Coxsackievirus B3 Infection Alters Plasma Membrane of Neonatal Skin Fibroblasts

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Replication of coxsackievirus B3 occurred for days in cultures of murine neonatal skin fibroblasts in the absence of cytopathology and resulted in alteration of the plasma membrane. Dual immunofluorescence studies showed that the lectin Ulex europaeus agglutinin I bound only to cells producing viral capsid antigens. Cultures of coxsackievirus B3-inoculated murine neonatal skin fibroblasts showed maximum binding of this lectin at 72 h postinoculation. These data show that in a nonlytic infection a picornavirus can alter the surface of an infected cell.

A hallmark of most picornavirus infections is extensive cytopathology of the host cell (3, 18); therefore, picornaviruses are generally thought not to alter the plasma membrane of infected cells (2). Here we report that replication of coxsackievirus B3 (CVB3) occurs for days in cultures of murine neonatal skin fibroblasts (MNSF) in the absence of cytopathology. We are studying this unusual picornavirus-cell interaction as an in vitro model of in vivo responses of murine heart tissue fibroblasts to this virus. Because the group B coxsackieviruses are the viruses most frequently associated with cardiomyopathies in infants and adults (1, 8, 9, 20) and because fibroblasts are readily detected in endomyocardial biopsy specimens from myocarditic lesions in human hearts (21), knowledge of any virus-induced cell surface neoantigens(s) may be important to our understanding of these diseases.

 Cultures of MNSF were prepared from CD-1 neonates (24 to 48 h old) (5, 19) and challenged with 100 to 500 PFU per cell of a myocarditic variant of CVB3 (CVB3m) (4, 5). Growth curves of CVB3m in MNSF show that titers ranging from 65 to 589 PFU per cell are produced between 24 and 156 h postinoculation (p.i.) and that greater than 95% of nascent virus is released at all times (data not shown). Gross cytopathology is not detected in CVB3m-infected cultures at the light microscope level at any time p.i. Immunofluorescence assays with rabbit hyperimmune anti-CVB3m antiseraum showed that CVB3m-inoculated MNSF contained a maximum proportion of virus-producing cells (10 to 12%) at 72 h p.i. (Table 1, experiments 1 and 2) and that this proportion declined thereafter. Cell viability was similar in both cultures, as were population doubling times of 36 to 44 h (in three experiments). Fibroblast cultures established from heart tissue of young (10 to 14 days old) and adolescent (4 to 6 weeks old) CD-1 mice also replicated CVB3m with similar parameters of infection (data not shown).

Among several fluorescein-labeled lectins examined for the ability to bind to infected but not uninfected cells in MNSF cultures (concanavalin A, wheat germ agglutinin, Dolichos communis agglutinin I [RCA 120], and peanut agglutinin), strong binding of Ulex europaeus agglutinin I (UEAI) and weak binding of soybean agglutinin were observed (Table 1). The similarities between percentages of lectin-binding cells and virus-infected cells in the cultures led to further examination of these cultures by dual fluorescence. The latter technique (Table 1, experiments 5 and 6) showed that UEAI-binding cells were also positive for production of CVB3m antigens. Photomicrographs of dual-fluorescence-positive cells showing cell surface-bound UEAI and internalized CVB3m antigens are presented in Fig. 1. The fluorescence patterns for surface-attached UEAI and internal CVB3-capsid antigens were different in the dually positive cells shown. Only a few cells (< 3/100) not showing detectable CVB3m immunofluorescence were positive for UEAI fluorescence. UEAI binding was minimal at 24 h p.i., maximal at 72 h p.i., and diminished thereafter (data not shown). Also, treatment of CVB3m-inoculated MNSF at 0 or 24 h p.i. with murine beta interferon (100 U/ml) reduced the level of UEAI binding (indirect fluorescence) to cells in MNSF cultures by half or greater (data not shown); CVB3m-inoculated MNSF cultures treated with murine beta interferon (100 U/ml) and the corresponding control cultures showed no differences.

<table>
<thead>
<tr>
<th>Binding agent</th>
<th>No. of fluorescent cells in different expts (total no. of cells)</th>
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<tbody>
<tr>
<td></td>
<td>1 (200)</td>
</tr>
<tr>
<td>Anti-CVB3a</td>
<td>25</td>
</tr>
<tr>
<td>Anti-UEAIb</td>
<td>ND</td>
</tr>
<tr>
<td>SBAa</td>
<td>ND</td>
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</tbody>
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* Cells were challenged with ~100 PFU of CVB3m, and adsorption was permitted for 3 h at 37°C. Complete minimal essential medium containing 10% fetal bovine serum was added, and incubation continued at 37°C until 72 h p.i. Cells were washed extensively with Dulbecco phosphate-buffered saline and fixed with cold methanol–ethanol (1:1) experiments 1 and 2) or incubated with UEAI or soybean agglutinin for 1 h at 37°C in a moist atmosphere before fixation with 0.01 M periodate–0.075 M lysine–1% paraformaldehyde–0.5% glutaraldehyde buffer (13) and then with cold methanol–ethanol (experiments 3–6).

* Dual fluorescence was performed on cells manipulated as in experiments 3-6 except that cells were incubated with UEAI, washed extensively, incubated with rabbit anti-UEAI antiserum and mouse anti-CVB3a in the primary mixture for 1 h at 37°C, washed extensively, and then incubated with goat anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate and sheep anti-mouse immunoglobulin G conjugated to tetramethyl rhodamine isothiocyanate in the dark for 1 h at 37°C. Controls, which included omission of the primary antibodies or heterospecific mixtures of primary-conjugated secondary antibody, were negative.

* Rabbit anti-CVB3a bound to cells was detected by fluorescein-conjugated goat anti-rabbit immunoglobulin G.

* Fluorescein-conjugated UEAI at 10 µg/ml.

* ND. Not done.

* Fluorescein-conjugated soybean agglutinin at 10 µg/ml.

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feron produce less virus (10). In other experiments (data not shown), fibroblasts cultured from heart tissues of adolescent CD-1 mice and inoculated with CVB3m showed coincident UEAI surface binding and internalized CVB3m antigens at 72 h p.i., and the dual-fluorescence staining patterns were indistinguishable from those observed in CVB3m-inoculated MNSF.

UEAI binds to α-L-fucosyl residues (7, 12), and since...
picornaviruses are not known to direct synthesis of any glycoproteins, the new surface residues are most likely cellular in origin. Also, a few (1 to 3%) cells were present in uninoculated MNSF cultures which displayed low-level binding of UEA1.

Previous studies from our laboratories with mouse (5, 6, 11, 15–17) and baboon (14) models of CVB3-induced myocarditis showed that heart tissues from CVB3-inoculated animals with myocarditis (but not from normal animals) contained a new nonviral KCl-extractable antigen(s). This antigen(s) evoked a specific immune response, i.e., production of migration-inhibition factor from sensitized peritoneal exudate cells taken from virus-inoculated animals in a cell migration inhibition assay (14–16), an in vitro correlate of cell-mediated immunity and delayed hypersensitivity. Studies are in progress to characterize and determine the relationship of the CVB3-induced UEA1-binding surface antigen(s) on MNSF to CVB3-induced KCl-extractable heart antigen(s).

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