Clinical Coxsackievirus B Isolates Differ from Laboratory Strains in Their Interaction with Two Cell Surface Receptors

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Coxsackie B viruses interact with two putative cell surface receptor molecules. Experiments with prototype laboratory strains suggest that all 6 coxsackie B serotypes interact with a 46-kDa protein recognized by the monoclonal antibody RmcB, whereas CB1, CB3, and CB5 may also bind to decay accelerating factor. Antireceptor monoclonal antibodies were used to study interactions between low-passage clinical coxsackie B virus isolates and the two receptors. In contrast to observations made with single prototype strains, these data indicate that receptor use by clinical isolates is not strictly related to serotype and that even prototype strains with different passage histories may differ in receptor use. Within a given serotype, variation exists in the capacity of individual virus isolates to bind to specific receptors, and variants with altered receptor specificity may arise during infection in humans and in tissue culture.

Viruses initiate infection by attaching to cell surface receptor molecules, and expression of specific receptors is an important determinant of viral host range and tissue tropism. Coxsackie B viruses, members of the picornavirus family, are human pathogens responsible for aseptic meningitis, myocarditis, and nonspecific febrile illnesses. Early attachment-interference experiments suggested that all 6 coxsackie B serotypes compete for a single cell surface receptor site [1]. However, subsequent work has suggested that the coxsackie B receptor may include multiple components, and no single receptor molecule has been conclusively identified.

Coxsackie B viruses interact with at least two HeLa cell surface molecules. A prototype strain, coxsackie B3 (Nancy), when attached to the HeLa cell surface, forms a detergent-stable complex with a 45- to 50-kDa protein [2], and radiolabeled CB3 (Nancy) identifies a protein of the same size on virus overlay blots [3]. A monoclonal antibody (MAb RmcB) raised against the putative virus/receptor complex recognizes a 46-kDa HeLa cell protein (unpublished data) and protects cells from infection by prototype strains of all 6 coxsackie B serotypes [4].

A CB3 variant adapted to growth in RD rhabdomyosarcoma cells, CB3-RD, shows an altered receptor specificity and binds to decay accelerating factor (DAF), a 70-kDa complement regulatory protein expressed on many human cells [5]. Anti-DAF antibodies protect RD cells from infection by CB3-RD [6] and protect HeLa cells from infection by prototype laboratory strains of CB1, CB3, and CB5 [6, 7], suggesting that these viruses—including some strains shown to interact with the 46-kDa receptor [2, 4, 6]—may interact with DAF on the cell surface. Consistent with this, CB3-RD [5] and some prototype strains of CB1, CB3, and CB5 [7] have been shown to bind to DAF on the surface of transfected rodent cells.

Results obtained with prototype strains have led some investigators to conclude that MAb RmcB identifies the specific receptor for all 6 coxsackie B serotypes [4]; other investigators have suggested that DAF is the receptor responsible for CB1, CB3, and CB5 attachment to cells, although additional factors may be required for subsequent steps in infection [7]. In preliminary experiments, we found that laboratory strains of CB3 differed in their capacity to bind DAF on transfected cells (unpublished data); these strains had been cultured under a variety of conditions over many years. To determine whether the results obtained with laboratory strains hold true for all coxsackie B viruses, we have used viruses recently isolated from clinical specimens to study virus interactions with two cell surface proteins identified by MAbs that block virus attachment.

Materials and Methods

Viruses. Isolation, identification, and serotyping of coxsackieviruses from clinical specimens during a prospective study of aseptic meningitis in infants have been described [8]. All viruses were isolated on Buffalo green monkey kidney cells and passed two or fewer times on HeLa cells. Prototype virus CB3 (Nancy) has been maintained in the laboratory of R. L. C. In addition, prototype strains CB3 (Nancy) and CB1 (Conn 5) were obtained from the American Type Culture Collection (ATCC; Rockville, MD).

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Antibodies. MAbs IF7 [9] and RmcB [4], used in plaque inhibition experiments, were prepared as ascites fluids. Antibody concentrations were determined by a murine immunoglobulin subclass-specific ELISA [10], with reagents purchased from Tago (Burlingame, CA). Isotype-matched control antibodies were purchased from Organon Teknika Cappel (West Chester, PA).

Plaque inhibition assays. HeLa cell monolayers in 6-well culture plates were washed in Hanks' balanced salt solution and then pretreated for 1 h at room temperature with MAb IF7, MAb RmcB, or control MAbs diluted in Earle's modified Eagle medium. Monolayers were then washed, incubated with virus for 1 h, and washed again before incubation at 37°C. After 24–72 h, plaques were developed essentially as described [11]. Plaque formation was calculated as a percentage of the total plaques in control wells, to which no antibody had been added.

Radiolabeled virus–binding assays. Viruses were radiolabeled and purified as described [11]. HeLa cell monolayers were preincubated with MAbs (10–20 μg/mL), exposed to radiolabeled viruses for 1 h at room temperature, and then washed and dissolved for determination of cell-bound radioactivity as described [12].

Results

MAb inhibition of infection by clinical group B coxsackievirus isolates. Experiments using anti-DAF MAbs to block infection, as well as radiolabeled virus attachment to transfected cells, have suggested that CB1, CB3, and CB5 prototype strains interact with DAF on the HeLa cell surface [6, 7]. RmcB has been shown to protect HeLa cells from infection by prototype strains of all 6 CB serotypes [4]. To determine whether clinical group B coxsackievirus isolates resembled prototype laboratory strains in their interaction with DAF and with the RmcB protein, we measured the ability of MAbs to inhibit plaque formation by low-passage clinical isolates. HeLa monolayers were pretreated with IF7, RmcB, or control antibodies at concentrations of 0.1–200 μg/mL; plaque assays were then performed. The concentration of antibody required to inhibit 50% of plaque formation was determined (table 1).

Twenty-seven clinical isolates and 3 prototype strains were tested. Four patterns of inhibition were seen. For most viruses, plaque formation was inhibited significantly by either IF7 or RmcB. Several viruses were inhibited by neither antibody at any concentration tested. One virus, the CB3 (Nancy) prototype strain used in the laboratory of R.L.C., was inhibited by both IF7 and RmcB, consistent with previous results [3, 4]. All 3 CB2 and all 7 CB4 isolates tested were inhibited by RmcB but not by IF7, consistent with results obtained with prototype viruses [4, 7]. However, for other viruses, the pattern of inhibition was less clearly related to serotype.

Three CB1 clinical isolates were inhibited only by RmcB, and 2 were inhibited only by IF7; the prototype CB1 (Conn 5) strain was not inhibited by either antibody. Five CB5 isolates were inhibited only by IF7, and 2 were inhibited by neither IF7 nor RmcB.

All 5 CB3 clinical isolates were inhibited by IF7 but not by RmcB. Results obtained with laboratory strains were more complex. CB3 (Nancy) obtained from two different sources behaved quite differently in these experiments. Virus maintained in the laboratory of R. L. C. was inhibited by low concentrations of either IF7 or RmcB, consistent with published results [4]. In contrast, CB3 (Nancy), obtained from the ATCC, was inhibited by IF7, but not by RmcB.

Table 1. Antibody inhibition of plaque formation by group B coxsackievirus isolates.

Group	Isolate	Monoclonal antibody	
		IF7	RmcB
CB1	86-1798	>100	0.4
	86-2298	>100	0.1
	86-2299	>100	0.3
	90-1112	3.8	>200
	94-0530	3.2	>100
	ATCC (Conn 5)	>100	>100
CB2	87-0566	>100	0.3
	90-1376	>100	0.6
	90-1445	>100	9.0
CB3	86-1799	4.2	>200
	86-1990	2.9	>100
	86-2153	27.0	>200
	86-2327	2.0	>200
	86-2424	2.8	>200
	CB3-N (R.L.C.)*	4.6	3.8
	CB3-N (ATCC)	38	>200
CB4	86-0566	>100	0.9
	87-1026	>100	0.3
	88-0566	>100	0.9
	88-0572	>100	0.5
	88-0658	>100	0.6
	88-0842	>100	0.9
	89-0731	>100	0.5
CB5	88-0542	>100	>200
	88-0578	7.5	>200
	88-0870	>100	>200
	88-0973	40	>200
	89-0416	2.0	>200
	89-0895	17	>200
	90-1378	3.0	>100

NOTE. Concentration of antibody required for 50% inhibition of plaque formation is shown in μg/mL.

* CBC (Nancy) from laboratory of R.L.C.

MAb inhibition of radiolabeled virus attachment to HeLa cells. IF7 prevented attachment of clinical CB3 isolates to HeLa cells, and little or no inhibition was seen with RmcB (figure 1). Attachment of CB3 (Nancy) from the laboratory of R. L. C. was inhibited by both IF7 and RmcB (figure 1), whereas attachment by CB3 (Nancy) obtained from the ATCC was prevented only by IF7 (figure 1). These results suggest that MAbs RmcB and IF7 prevented infection by these viruses by blocking virus attachment and indicate that e.en within a single serotype, viruses interact differently with the two cellular receptors.

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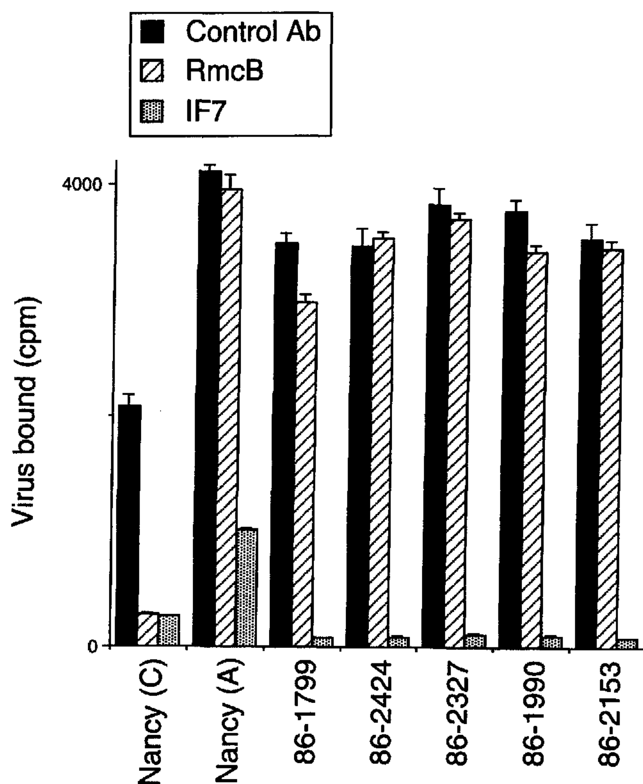

Figure 1. Inhibition of coxsackievirus B3 attachment by monoclonal antibodies (Ab) RmcB and IF7. HeLa cell monolayers were preincubated with RmcB, IF7, or control antibody MOPC 195 for 1 h at room temperature, washed, and then exposed to radiolabeled viruses for 1 h. CB3-Nancy strains maintained in laboratory of R. L. C. or obtained from American Type Culture Collection, and 5 clinical CB3 isolates were tested (6000–8000 cpm added/well). After extensive washing, monolayers were dissolved for determination of cell-bound radioactivity.

Discussion

Work with prototype virus strains suggests that all 6 coxsackie B virus serotypes interact with the 46-kDa protein recognized by MAb RmcB [3, 4] and that prototype CB1, B3, and B5 strains also interact with a 70-kDa protein [3, 6] now identified as DAF [5, 7]. In the experiments described here, we have examined for the first time the interaction of low-passage clinical isolates with these two cell surface proteins, and the results differ from those obtained with prototype strains.

In previous studies, MAb RmcB inhibited prototype strains of all 6 serotypes, whereas an anti–DAF antibody inhibited only CB1, CB3, and CB5 [4, 6]. Consistent with these observations, we found that clinical isolates of CB2 and CB4 were inhibited by RmcB but not by the anti–DAF antibody IF7. However, in contrast to results obtained with prototype strains, we found that plaque formation by most, but not all, clinical isolates of CB1, CB3, and CB5 was inhibited by the anti–DAF antibody but not by RmcB.

The interactions between these viruses and the putative receptor molecules do not correlate strictly with virus serotype. Although all clinical and laboratory strains of CB2 and CB4 thus far examined appear to interact with the 46-kDa protein identified by RmcB and not with DAF, results differed dramatically for different strains of CB1, CB3, and CB5. For example, some CB1 strains were inhibited exclusively by RmcB, while others were inhibited exclusively by the anti–DAF antibody. We also found that 2 CB3 strains, both derived from CB3 (Nancy) but obtained from different sources, were inhibited differently by the antireceptor MAbs. Consistent with this, although CB3 (Nancy) maintained in the laboratory of R. L. C. shows little or no capacity to bind DAF on the surface of transfected cells [5], CB3 (Nancy) obtained from the ATCC binds efficiently to DAF transfectants (unpublished observations [7]). Although many investigators have assumed that viruses within a given serotype will bind to the same receptor, for other picornaviruses, the sites for receptor attachment and the neutralization epitopes that determine serotype are known to be structurally distinct [13]. Variants with altered receptor specificity may arise spontaneously, either in the body or in tissue culture, and interaction with cells that do or do not express specific receptor molecules may exert powerful selective pressures on a virus population. A variant strain of CB3 (Nancy) selected for its capacity to grow in rhabdomyosarcoma cells [14]—which express DAF but not the 46-kDa protein recognized by RmcB [3, 4]—gained the capacity to bind DAF on transfected cells [5]. Similar RD-adapted variants of CB1 and CB5 have also been isolated in vitro [9]. The observation that low-passage clinical isolates of CB3 bind to DAF on transfected cells suggests that such variants may also arise in vivo. The clinical isolates used in these studies were isolated in Buffalo green monkey kidney cells, which do not express DAF [3, 4]; the capacity of these viruses to bind DAF thus does not result from selection in tissue culture.

Interactions between coxsackie B viruses and their cell surface receptors appear to be quite complex. Molecular cloning of individual receptor components and their expression in nonpermissive cells, singly and in combination, may be required before many complexities can be resolved. The results described here suggest that within a given serotype, variation exists in the capacity of individual virus isolates to bind to specific receptors, and that variants with altered receptor specificity may arise during infection in humans as well as in tissue culture. The role for such variation in the pathogenesis of illness caused by these viruses remains to be explored.

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References

1. Crowell RL, Landau MA. Receptors in the initiation of picornavirus infections. In: Fraenkel-Conrat H, Wagner RR, eds. Comprehensive virology. New York: Plenum Publishing, **1983**:1–42.

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2. Mapoles JE, Krah DL, Crowell RL. Purification of a HeLa cell receptor protein for group B coxsackieviruses. J Virol **1985**;55:560–6.
3. Hsu KHL, Paglini S, Alstein B, Crowell RL. Identification of a second cellular receptor for a coxsackievirus B3 variant, CB3-RD. In: Brinton M, Heinz F, eds. New aspects of positive-strand RNA viruses. Washington, DC: American Society for Microbiology, **1990**:271–7.
4. Hsu KHL, Lonberg-Holm K, Alstein B, Crowell RL. A monoclonal antibody specific for the cellular receptor for the group B coxsackieviruses. J Virol **1988**;62:1647–52.
5. Bergelson JM, Mohanty JG, Crowell RL, St. John NF, Lublin DM, Finberg RW. Coxsackievirus B3 adapted to growth in RD cells binds to decay-accelerating factor (CD55). J Virol **1995**;69:1903–6.
6. Crowell RL, Field AK, Schlief WA, et al. Monoclonal antibody that inhibits infection of HeLa and rhabdomyosarcoma cells by selected enteroviruses through receptor blockade. J Virol **1986**;57:438–45.
7. Shafren DR, Bates RC, Agrez MV, Herd RL, Burns GF, Barry RD. Coxsackieviruses B1, B3, and B5 use decay accelerating factor as a receptor for cell attachment. J Virol **1995**;69:3873–7.
8. Berlin L, Rorabaugh M, Heldrich F, Roberts K, Doran T, Modlin J. Aseptic meningitis in infants <2 years of age: diagnosis and etiology. J Infect Dis **1993**;168:888–92.
9. Bergelson JM, Chan M, Solomon K, St. John NF, Lin H, Finberg RW. Decay-accelerating factor, a glycosylphosphatidylinositol-anchored complement regulatory protein, is a receptor for several echoviruses. Proc Natl Acad Sci USA **1994**;91:6245–8.
10. Brubaker JO, Thompson CM, Morrison LA, Knipe DM, Siber GR, Finberg RW. Th1-associated immune responses to β-galactosidase expressed by a replication-defective herpes simplex virus. J Immunol **1996**;157:1598–604.
11. Bergelson JM, St. John N, Kawaguchi S, et al. Infection by echoviruses 1 and 8 depends on the $\alpha 2$ subunit of human VLA-2. J Virol **1993**;67:6847–52.
12. Bergelson JM, Chan BMC, Finberg RW, Hemler ME. The integrin VLA-2 binds echovirus 1 and extracellular matrix ligands by different mechanisms. J Clin Invest **1993**;92:232–9.
13. Rossmann MG, Arnold E, Erickson JW, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature **1985**;317:145–53.
14. Reagan KJ, Goldberg B, Crowell RL. Altered receptor specificity of coxsackie B3 after growth in rhabdomyosarcoma cells. J Virol **1984**;49:635–40.

Note Added in Proof

We have cloned the 46-kDa protein recognized by MAb RmcB. Prototype and clinical isolates of CB3, CB4, and CB5—including some that bind to DAF and some that were not inhibited by RmcB in plaque assays—bind to and infect nonpermissive hamster cells transfected with cDNA encoding the 46-kDa receptor.

Detection of Enterovirus by Polymerase Chain Reaction and Culture in Cerebrospinal Fluid of Children with Transient Neurologic Complications Associated with Acute Febrile Illness

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Cerebrospinal fluid samples collected from 23 children with neurologic symptoms, such as febrile seizures, status epilepticus, and transient altered states of consciousness, associated with acute febrile illness, were examined for infectious virus by cell culture. Enteroviruses (echovirus type 9 and coxsackievirus B3) were isolated from 2 of the cerebrospinal fluid samples. The samples were also examined for enterovirus by use of the polymerase chain reaction, which could detect nearly the whole human enterovirus group: Enteroviral RNA was detected in 9 of the 23 samples. The findings suggest that transient neurologic complications during the febrile phase of acute febrile illness are caused, in part, by enteroviral infection of the central nervous system.

Polymerase chain reaction (PCR) has been used to detect enteroviral RNA for a direct and rapid diagnosis of clinically important enteroviral infections [1–3]. Enteroviral RNA has been found in myocardial tissue from patients with myocarditis and cardiomyopathy [4] and in cerebrospinal fluid (CSF) of patients with aseptic meningitis [5, 6]; enteroviruses are believed to be the most common viral pathogenic agents for these diseases. Enteroviral infections, such as herpangina, hand-foot-and-mouth disease, and aseptic meningitis, have not been associated commonly with neurologic abnormalities [7]. The association of transient neurologic complications with central nervous system (CNS) infection due to enteroviruses has not been studied.

CSF samples were collected from children who were suspected of having a CNS infection because of neurologic symptoms: 23 patients were subsequently diagnosed as having febrile seizures, status epilepticus, or transient altered states of consciousness associated with acute febrile illness. CSF samples from these patients were examined for infectious virus by cell culture and for enteroviral RNA by PCR.

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Informed consent was obtained from the subjects or their parents.

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