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Monoclonal Antibodies That Inhibit Attachment of Group B Coxsackieviruses

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Hybridoma cell lines that secrete monoclonal antibodies which react with HeLa cell surface antigens were produced. The monoclonal antibodies prevented cytopathic effects caused by coxsackievirus B1 and significantly reduced the amounts of coxsackieviruses B1, B5, and B6 that absorb to HeLa cells. These antibodies did not protect the cells from poliovirus cytopathic effects, and they had no effect on the attachment of other picornaviruses to HeLa cells.

The first step in the infection of cells by picornaviruses is the binding of virus to a cell membrane receptor. Virus competition studies show that various picornaviruses attach to different sites on the cell surface, suggesting that there are distinct families of receptors for picornaviruses (4, 9, 10, 13). Specificity among picornavirus receptors has also been demonstrated in studies which show that preadsorbed anticellular serum specifically prevents attachment of group B coxsackieviruses (1) or polioviruses (11) to HeLa cells. The antisera used in these reports were not strictly monospecific and, consequently, have limited utility for detailed biochemical characterization of viral receptors.

We have chosen to study picornavirus receptors by using monoclonal antibodies. Female C57BL/6 mice were immunized with three subcutaneous injections of 10^7 washed HeLa cells given at 2-week intervals. The animals were boosted every 4 weeks thereafter by intraperitoneal injection. Mouse anticellular serum was obtained by bleeding the animals at various times during the immunization procedure. About 3 to 4 weeks after the last injection, the mice received 10^7 cells intraperitoneally. Mice were sacrificed 2 days later, and their spleen cells were fused with P3/X63-Ag8 (P3X) myeloma cells in the presence of polyethylene glycol (7). Hybrid cells were selected by incubation in Dulbecco modified Eagle medium (20% heat-inactivated horse serum, 4.5 mg of glucose, 100 U of penicillin, and 100 μg of streptomycin per ml) containing 1 μM hypoxanthine, 0.4 μM aminopterin, and 0.16 μM thymidine. Hybridomas were grown for 2 weeks and screened for the production of antibodies reactive with the group B coxsackievirus receptor.

The test we developed for screening hybridomas for antireceptor antibodies was based on the rationale that such antibodies should prevent attachment and, therefore, prevent cytopathic effect (CPE) of those picornaviruses which use the receptor to which the antibody was directed but that such antibodies should have no effect on adsorption or CPE of other picornaviruses which use receptors belonging to different receptor families. Briefly, triplicate wells (3034 Microtest plates; Falcon Plastics, Oxnard, Calif.), each containing about 1,000 cells, were treated for 1 h at 37°C with 10 μl of hybridoma culture fluid. After being washed, the cells were challenged with either coxsackievirus B1 or poliovirus 1 (0.1 PFU per cell). The plates were incubated at 37°C and screened at 24 h intervals for the presence of typical picornavirus CPE.

Approximately 200 hybridomas were screened, and culture fluids from three of them (designated DB5, ED9, and EE8) protected HeLa cells from CPE caused by coxsackievirus B1 but did not protect cells against infection by poliovirus 1. Another hybridoma culture fluid (DD7) did not yield a positive result in the screening assay but was retained for use as a negative control in some experiments. Each hybridoma was cloned three times by limiting dilution on mouse thymocyte feeder layers. Direct immunofluorescence of hybrids DB5, ED9, and EE8 by reaction with fluorescein-conjugated antisera (monospecific for mouse immunoglobulin heavy chains; Meloy Laboratories, Inc., Springfield, Va.) revealed the presence of mouse γ1, μ, and κ immunoglobulin chains on the cell surface (data not shown). The DB5, ED9, and EE8 monoclonal antibodies were immunoglobulin M class antibodies because hybrids constructed by fusion of P3X myeloma cells with spleen cells produced the myeloma heavy (γ1) and light (κ) chains in addition to the splenic lymphocyte heavy and light chains (8).

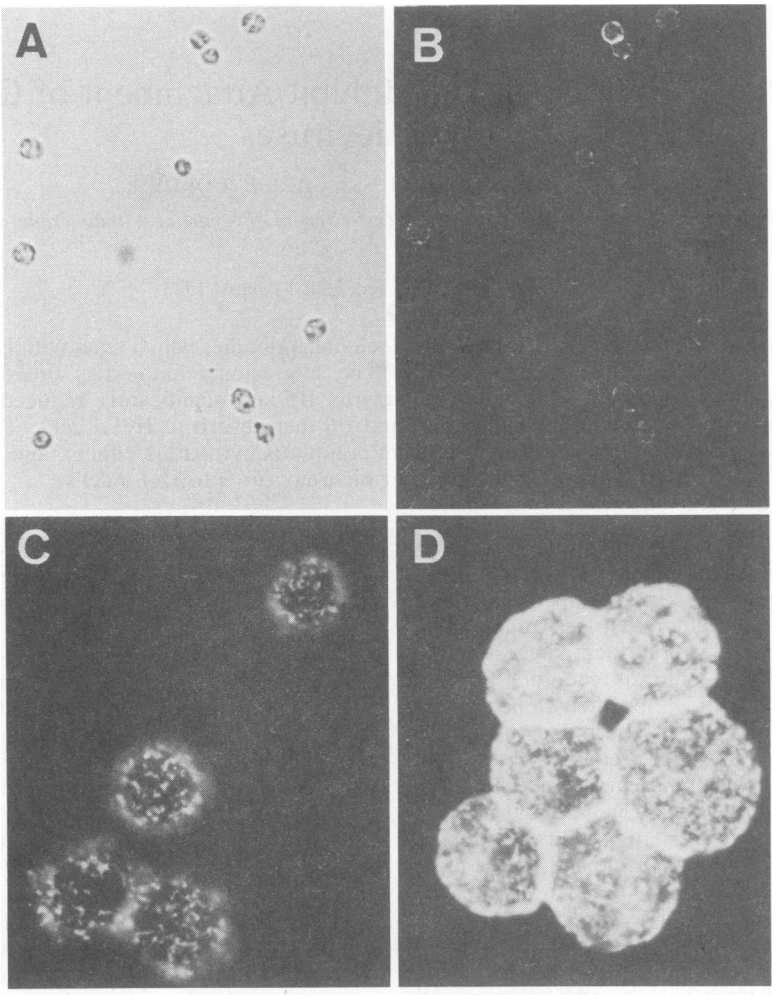

FIG. 1. Indirect immunofluorescence membrane staining of HeLa cells. HeLa cells were incubated with DB5 hybridoma culture fluid (A to C) or a 10^{-2} dilution of mouse anticellular serum (D) and stained in the second step with a fluorescein-conjugated goat antiserum reactive with mouse immunoglobulin. Phase microscopy (A) and fluorescence microscopy (B) of the same field (×100). (C and D) Fluorescent micrographs (×400).

Since culture fluids from the parent P3X myeloma cells had no effect on any of the parameters examined, we suspect that the immunoglobulin M class antibodies were responsible for the results obtained.

The assay for selection of antireceptor monoclonal antibodies revealed that hybridoma culture fluids selectively inhibited CPE of coxsackievirus B1 but not that of poliovirus 1. This functional procedure yielded no information about what caused the inhibition of CPE or at what cellular level the inhibition occurred. The possibility that mouse antibody binding to a HeLa cell surface antigen was responsible for these results was examined by indirect immunofluorescence of monoclonal antibody-treated HeLa cells (2). Under the conditions of the experiment, all cells fluoresced (cf. Fig. 1A and B). The fluorescence pattern observed on the HeLa cell membrane was similar for cells treated with each of the three monoclonal antibodies and was characterized by bright patches of fluorescence punctuated with nonstaining regions (Fig. 1C). In contrast, cells stained after treatment with anticellular serum fluoresced uniformly (Fig. 1D). HeLa cells did not fluoresce when treated with P3X culture fluids, DD7 culture fluids, or Dulbecco modified eagle medium (data not shown). Results of the indirect immunofluorescence studies suggested that mouse immunoglobulin, reacting with a HeLa cell membrane antigen, was responsible for the specificity of inhibition of CPE. Additionally, these results suggested that the antigen recognized by the monoclonal antibodies may be the receptor for the group B coxsackieviruses be-

cause coxsackievirus receptors are limited to the surfaces of susceptible cells (12, 15) and because ultrastructural studies show that coxsackievirus B3 virions attach to HeLa cells with a similar patchy distribution (12).

If the monoclonal antibodies are specific for the group B coxsackievirus receptor, the antibodies might be expected to react with all cell lines susceptible to infection by group B coxsackieviruses. Eight continuous cell lines from various species (human WI38, M7, and RD cells; African green monkey Vero cells; mouse L-929 cells; baby hamster kidney cells; rabbit skin cells; and Madin-Darby canine kidney cells) and one primary human fibroblast line were examined for fluorescence after treatment with hybridoma culture fluids and fluorescein-conjugated goat antiserum against mouse immunoglobulin. No fluorescence was detected on any of the cells tested, although a suspension strain of HeLa cells did show the typical fluorescence pattern (data not shown). These results suggest that the epitopes recognizd by the monoclonal antibodies were HeLa cell specific. Receptors on HeLa cells for polioviruses are antigenically homogeneous to receptors on other primate cell lines when analyzed with polyclonal antisera (11). Nevertheless, monoclonal antibodies are extraordinarily specific, and cross-reactivity is not a general characteristic of these molecules (14). For example, monoclonal antibodies reactive with the baboon endogenous virus receptor of human cells do not react with canine cells that also possess receptors, showing that differences exist among epitopes of the site of attachment for a given virus on cells derived from different animal species (3).

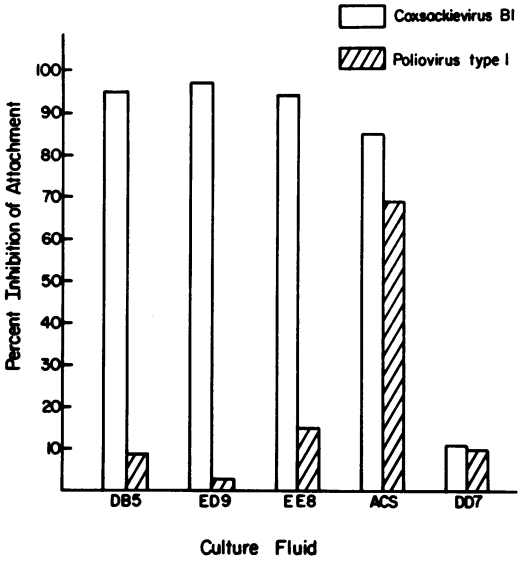

FIG. 2. Attachment of coxsackievirus B1 and poliovirus 1 to HeLa cells treated with hybridoma culture fluids. HeLa cell monolayers were treated with undiluted hybridoma culture fluid (DB5, ED9, EE8, or DD7 hybridomas), mouse anticellular serum (ACS) at a 1:20 dilution, or balanced salt solution as a control. The monolayers were challenged with virus, and the amount of virus remaining unattached was determined (1, 11). The percent inhibition of attachment was calculated from the following equation: 100 − [100 × (number of PFU recovered from balanced salt solution-treated monolayers/number of PFU recovered from hybridoma culture fluid-treated monolayers)].

TABLE 1. Effect of hybridoma ascites fluids on the attachment of picornaviruses to HeLa cell monolayers[a]

Ascites fluid	% Inhibition of attachment[b] of coxsackievirus					
	A13	A21	B5	B6	EMC[c]	PT3[d]
DB5	0	9	95	39	1	0
ED9	21	16	63	45	15	6
EE8	23	0	98	50	25	19

[a] HeLa cell monolayers were treated with a 1:20 dilution of the designated hybridoma ascites fluid and were subsequently challenged with the indicated virus as previously described (1, 11).

[b] The percent inhibition of attachment was calculated as described in the legend to Fig. 2. Values are the average of two determinations When negative averages were calculated, the value was registered as 0.

[c] Encephalomyocarditis virus.

[d] Poliovirus 3.

The immunofluorescence studies suggested that the effects of the monoclonal antibodies were exerted at the level of the HeLa cell membrane, perhaps by inhibiting virus attachment. The ability of monoclonal antibodies to prevent the attachment of picornaviruses to HeLa cells was evaluated essentially as previously described (1, 11). Treatment of HeLa cells with monoclonal antibodies inhibited the adsorption of over 90% of the inoculum of coxsackievirus B1; however, the adsorption of poliovirus 1 was not significantly affected (Fig. 2).

Picornaviruses are classified into receptor families based upon virus competition studies (4–6, 9). To determine whether the DB5, ED9, and EE8 monoclonal antibodies would show a similar viral specificity, we extended the attachment interference studies to determine the effect of hybridoma ascites fluids on the attachment of selected picornaviruses to HeLa cell monolayers. The results of these experiments showed that ascites fluids from all three hybridomas inhibited the adsorption of coxsackieviruses B5 and B6 (Table 1). Hybridoma ascites fluids did not affect the attachment of any other virus tested.

The ability to selectively prevent attachment of, and infection by, group B coxsackieviruses

suggests that the DB5, ED9, and EE8 monoclonal antibodies recognize the HeLa cell surface receptor for group B coxsackieviruses. Nevertheless, this conclusion is not unequivocal, since it is possible that the monoclonal antibodies react with a nonreceptor antigen, and this interaction may selectively affect the attachment of group B coxsackieviruses.

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LITERATURE CITED

1. **Axler, D. A., and R. L. Crowell.** 1968. Effect of anticellular serum on the attachment of enteroviruses to HeLa cells. J. Virol. **2**:813–821.
2. **Chesebro, B., K. Wehrly, M. Cloyd, W. Britt, J. Portis, J. Collins, and J. Nisho.** 1981. Characterization of mouse monoclonal antibodies specific for Friend murine leukemia virus-induced erythroleukemia cells: Friend-specific and FMR-specific antigens. Virology **112**:131–144.
3. **Cogniaux, J., R. Olislager, S. Sprecher-Goldberger, and L. Thiry.** 1982. Monoclonal antibodies against baboon endogenous virus and against host cell antigens. J. Virol. **43**:664–672.
4. **Crowell, R. L.** 1966. Specific cell-surface alteration by enteroviruses as reflected by viral-attachment interference. J. Bacteriol. **91**:198–204.
5. **Crowell, R. L.** 1976. Comparative generic characteristics of picornavirus-receptor interactions, p. 179–202. *In* R. F. Beers, Jr., and E. G. Bassett (ed.), Cell membrane receptors for viruses, antigens and antibodies, polypeptide hormones, and small molecules. Raven Press, New York.
6. **Crowell, R. L., and J.-S. Siak.** 1978. Receptor for group B coxsackieviruses: characterization and extraction from HeLa cell plasma membranes, p. 39–55. *In* M. Pollard (ed.), Perspectives in virology. Raven Press, New York.
7. **Gefter, M. L., D. H. Margulies, and M. D. Scharff.** 1977. A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. Somatic Cell Genet. **3**:231–236.
8. **Kohler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
9. **Lonberg-Holm, K., R. L. Crowell, and L. Philipson.** 1976. Unrelated animal viruses share receptors. Nature (London) **259**:679–681.
10. **Morishima, T., P. R. McClintock, G. S. Aulakh, L. C. Billups, and A. L. Notkins.** 1982. Genomic and receptor attachment differences between mengovirus and encephalomyocarditis virus. Virology **122**:461–465.
11. **Much, D. H., and I. Zajac.** 1973. Homology of surface receptors for poliovirus on mammalian cell lines. J. Gen. Virol. **21**:385–390.
12. **Roesing, T. G., P. A. Toselli, and R. L. Crowell.** 1975. Elution and uncoating of coxsackievirus B3 by isolated HeLa cell plasma membranes. J. Virol. **15**:654–667.
13. **Sekiguchi, K., A. J. Franke, and B. Baxt.** 1982. Competition for cellular receptor sites among selected aphthoviruses. Arch. Virol. **74**:53–64.
14. **Yewdell, J. W., and W. Gerhard.** 1981. Antigenic characterization of viruses by monoclonal antibodies. Annu. Rev. Microbiol. **35**:185–206.
15. **Zajac, I., and R. L. Crowell.** 1965. Location and regeneration of enterovirus receptors of HeLa cells. J. Bacteriol. **89**:1097–1100.