 receptor for CVB resembles that of the HeLa cell receptor.

The initial event in the life cycle of a virus is attachment to receptors at the cell surface (14). The presence or absence of specific cellular receptors influences the host range of viruses (7, 16). Identification of cellular receptors and understanding of virus-receptor interactions are therefore important in understanding viral tropism and subsequent pathogenesis.

It is known that six serotypes of group B coxsackieviruses (CVB) compete for a binding site on HeLa cells that is distinct from the site for other picornaviruses (3, 4). A receptor protein (Rp-a) of approximately 50 kilodaltons was purified from HeLa cells and shown to combine specifically with CVB (15).

Since inbred mice have been used as models for studying CVB-induced pathogenesis (10-12), it is important to determine the relationship of the mouse cell receptor to the HeLa cell receptor for CVB. We now report preliminary studies on the characterization of a YAC-1 mouse T-lymphoma cell receptor for CVB and a comparison of this receptor with that on HeLa cells.

MATERIALS AND METHODS

Cells and viruses. The mouse T-lymphoma cell lines YAC-1 (American Type Culture Collection, Rockville, Md.), MKDG, and TK-11 were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Mouse fibroblast L cells, 3T3-L1 adipocytes, C57MG epithelial cells, the BGM kidney cell line, and the JJH strain of HeLa cells were grown in monolayers in minimal Eagle medium in Hanks balanced salt solution supplemented with 10% fetal calf serum. Mouse myeloma cells Sp2/0 were grown in the same medium in suspension. Human HeLa cells (Mandel strain) were grown in suspension as described previously (15).

The prototype strains of CVB, CVB1 to CVB6, that were used in this study have been reported previously (2). The variant coxsackieviruses CVB1-RD and CVB3-RD were derived by Reagan et al. (17) by serial passages of the parental viruses on human rhabdomyosarcoma (RD) cells.

Poliovirus type 1 was purchased from the American Type Culture Collection. Virus PFU was determined on either BGM or HeLa (JJH) cells. Methods for the growth and purification of unlabeled viruses, [³H]uridine-labeled viruses, and ¹⁴C-amino acid-labeled viruses were detailed previously (6, 15). An estimate of the number of particles per milliliter in purified pools of virions was based on the assumption that 1 A_{260} unit is equivalent to 10^{13} particles. We consistently obtained 1 PFU per 40 to 70 virions of CVB3 on BGM cells, which was approximately 10 times closer to a 1:1 ratio than for assays in HeLa cells (9). The specific activity of [³H]uridine-labeled CVB3 was found to be 4×10^5 virions cpm⁻¹.

Virus infectivity. Cells were infected with viruses at a multiplicity of 10 to 100 PFU cell⁻¹, and the cytopathic effect was determined daily for 3 days. For suspension cell cultures, virus-caused cell death was determined after trypan blue staining. The viable cell counts were compared with those of control cultures. Cell cultures resistant to viruses showed no significant difference from control cultures. CVB3 consistently produced 25 to 95% cell reduction overnight in YAC-1 cells, depending on the initial cell concentration and the amount of virus in the inoculum.

Virus attachment. Cells grown in suspension or removed from monolayers by trypsin were washed twice and suspended in phosphate-buffered saline containing 3% fetal calf serum and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7.4). The cells, at a final concentration of 2×10^7 cells ml⁻¹, were mixed at room temperature (RT) with radioactive virus. Directly after the addition of radioactive ligand, a portion of 50 μ l was removed and diluted with 2 ml of the ice-cold buffer described above. Other portions were removed at intervals, diluted, and then centrifuged. The supernatant fluids were removed, the pellets were dissolved in 0.5 ml of 0.5 M NH₄OH, and radioactivity was determined by liquid scintillation counting. Portions of the suspension of radioactive ligand plus cells were also counted without washing. The pseudo-first-order rate constant of attachment (k) is the fraction of virus which attaches per unit time per cell, at zero time when the cellular receptors are vacant. It was determined from the linear, initial attachment portion of the attachment kinetics curve

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TABLE 1. Correlation between susceptibility of cell lines to infection and their capacity to attach CVB3

Mouse cell line	% Virus attachment ^a	CPE ^b
L (fibroblast)	0.1	—
3T3-L1 (adipocyte)	0.2	—
C57MG (epithelial cell)	0.4	—
Sp2/0 (myeloma)	0	—
YAC-1 (T lymphoma)	9.8	+
MKD6 (T lymphoma)	0	—
TK-11 (T lymphoma)	0.2	—

^a Percentage of virus label which became cell associated at RT within 1 h of addition of 2×10^4 cpm of [³H]uridine-labeled CVB3 per 2×10^7 cells per ml.

^b Cytopathic effect (CPE) was determined as detailed in Materials and Methods following infection with 10 to 100 PFU cell⁻¹.

(13). Virus attachment was also determined by adding radioactive virus to confluent monolayers in 24-well culture plates. Unbound virus was washed off at intervals, and cell-associated counts were determined. No difference was found between trypsinized cells and cells in monolayers.

In the virus attachment competition assay, cells were incubated at RT with nonradioactive competing viruses for 1 h before being washed once, and radioactive virus was then added. Portions were removed at intervals and assayed as described above. To find the number of binding sites per cell, we assayed samples at 60 min with increasing amounts of labeled virus. The percent cell-associated label was used to calculate the number of particles bound per cell, and this number was plotted against the number of labeled particles added per cell. The intercept of the slope of the nonspecific binding at zero input was considered to be the approximate number of "specific" sites per cell (13).

Purification of the VRC. The virus-receptor complex (VRC) was formed by using detergent-solubilized YAC-1 cells and purified by procedures described previously (15), except that the initial cell concentration was 2×10^8 cells ml⁻¹ instead of 4×10^7 cells ml⁻¹, to compensate for the smaller number of receptors on YAC-1 cells. The purified VRC was iodinated by the chloramine-T procedure and identified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (15).

RESULTS

Susceptibility of mouse cell lines to CVB3 infection. A panel of readily available mouse cell lines, including fibroblasts, adipocytes, epithelial cells, and myeloma and lymphoma cells, were screened for susceptibility to infection by CVB3. In addition, each cell line was assayed for the capacity to attach [³H]uridine-labeled CVB3 (Table 1). Only YAC-1 cells were positive for each assay, and they also produced infectious virus (1). With an inoculum of 4×10^7 PFU per 2×10^6 cells per ml, a total of 2.8×10^8 PFU was produced after 18 h of incubation at 37°C.

Furthermore, YAC-1 cells were found to be susceptible to infection by each of the CVB1 to CVB6 prototype strains and the CVB3-RD strain, but not to poliovirus T1. In these experiments 2×10^6 YAC-1 cells in 100 µl of culture medium were incubated separately with 100 µl of each virus (containing 1×10^7 to 2×10^8 PFU) in 24-well culture plates at RT for 1 h, after which 0.8 ml of culture medium was added. The number of viable cells was determined by trypan blue staining after 18 h of incubation at 37°C. The results showed that each of the CVB strains tested reduced the cell viability

TABLE 2. Virus competition among CVB for attachment to YAC-1 cells^a

Competing virus	Serotype	Amt (10 ⁸ PFU)	% Inhibition following challenge virus:	
			CVB1	CVB3
CVB1		2.5	35	46
CVB2		1.5	49	31
CVB3		4.0	15	20
CVB4		4.0	65	65
CVB5		23.0	40	22
CVB6		3.5	48	30
CVB3-RD		30.0	38	54
Poliovirus T1		1.3	2	-6

^a YAC-1 cells at 2.5×10^7 cells ml⁻¹ were preincubated with unlabeled competing virus at RT for 1 h and then washed once with phosphate-buffered saline. The cells were suspended in 0.2 ml of [³H]CVB1 or [³H]CVB3 (2×10^4 cpm), and the amount of radioactivity which became cell associated after 30 min was determined. Cells not receiving unlabeled virus bound 2,100 cpm for CVB1 and 1,850 cpm for CVB3.

by 26 to 63%. Cells which received poliovirus T1 resembled the uninoculated control cells, which was consistent with the lack of receptors on mouse cells for prototype polioviruses (16).

Characterization of the cellular receptors on YAC-1 cells.

The cellular receptors on YAC-1 cells were characterized by determining the receptor specificity, virus attachment kinetics, and number of attachment sites per cell. The receptor specificity of YAC-1 cells was determined by virus competition assays. Cells were incubated at RT with unlabeled virus (competing virus), washed, and assayed for the capacity to bind a second virus at RT ([³H]uridine-labeled CVB1 or CVB3 [challenge virus]). The results showed that each of the six serotypes of CVB, as well as the variant virus CVB3-RD, competed for the receptor shared by CVB1 and CVB3 on YAC-1 cells, whereas poliovirus T1 was not blocked (Table 2). The results were similar to those found for HeLa cells (3, 4). The attachment kinetics of CVB3 to YAC-1 also were determined at RT (Fig. 1). The pseudo-first-order rate constant of attachment, k , was 4×10^{-11} min⁻¹ cell⁻¹. This value is 150 times lower than that with HeLa cells ($k = 6 \times 10^{-9}$ min⁻¹ cell⁻¹) (4). The number of specific, saturable attachment sites for CVB3 per YAC-1 cell

FIG. 1. Attachment kinetics of CVB3 to YAC-1 cells. The cells, at a final concentration of 2×10^7 cells ml⁻¹, were mixed with [³H]CVB3. Portions were removed at intervals, and attached virus was determined as described in Materials and Methods.

FIG. 2. Determination of the number of receptors for CVB3 on YAC-1 cells. Dilutions of [^3H]CVB3 were incubated with 2×10^7 cells ml^{-1} for 60 min. The cells were washed twice and counted for radioactivity. The number of virus particles bound per cell was determined and plotted against the number of input virus particles per cell.

was determined to be 5×10^2 (Fig. 2), which is about 40-fold less than that for HeLa cells (2×10^4 sites cell^{-1}) (13). The relatively few attachment sites available on YAC-1 cells would account for the relatively low virus attachment rate determined above.

Protection of YAC-1 and HeLa cells against virus infection by polyclonal anti-VRC antibody. A polyclonal antiserum against the HeLa cell receptor for CVB was prepared previously by immunizing a rabbit with a VRC (13). This anti-HeLa VRC serum was assayed for its capacity to protect HeLa cells and YAC-1 cells against the virus-

FIG. 3. Cosedimentation of [^{14}C]CVB3 marker with [^3H]VRC on a 5 to 30% sucrose gradient. Sedimentation was from right to left.

induced cytopathic effect (Table 3). The antiserum protected YAC-1 cells against each of the six serotypes of CVB and the CVB3-RD variant virus to indicate that the CVB receptors on YAC-1 cells and HeLa cells have common determinants. The reason that anti-HeLa VRC serum did not protect HeLa cells against CVB3-RD may be the existence of a variant receptor, HR2, on HeLa cells. This possibility was discussed in detail previously (13).

Isolation of VRC. To further characterize the CVB receptor from YAC-1 cells, we prepared a VRC from detergent-solubilized YAC-1 cell plasma membranes and purified it as described in Materials and Methods. The VRC formed from [^3H]uridine-labeled CVB3 was identified by its slower sedimentation in sucrose gradients compared with marker ^{14}C -amino-acid-labeled virus (Fig. 3). The purified YAC-1 cell-derived VRC was iodinated, repurified, and analyzed by SDS-polyacrylamide gel electrophoresis, as done previously for a HeLa cell-derived VRC (15) (Fig. 4). The autoradiogram shows a band of approximately 50 kilodaltons, which indicates the same mobility as that of the receptor protein, Rp-a, derived from HeLa cells.

DISCUSSION

Evidence has been presented that the mouse T-cell lymphoma cell line YAC-1 has a specific receptor for CVB that resembles the receptor found previously on human HeLa

TABLE 3. Cell protection assays of a rabbit antiserum prepared against a HeLa cell-CVB3 receptor complex

Challenge virus	HeLa cells (CPE) ^a			YAC-1 cells (10 ⁶ viable cells/ml) ^b		
	Control wells	1/30 serum dilution	1/10 serum dilution	Control wells	1/30 serum dilution	1/10 serum dilution
None	0	0 (0)	0 (0)	3.0	2.9 (3.0)	3.2 (3.2)
CVB1	4	2 (4)	0 (3)	1.4	2.8 (1.6)	2.9 (1.7)
CVB2	4	4 (4)	2 (3)	1.1	2.0 (1.0)	2.4 (1.1)
CVB3	3	1 (4)	0 (3)	1.6	2.9 (1.7)	3.0 (1.7)
CVB4	4	2 (4)	1 (3)	1.2	2.3 (1.2)	2.6 (1.1)
CVB5	4	2 (4)	0 (4)	1.3	2.2 (1.4)	3.0 (1.2)
CVB6	4	4 (4)	3 (3)	1.5	2.5 (1.4)	2.7 (1.4)
CVB3-RD	3	3 (3)	2 (2)	1.8	2.7 (1.8)	2.7 (1.6)
Poliovirus T1	3	3 (3)	3 (3)	NA ^c	NA	NA

^a Confluent HeLa monolayers in 96-well culture plates were pretreated with anti-HeLa VRC for 30 min at RT and then washed once with phosphate-buffered saline. Viruses were added which gave the cytopathic effect (CPE) in control wells within 48 h. Scores were from 0 to 4, representing 0, 25, 50, 75, and 100% cytopathic effect, respectively. Scores in parentheses are for controls treated with prebleed serum.

^b YAC-1 cells (2×10^6 cells ml^{-1}) were pretreated with anti-VRC and washed as described in footnote a. One milliliter of virus (10-fold dilutions of viruses were used from those indicated in Table 2) was added, and viable cells were counted after 18 h of incubation. Numbers in parentheses are for controls, treated with prebleed serum.

^c NA, Not applicable.

FIG. 4. Autoradiogram of the iodinated CVB3-HeLa cell receptor complex and CVB3-YAC-1 cell receptor complex after SDS-polyacrylamide gel electrophoresis. Samples were run on an SDS-polyacrylamide gel (10% acrylamide) in a standard Laemmli system. In this gel system, virion polypeptides 1 and 2 of CVB3 comigrated.

cells (15). Virus competition studies showed that each of the CVB attached to the same YAC-1 receptor and that rabbit antiserum prepared against the HeLa cell receptor for CVB specifically inhibited the attachment of CVB1 and CVB3 to the mouse cells. In addition, SDS-polyacrylamide gel electrophoresis of the VRC derived from YAC-1 cells revealed a band with a molecular size (50 kilodaltons) similar to that of Rp-a, which is derived from HeLa cells.

We have recently characterized two noncompeting monoclonal antibodies (RmcA and RmcB), which recognized two distinct saturable receptors (HR1 and HR2) on HeLa cells (13). One receptor (HR1) is the CVB group-specific receptor which binds each of the six prototype strains of CVB and RmcB. The other receptor binds CVB3-RD and RmcA. Neither monoclonal antibody, however, binds to or protects YAC-1 cells against CVB3 or CVB3-RD infections (13). It is likely that the epitopes for these monoclonal antibodies are not shared between human and mouse cells. Nevertheless, the rabbit anti-HeLa cell polyclonal antiserum blocked the mouse cell receptors for CVB3 and CVB3-RD. It should be possible to obtain additional monoclonal antibodies with specificity for blocking receptors that occur in the different animal species, provided that tolerance can be surmounted in the mouse.

Although CVB are human pathogens, they cause lethal infections of newborn mice and produce a number of different diseases in adult mice from selected inbred strains (10–12). Results of comparative studies of the effects of CVB and the host range variants CVB-RD on three inbred strains of young adult mice indicated that the variant viruses produce a different pattern of pathogenesis from their respective parental viruses (8, 20). In addition, variability in the extent and severity of tissue damage in mice of different strains was observed. Whether such differences reflect qualitative or quantitative variations in the distribution of receptors on a specific organ within a mouse strain, as well as between mouse strains, remains to be determined. It is also possible that more than one type of receptor can serve for virus entry into cells (5, 13, 17). Further studies of cellular receptors on murine cells are in progress, so that a better understanding of their role in the pathogenesis of coxsackievirus infections in the mouse model can be obtained.

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