A Monoclonal Antibody Specific for the Cellular Receptor for the Group B Coxsackieviruses

KUO-HOM LEE HSU, KARL LONBERG-HOLM, BARBARA ALSTEIN, AND RICHARD L. CROWELL*

*Department of Microbiology and Immunology, Hahnemann University School of Medicine,
Philadelphia, Pennsylvania 19102-1192*

Received 24 September 1987/Accepted 27 January 1988

A 50-kilodalton receptor protein (Rp-a) for the group B coxsackieviruses (CB) was isolated in a virus-receptor complex from detergent-solubilized HeLa cells (J. E. Mapoles, D. L. Krah, and R. L. Crowell, *J. Virol.* 55:560-566, 1985). It was used as an immunogen for preparation of a mouse monoclonal antibody (RmcB) which protected HeLa cells and Buffalo green monkey kidney cells from infection by all six serotypes of CB. RmcB did not protect HeLa cells from infection by poliovirus, echovirus 6, or coxsackievirus A18. This monoclonal antibody differed in receptor epitope specificity from a previously isolated antibody (RmcA) (R. L. Crowell, A. K. Field, W. A. Schleif, W. L. Long, R. J. Colonno, J. E. Mapoles, and E. A. Emini, *J. Virol.* 57:438-445, 1986) which blocked receptors only for type 1 CB (CB1), CB3, CB5, and echovirus 6. RmcA and RmcB recognized two distinct saturable receptors on HeLa cells, designated HR2 and HR1, respectively. Human rhabdomyosarcoma (RD) cells have the HR2 receptor for CB3-RD (a variant of CB3), but lack the HR1 receptor for CB3. Therefore, RD cells were resistant to infection by CB3. Although binding of CB3-RD to the HR2 receptor on RD cells can lead to infection, binding of CB3-RD to the HR2 receptor on HeLa cells did not lead to infection. Apparently, both CB3 and CB3-RD use only the HR1 receptor for infection of HeLa cells. Thus, a given virus may use two distinct receptors to bind to cells when only one virus-receptor interaction leads to infection.

Picornaviruses are among the smallest and simplest human pathogens, and yet they produce a bewildering array of diseases. It is clear that among the factors contributing to disease diversity are the specific requirements of viruses for host cell receptors. During the past two decades there has been a continuous interest in receptors and in the early events of picornavirus infection (for reviews, see references 4-6, 12, and 13). However, relatively little is known about the structures of these receptors or their roles in initiating virus infection. Receptor specificity was first demonstrated by binding competition between two virus serotypes which shared the same receptor (1, 2, 9, 11, 16). Different HeLa cell receptors for polioviruses, human rhinoviruses of two separate families, and group B coxsackieviruses (CB) have been discovered (11).

More recently, it was discovered (17) that a variant of a type 3 CB virus (CB3-RD) that was selected by growth in human rhabdomyosarcoma (RD) cells recognized both a new human cellular receptor (designated HR2) found on RD cells and the receptor (HR1) recognized by parental virus on HeLa cells. It was postulated that HeLa cells have both HR1 and HR2 and that RD cells lack HR1. Partial evidence for two receptors on HeLa cells was provided by the observation of separate specificities during the screening of monoclonal antibodies (3). Here we report a new monoclonal antibody (RmcB) prepared against the HeLa cell receptor HR1 which protects HeLa cells and Buffalo green monkey kidney cells (BGM) from infection by all six serotypes of CB. This monoclonal antibody shows a different receptor epitope specificity than an antibody obtained previously (3), now designated RmcA, which did not protect HeLa cells against the even-numbered CB serotypes. Our data reveal that RmcA and RmcB recognize two distinct and saturable

receptors (HR2 and HR1) for CB3-RD on HeLa cells and that only RmcB consistently protects the cells against infection by all six CB and their RD variants.

MATERIALS AND METHODS

Cells and viruses. HeLa cells (Mandel strain) and RD cells were cultured in suspension and in monolayers, respectively, as described previously (7, 18). The BGM cell line was obtained from Marilyn Menegus (15), and a mouse T-lymphoma cell line, YAC-1, was obtained from the American Type Culture Collection.

The prototype strains of CB (serotypes CB1 through CB6) and echovirus 6 have been described previously (2). The variant coxsackieviruses CB1-RD and CB3-RD were derived by Reagan et al. (17) by serial passages of the parental viruses on RD cells. Coxsackievirus A18 (CA18) was obtained originally from the University of Minnesota stock collection (8). Poliovirus type 1 was purchased from the American Type Culture Collection, Rockville, Md. Methods for the growth and purification of unlabeled, [³H]uridine-labeled, and [³⁵S]methionine-labeled viruses have been detailed previously (3, 14). The concentration of virions was estimated from the optical density at 260 nm, at which a value of 1.0 corresponds to approximately 10¹³ particles per ml.

Purification of VRC. Virus-receptor complex (VRC) was formed by using detergent-solubilized HeLa cells and purified by previously described methods (14) with modifications. In brief, HeLa cells were washed twice in phosphate-buffered saline (PBS) and suspended in TD1 buffer (1% Triton X-100 and 0.5% sodium deoxycholate in PBS with protease inhibitors, 2 mM phenylmethylsulfonyl fluoride, 2 mM N-ethylmaleimide, and 5 mM EDTA) at a concentration of 4 × 10⁷ cells per ml at 4°C for 15 min. The preparation was centrifuged at 1,000 × g for 5 min to remove nuclei and then

* Corresponding author.

ultracentrifuged at $55,000 \times g$ for 4.5 h. Purified CB3 and [^3H]CB3 were added to the supernatant fluid at a concentration of 4×10^{11} particles per ml (5 $\mu\text{g}/\text{ml}$). After incubation overnight at 4°C , the VRC preparation was warmed to room temperature, and sodium dodecyl sulfate was added to a final concentration of 1%. The VRC preparation (25 ml) was overlaid onto a step gradient containing 1.5 ml of 54% metrizamide and 5 ml of 25% sucrose, both of which were prepared in TD1 buffer. The preparation was centrifuged in an SW27 rotor for 4.5 h at 24,000 rpm. The gradient was fractionated from the bottom of the tube. The VRC peak fractions (at the metrizamide-sucrose interface, identified by liquid scintillation counting) were pooled and dialyzed overnight at 4°C against TD2 buffer (0.2% Triton X-100 and 0.2% sodium deoxycholate in PBS with 0.4 mM phenylmethylsulfonyl fluoride, 0.4 mM *N*-ethylmaleimide, and 1 mM EDTA). The VRC dialysate (4 ml) was overlaid onto a 30-ml 5 to 35% sucrose gradient in TD2 buffer and centrifuged for 4 h at 24,000 rpm in an SW27 rotor. The VRC peak fractions were pooled and dialyzed overnight against TD2 buffer. The VRC was pelleted by centrifugation at $200,000 \times g$ for 45 min through a 25% sucrose cushion in TD2 buffer, suspended in a small volume of TD2 buffer, and stored at -70°C until used.

Preparation of polyclonal antibodies. Polyclonal antiserum to VRC was prepared in a New Zealand White rabbit by subcutaneous immunization with approximately 40 μg of purified VRC in complete Freund adjuvant. Two booster injections with 40 μg of VRC in incomplete Freund adjuvant were given at monthly intervals.

Preparation of monoclonal antibodies. A/J mice were injected intraperitoneally at monthly intervals with 20 μg of VRC which had been heat treated (60°C for 30 min) to inactivate the virus. The first injection was of VRC in complete Freund adjuvant, and the second and third were of VRC in incomplete Freund adjuvant. Following the second injection, mice were bled and sera were examined for evidence of polyclonal antibodies by the cell protection assay (see below). The titers of the antisera ranged from 1:100 to 1:300. The mice were given a final boost of VRC without Freund adjuvant 5 days before the cell fusion. The spleen cells from immunized mice were collected and fused with sp2/o myeloma cells as described previously (10). Supernatants from clones with approximately 40% hybridoma cell confluency were assayed by the cell protection assay 10 to 14 days after cell fusion. The clones producing antibody that protected cells from infection were subcloned by limiting dilution.

Purification of monoclonal antibody. Fluids from subcloned RmcB hybridoma cell cultures were found to contain the mouse immunoglobulin G1 isotype as determined by gel diffusion assays with isotype-specific antiserum. The antibody was concentrated from culture fluids by 45% $(\text{NH}_4)_2\text{SO}_4$ precipitation. The precipitate was dissolved in a small volume and dialyzed overnight against PBS. Antibody was then purified on a protein A-Sepharose (Pharmacia, Inc., Piscataway, N.J.) column. The dialysate was mixed with an equal volume of loading buffer (3.0 M NaCl, 1.0 M glycine [pH 8.8]) and passed over the column, which was then washed with loading buffer until A_{280} was equal to zero. The immunoglobulin G1 was eluted with 0.2 M NaH_2PO_4 (pH 6.0) and then dialyzed overnight against PBS. The purified antibody was iodinated by a procedure using low concentrations of chloramine-T at 0°C (14).

Cell protection assay. Cells were seeded in 96-well, flat-bottom microdilution plates at 4×10^4 cells per well and

incubated overnight at 37°C in a humidified incubator with 5% CO_2 . The cell monolayers were pretreated with 50 to 100 μl of hybridoma culture fluid for 30 min at room temperature before addition of the virus. A minimum amount of virus was added to yield a cytopathic effect within 24 to 48 h. Cytopathic effect was detected by crystal violet staining of the cells.

Virus or antibody binding. Cells grown in suspension or removed from monolayers by trypsin were washed twice and resuspended in PBS with 3% fetal calf serum–20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4). Receptors for CB3 and CB3-RD were insensitive to the trypsin treatment. The cells were mixed with radioactive virus or antibody to a final concentration of 2×10^7 cells per ml and were maintained in suspension with a reciprocating shaker. Directly following addition of radioactive ligand, a 50- μl sample was removed and diluted with 2 ml of the ice-cold buffer described above. Other portions were removed and diluted at intervals and then centrifuged to remove unattached virus or antibody. The samples were dissolved in 0.5 ml of 0.5 M NH_4OH , and radioactivity was determined by liquid scintillation counting. When ^{125}I -labeled antibody was employed as the ligand, samples were counted directly in a gamma counter. Samples of the suspension of radioactive ligand plus cells were also counted without washing. Results were expressed as the fraction of total counts per minute found in the washed cells. In the binding inhibition assay, cells were incubated with nonradioactive competing ligands for 30 min prior to addition of radioactive virus or antibody. Portions were then removed at intervals and assayed as described above.

For titration to determine the number of binding sites per cell at ligand saturation, samples were assayed at a fixed time (30 to 60 min) with increasing amounts of labeled virus or antibody. The percentage of cell-associated label was used to calculate the number of molecules or particles bound per cell. This number was then plotted against the number of labeled molecules or particles added per cell. We did not use the scatchard plot to linearize the binding data, since that method assumes that binding is monovalent, which clearly is not the case for virions or bivalent antibodies. Also, the binding of CB3 to HeLa cells has a very low reversibility (14). Thus, we have assumed that the adsorption isotherms for antibodies or coxsackieviruses on cells are composed of two components. The first is a specific saturable attachment; superimposed upon this is a nonspecific attachment, which appears not to be saturable but to be proportional to input multiplicity. The intercept of the slope of the nonspecific binding at zero input was considered to be the approximate number of specific sites per cell.

RESULTS

Polyclonal anti-VRC. A rabbit was immunized with purified VRC. To obtain larger amounts of the VRC for use as an immunogen, the method reported previously (14) was modified as detailed in Materials and Methods. Although the antiserum obtained was not specific for Rp-a, it recognized Rp-a and protected HeLa cells against CB3 infection. The antiserum immunoprecipitated four polypeptides from detergent-solubilized ^3H -labeled HeLa cells as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). The protein at 50 kilodaltons is Rp-a; the others remain to be identified. In cell protection assays, this rabbit antiserum had a titer of 1:100.

Selection of monoclonal antibody. Hybridomas were prepared by immunizing mice with VRC, and the antibodies

FIG. 1. Pattern of immunoprecipitation of ^3H -amino-acid-labeled HeLa cell proteins by rabbit anti-VRC antibody. ^3H -amino-acid-labeled HeLa cells were solubilized with TD buffer (1% Triton X-100 and 0.5% deoxycholate in PBS) and immunoprecipitated with rabbit anti-VRC followed by goat anti-rabbit immunoglobulin. The immunoprecipitates were solubilized and assayed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% gel). The gel was sliced for measurement of radioactivity (given as counts per minute on the ordinate). Positions of molecular weight markers are indicated.

produced were screened for blocking of CB3 infection of HeLa cells. From 1,200 cultures, a single positive culture was found and subcloned. The antibody was determined to be a murine immunoglobulin G1 isotype and was designated RmcB to distinguish it from the antibody obtained previously (3), which has now been redesignated RmcA.

Receptor specificity of monoclonal antibodies. RmcB protected HeLa cells against infection by all six serotypes of CB as well as by the CB-RD variants (Table 1). It did not protect HeLa cells against echovirus 6, poliovirus type 1, or coxsackievirus A18. Thus, RmcB showed a different specificity than RmcA, which protected only against infection by CB1, CB3, and CB5 as well as by echovirus 6 (Table 1).

To further characterize the receptor specificity, binding studies with HeLa cells were performed using iodine-labeled RmcA and RmcB. No competition was found between these two monoclonal antibodies (Fig. 2A). Interestingly, RmcB blocked only a low percentage of the binding of [^{35}S]CB3-RD, whereas RmcA showed extensive blockade of CB3-RD binding (Fig. 2B). These results are consistent with the number of receptors on HeLa cells for these two antibodies (see below). Pretreatment of HeLa cells with both RmcA

TABLE 1. RmcA and RmcB protection of HeLa cells from virus infection^a

Challenge virus ^b	Protection by ^c :	
	RmcA	RmcB
CB1, CB3, CB5	+	+
CB2, CB4, CB6	—	+
CB1-RD, CB3-RD	—	+
Echovirus 6	+	—
Poliovirus T1	—	—
CA18	—	—

^a Cell protection assays were as described in Materials and Methods.

^b A minimum amount of virus was added to yield a cytopathic effect within 24 to 48 h.

^c +, Protected against infection; —, did not protect against infection.

and RmcB totally blocked binding of CB3-RD to the cells. These results confirmed the hypothesis, based on previously reported virus competition studies (17), that CB3-RD recognizes two separate receptors on HeLa cells. The finding that RmcB protected HeLa cells against CB3-RD infection (Table 1) indicated that the attachment site which RmcA identified does not lead to productive infection by this virus. Both RmcA and RmcB inhibited attachment of the parental virus CB3 to HeLa cells (Fig. 2C). However, we do not know why RmcA was slightly more effective than RmcB in inhibiting the attachment of CB3.

Cell specificity of monoclonal antibodies. The differences between RmcA and RmcB in receptor specificities and in protection against infection by CB3 and CB3-RD were further investigated with different cells. In addition to the RD cells, which cannot be infected by the parental CB, BGM, a monkey kidney cell line, and YAC-1, a mouse T-lymphoma cell line, were studied. The latter two cells can be infected by all members of CB as well as by the RD virus variants. RmcA protected RD cells against CB3-RD infection (Table 2), but did not protect BGM or YAC-1 cells. RmcB did not protect RD or YAC-1 cells, but protected BGM against both CB3 and CB3-RD.

Number of antibody and virus binding sites on cells. Experiments with labeled monoclonal antibodies were performed to determine the number of antibody binding sites on different cell types (3). Titrations to determine the number of binding sites per cell were done (Fig. 3). As described in Materials and Methods, the number of molecules bound per cell was determined and plotted versus the number of labeled molecules added per cell. The intercept of the slope

FIG. 2. Attachment of labeled antibodies and viruses to HeLa cells. (A) Binding of RmcA and RmcB to different sites on HeLa cells. (B) RmcA blocks the binding of CB3-RD to the HR2 receptors on HeLa cells, whereas RmcB blocks the binding of CB3-RD to the HR1 receptors. Together, RmcA and RmcB totally block binding of CB3-RD to HeLa cells. (C) RmcA and RmcB block CB3 binding to HeLa cells.

TABLE 2. Summary of RmcA and RmcB protection of different cell types against infection by CB3 and CB3-RD^a

Cell type	RmcA		RmcB	
	CB3	CB3-RD	CB3	CB3-RD
HeLa	+	—	+	+
RD	NA ^b	+	NA	—
BGM	—	—	+	+
YAC-1	—	—	—	—

^a Protection assays were as described in Materials and Methods. +, Protected against infection; —, did not protect against infection.

^b NA, Not applicable; virus produced no cytopathic effect in control cells.

of the nonspecific binding at zero input was used as a measure of the number of specific binding sites per cell. In experiments of similar design, the number of virion binding sites per cell also was determined (2). The number of binding sites for CB3, CB3-RD, RmcA, and RmcB on different cells was determined (Table 3). HeLa cells were found to be rich in receptors for CB3-RD (5.6×10^5 per cell) compared with RD cells (3.5×10^3 per cell) and BGM cells (3.3×10^3 per cell). The monoclonal antibodies consistently bound to between 3- to 10-fold more sites than did the virions. This observation is in keeping with the different sizes and valences of the ligands.

DISCUSSION

The HeLa cell plasma membrane receptor for CB was solubilized by detergents, purified as a VRC, and used as an immunogen to prepare a murine hybridoma. The monoclonal antibody thus obtained, RmcB, was found to protect HeLa cells and BGM cells from infection by CB1 through CB6.

TABLE 3. Comparative number of binding sites for CB3, RmcA, and RmcB on different cells

Ligand	Number of binding sites on:		
	HeLa	RD	BGM
CB3	1.8×10^4	— ^a	2.7×10^3
CB3-RD	5.6×10^5	3.5×10^3	3.3×10^3
RmcA	2.5×10^6	2.7×10^4	—
RmcB	5.0×10^4	—	3.6×10^4

^a —, Less than 100 per cell (limit of detection of this assay; binding considered absent).

Cell protection correlated with blockage of specific receptors. It is remarkable that the receptor specificity of RmcB is distinct from that of another receptor-specific monoclonal antibody (RmcA) described previously (3). RmcA protected HeLa cells against infection by only the odd-numbered CB serotypes (CB1, CB3, and CB5) and by echovirus 6 and CA21. It also protected RD cells against infection by CB1-RD, CB3-RD, and CB5-RD virus variants, but not by 16 other picornaviruses. RmcA did not protect the BGM cells of simian origin, even though these cells are highly susceptible to infection by all six CB serotypes (15).

The findings that CB3-RD virus saturated the receptors on HeLa cells and blocked binding of CB3, while saturating amounts of CB3 did not block binding of CB3-RD, indicated that a second receptor site was utilized for binding the CB3-RD variant (17). The results of binding studies with RmcA and RmcB are compatible with the existence of two distinct receptors on HeLa cells which bind the CB3-RD variant virus.

To help clarify discussion of the receptors for coxsackieviruses, we have developed a system of nomenclature in

FIG. 3. Titration of binding sites for monoclonal antibodies on cells. Dilutions of ¹²⁵I-labeled monoclonal antibodies were incubated with cell suspensions for 60 min at room temperature. The cells were washed and counted for radioactivity. The number of molecules bound per cell was determined and plotted versus input virus multiplicity. Shown are binding of RmcA to (A) HeLa cells (B) RD cells and binding of RmcB to (C) HeLa cells and (D) BGM cells.

FIG. 4. Schematic model for CB receptors and effects of RmcA on infection. Symbols: $\downarrow$, HR2 (functional); $\blacksquare$, HR2 (nonfunctional); $\circ$, HR1 (functional); P, parental CB3 virus. (A) RD cells with CB3-RD virus; RmcA blocks infection. (B) HeLa cells with parental CB3 virus; RmcA blocks attachment and infection. (C) HeLa cells with CB3-RD virus; RmcA blocks most of attachment, but permits infection. See text for details.

which the first letter refers to the species of origin of the cell, (e.g., H for human), R indicates receptor, and the arabic numerals indicate distinct, saturable receptors in their chronological order of discovery. For example, HR1 refers to the HeLa cell receptor which binds the parental CB viruses, and HR2 refers to the receptor on RD cells which binds the CB3-RD variant virus. HeLa cells appear to possess both HR1 and HR2, whereas BGM cells have simian receptor 1 (SR1), a receptor which closely resembles HR1 in both virus and monoclonal antibody specificity.

The virus and cell specificity of monoclonal antibodies RmcA and RmcB have been compared (Tables 1 and 2; Fig. 2). We have concluded that RmcA specifically combines only with HR2 and RmcB combines only with HR1. By use of radioiodinated antibodies it was found that there are approximately 3×10^6 and 5×10^4 binding sites on HeLa cells for RmcA and RmcB, respectively (Table 3). These values may be increased by a factor of 2 in estimating the number of receptor sites per HeLa cells, since the antibodies are divalent. Thus, there are approximately 10 times more receptor sites for RmcA than for CB3-RD. Likewise there are approximately five times more receptor sites for RmcB than for CB3. These values are consistent with the concept that virions have multiple binding sites for cellular receptors (12, 13). Also, the results which showed no competition between RmcA and RmcB in binding to HeLa cells did show additive inhibition of attachment of CB3-RD when the two antibodies were used in combination (Fig. 2).

The separate specificities of RmcA and RmcB are evident, since RD cells bind neither RmcB nor CB3, and BGM cells bind CB3 but not RmcA. In addition, RmcA and RmcB were found to differ significantly in their abilities to protect cells against infection, and this has led us to some interesting conclusions. RmcA saturated a receptor on HeLa cells (HR2) but did not protect the cells against infection by CB3-RD, although it caused significant inhibition of attachment (Tables 1 and 3; Fig. 2). In contrast, RmcB saturated HR1 and protected HeLa cells against infection by CB3 and CB3-RD. Thus, HR1 on HeLa cells serves as the functional receptor for binding, internalizing, and uncoating of both CB3 and CB3-RD, whereas HR2 can attach CB3-RD but is unable to facilitate the succeeding early events in virus infection. It is possible that the HeLa cell HR2 lacks a cytoplasmic domain required for internalization of bound

ligand via endosomes. The limited function of HR2 on HeLa cells is in sharp contrast to its role on RD cells, where the HR2 receptors are fully functional in facilitating infection by CB3-RD.

The separate functional specificities of RmcA and RmcB are evident, since RmcB protects BGM cells against parental virus infection and attachment and RmcA protects RD cells. However, the paradoxical effects of RmcA and RmcB on HeLa cells require explanation. It is possible that RmcA prevents attachment of CB3 to the HR1 receptor by a secondary steric blockade (Fig. 4B). This requires that the HR2 receptors (present in roughly 50-fold excess over HR1) be associated with HR1 molecules on the cell surface. A possible explanation for the ability of RmcA to inhibit attachment of CB3-RD to HeLa cells without preventing infection is that the affinity of RD virus for the cell may be sufficient to compete with binding of RmcA to HR2 sites, since RD virus can bind to both HR1 and HR2 (Fig. 4C). This could lead to interactions promoting the binding of infecting virus to the HR1 receptors and then to internalization of the virus and infection.

More information, such as binding constants of virus and antibodies to HR1 and HR2, will be needed before our models for the interactions of viruses and antibodies with HeLa cells can be accepted. The results presented here, however, illustrate the complexity which can be encountered in investigations of cellular receptors for viruses with monoclonal antibodies. Further investigations in this system using adenovirus type 2 fiber protein as a probe (11) for the HR1 receptor will be reported elsewhere.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research support grant AI-03771 from the National Institute of Allergy and Infectious Diseases and by our institutional Biomedical Research Support grant 2S07RR05413.

LITERATURE CITED

1. Crowell, R. L. 1963. Specific viral interference in HeLa cell cultures chronically infected with coxsackie B5 virus. *J. Bacteriol.* **86**:517-526.
2. Crowell, R. L. 1966. Specific cell-surface alteration by enteroviruses as reflected by viral-attachment interference. *J. Bacteriol.* **91**:198-204.

3. Crowell, R. L., A. K. Field, W. A. Schleif, W. L. Long, R. J. Colunno, J. E. Mapoles, and E. A. Emini. 1986. Monoclonal antibody that inhibits infection of HeLa and rhabdomyosarcoma cells by selected enteroviruses through receptor blockade. *J. Virol.* 57:438-445.
4. Crowell, R. L., K.-H. L. Hsu, M. Schultz, and B. J. Landau. 1987. Cellular receptors in coxsackievirus infections, p. 453-466. *In* M. A. Brinton and R. R. Rueckert (ed.), Positive strand RNA viruses. Alan R. Liss, Inc., New York.
5. 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. Virus-host interaction: receptors, persistence, and neurological diseases. Plenum Publishing Corp., New York.
6. Crowell, R. L., and K. Lonberg-Holm (ed.). 1986. Virus attachment and entry into cells. American Society for Microbiology, Washington, D.C.
7. Crowell, R. L., and L. Philipson. 1971. Specific alterations of coxsackievirus eluted from HeLa cells. *J. Virol.* 8:509-515.
8. Crowell, R. L., and J. T. Syverton. 1955. The viral range *in vitro* of a malignant, human epithelial cell (strain HeLa, GEY). IV. The cytopathogenicity of C viruses. *J. Immunol.* 74:169-177.
9. Crowell, R. L., and J. T. Syverton. 1961. The mammalian cell-virus relationship. VI. Sustained infection of HeLa cells by coxsackie B3 virus and effect on superinfection. *J. Exp. Med.* 113:419-435.
10. Hughes, J. V., L. W. Stanton, J. E. Tomassini, W. J. Long, and E. M. Scolnick. 1984. Neutralizing monoclonal antibodies to hepatitis A virus: partial localization of a neutralizing antigenic site. *J. Virol.* 52:465-473.
11. Lonberg-Holm, K., R. L. Crowell, and L. Philipson. 1976. Unrelated animal viruses share receptors. *Nature (London)* 259: 679-681.
12. Lonberg-Holm, K., and L. Philipson. 1974. Early interaction between animal viruses and cells. *Monogr. Virol.* 9:1-148.
13. Lonberg-Holm, K., and L. Philipson (ed.). 1981. Receptors and recognition series B, Vol. 8. Virus receptors, part 2. Animal viruses. Chapman and Hall, Ltd., London.
14. Mapoles, J. E., D. L. Krah, and R. L. Crowell. 1985. Purification of a HeLa cell receptor protein for the group B coxsackieviruses. *J. Virol.* 55:560-566.
15. Menegus, M. A., and G. E. Hollick. 1982. Increased efficiency of group B coxsackievirus isolation from clinical specimens by use of BGM cells. *J. Clin. Microbiol.* 15:945-948.
16. Quersin-Thiry, L., and E. Nihoul. 1961. Interaction between cellular extracts and animal viruses. II. Evidence for the presence of different inactivators corresponding to different viruses. *Acta Virol.* 5:282-293.
17. Reagan, K. J., B. Goldberg, and R. L. Crowell. 1984. Altered receptor specificity of coxsackievirus B3 after growth in rhabdomyosarcoma cells. *J. Virol.* 49:635-640.
18. Schultz, M., and R. L. Crowell. 1983. Eclipse of coxsackievirus infectivity: the restrictive event for a non-fusing myogenic cell line. *J. Gen. Virol.* 64:1725-1734.