Ventricular Aneurysms Complicating Coxsackievirus Group B, Types 1 and 4 Murine Myocarditis

M. Riad El-Khatib, M.D., Jacob L. Chason, M.D., Ph.D., and A. Martin Lerner, M.D.

SUMMARY Suckling Swiss Webster mice were inoculated with $10^4\text{TCD}_{50}$ of coxsackieviruses, group B types 1 or 4. Virulent necrotizing myocarditis resulted in 185 infected mice. Of the latter group, three (14.3%) nurslings on the 17th and 23rd day after inoculations had left ventricular aneurysms postmortem. None of 61 concurrently matched control mice developed aneurysms. Ventricular aneurysm is a suggested but previously undocumented complication of murine, and possibly human necrotizing transmural coxsackievirus myocarditis.

The major cause of ventricular aneurysms is coronary arterial atherosclerosis progressing to occlusion and transmural myocardial infarction. Occasionally, rheumatic heart disease, bacterial endocarditis, trauma, syphilitic gumma, sarcoidosis or congenital anomaly (e.g., origin of left coronary artery from pulmonary artery or defect in fibrous band of mitral ring) are responsible. Although abnormal left ventricular motion and ventricular aneurysms have also been documented in humans with primary myocardial disease in the Bantu native, in Nigeria and other parts of Africa, in alcoholics, and in sarcoid, there is no conclusive evidence that viruses produce a continuing cardiomyopathy or ventricular aneurysm. 1–10 Nor does proof exist for this thesis in an experimental model. 11, 12 We report the experimental production of three instances of aneurysmal dilatation of the left ventricle 17–23 days after the onset of acute necrotizing myocarditis in suckling mice with coxsackieviruses, group B, types 1 or 4.

Materials and Methods

Viruses

We received a strain (#6631) of coxsackievirus B1. This virus had been recovered from a rectal swab in monkey kidney tissue cultures. It was sent to us after several passages in the same cells. Coxsackievirus B4 (Dowell) had been isolated in rhesus monkey kidney tube cultures at autopsy from the heart of an infant dying with myocarditis. Both stocks were kept ($-70^\circ \text{C}$) in screw-capped vials until use.

Coxsackieviruses were passaged twice in vero renal tube cultures. Using standard methods, pools were titered and re-identified as coxsackieviruses B1 or B4 by neutralization tests in tube cell cultures with hyperimmune sera (Microbiologic Associates, Bethesda, Maryland). Maintenance medium for these cultures was Eagle's basal medium (EM) with 2% fetal calf serum and antibiotics (50 μg penicillin G and 50 μg streptomycin per ml).

Animals

Pregnant Swiss Webster mice were obtained from Spartan Research Animals Inc. (Hasslet, Michigan). After delivery, litters were randomly separated into control (61 mice) or infected groups (418 mice). Every litter, either infected or control, was cared for in separate cages.

Experimental Infection, Isolation of Viruses and Pathologic Findings

When nurslings were 2 days old, stock viruses were thawed, diluted in EM, and 0.1 ml containing $10^5-10^4 \text{TCD}_{50}$ of coxsackievirus B1 or B4 was inoculated intraperitoneally into each baby mouse. Two hundred twenty-four animals were inoculated with coxsackievirus B1 and 194 with coxsackievirus B4. Control animals were simultaneously inoculated with virus-free suspensions of sonicated vero tissue cultures. For any experiment, either coxsackievirus B1 or B4, but not both, was used. All mice were observed daily, their condition recorded, and, after ether, on days 2, 3, 5, 7, 10, 14–17, and 20–23, four to eight animals from paired control and infected groups were sacrificed by exsanguination, cutting deeply into an axillary vein. Hearts were removed aseptically from the thorax, and gross findings were noted.

At autopsy, 1–4 hearts were minced, and 10% suspensions in EM prepared for attempts at virus isolation in rhesus renal tube tissue cultures. Similarly, bloods were assayed for virus. Cytopathogenic agents were passaged 1×, pools of infected fluids quantitated, and viruses were identified again as coxsackievirus B1 or B4 by standard neutralization tests.

Remaining hearts were coded and cut coronally at two levels parallel to the base, thus producing three blocks for serial study. After embedding in paraffin, 10 serial $6-\mu$ sections were made from the upper surface of each block. Sections were stained with hematoxylin and eosin, and examined for histologic changes.

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*\text{TCD}_{50}$, fifty percent tissue culture infective doses.
TABLE 1. Clinical and Virological Findings Among Suckling Mice with Coxsackievirus Group B Type 1 or Type 4 Myocarditis

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>A. Coxsackievirus, B1</th>
<th>B. Coxsackievirus, B4</th>
<th>C. Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice, sick (s)/dead (d)/well (w)</td>
<td>No. of mice, sick (s)/dead (d)/well (w)</td>
<td>No. of mice, sick (s)/dead (d)/well (w)</td>
</tr>
<tr>
<td>0</td>
<td>0 8†† 22†† 49†† 35†† 26 0</td>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>2</td>
<td>100 85 49 0 0 0 18</td>
<td>100 100 100 100 100 100 100</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td>s</td>
<td>8 30.56 84.48 97.1 96.3 0</td>
<td>85 82 55 0 0 3 3</td>
<td>100 96.5 79.71 0 0 100 100</td>
</tr>
<tr>
<td>d</td>
<td>7 1.39 15.52 2.9 3.7 0</td>
<td>0 0 0 0 0 0 0</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td>w</td>
<td>100 85 68.05 0 0 0 100</td>
<td>100 100 100 100 100 100 100</td>
<td>100 100 100 100 100 100 100</td>
</tr>
</tbody>
</table>

*Inoculum in each case was 10^4 TCD50 of coxsackievirus B1 or B4.
†Virus isolated from four of four hearts.
‡Virus isolated from four of four blood samples.
§Virus isolated from one of four blood samples.

Results

Three days after the inoculations, infected mice developed seizures and spastic paralyses (table 1). Signs were maximum on days 5–10. Some sick mice survived and appeared well after day 10, but fore- or hindlimb weakness persisted. No deaths occurred after the tenth day of infection. All control mice remained well, and had no gross or microscopic lesions.

Coxsackievirus B1 or B4, respectively, was isolated from every blood or heart of infected animals on days 2–7, but cytopathogenic agents were never recovered after inoculation from hearts or blood of controls. Necrotizing myocarditis appeared in baby mice inoculated with either virus beginning the second day after infection (table 2). Lesions were maximal on days 3–5 with coxsackievirus B1 and on day 5 with coxsackievirus B4. Residual inflammation, fibrosis and calcification were observed through day 23.12,13 None of the 61 controls showed any evidence of myocarditis (table 2). These mortality and pathologic findings are not unusual, and were expected.13

The hearts of three mice were distinctive. At necropsy, on day 17, the heart of one mouse infected with coxsackievirus B1 had a left ventricular aneurysm. On day 23, two additional hearts from mice infected with coxsackievirus B4 had left ventricular aneurysms (figs. 1 and 2). Indeed, one of the latter mice had two aneurysmal dilatations in its left ventricle. All three of these nurslings had been inoculated with 10^4 TCD50 of the respective coxsackievirus (tables 1 and 2). Of the 185 suckling mice which received an inoculum of 10^4 TCD50, only 21 survived. Eighteen developed coxsackievirus B1 infections, and three had coxsackievirus B4 infections. Thus, of the combined survivors beyond the tenth day of infection, three of 21 mice (14.3%) developed aneurysms.

At aneurysmal sites, myocardial thickness was strikingly reduced (fig. 2). Severe necrosis of myocardial fibers, mononuclear cell infiltration and beginning fibrosis were present in involved and adjacent areas, but calcification was absent. Mural thrombi were not seen. Coronary vessels were patent and without vasculitis.

In parallel experiments not reported here, none of 129 suckling mice inoculated with coxsackieviruses group B, types 2, 3 or 5 developed ventricular aneurysms. Similarly, 378 older mice, weanlings or adults, either infected with coxsackieviruses group B, types 1–5, or controls had no ventricular aneurysms.

Discussion

These initial findings of three ventricular aneurysms among 21 suckling mice who survived a virulent necrotizing myocarditis induced by intraperitoneal injections of 10^4 TCD50 of coxsackieviruses, group B, types 1 or 4 seem important. In 1969, we found that a chronic continuing murine cardiomyopathy might follow acute myocarditis induced by coxsackievirus B3.19 Previously, only the acute phase of experimental
TABLE 2. Gross and Microscopic Findings of Necrotizing Myocarditis Among Suckling Mice with Coxsackievirus, Group B, Type 1 or Type 4 Myocarditis

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>No. of mice with/without myocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>A. Coxsackievirus, B1</td>
<td></td>
</tr>
<tr>
<td>Gross findings</td>
<td>0/13</td>
</tr>
<tr>
<td>Microscopic findings</td>
<td>3/2</td>
</tr>
<tr>
<td>% Gross findings</td>
<td>0</td>
</tr>
<tr>
<td>% Microscopic findings</td>
<td>60</td>
</tr>
<tr>
<td>B. Coxsackievirus, B4</td>
<td></td>
</tr>
<tr>
<td>Gross findings</td>
<td>0/13</td>
</tr>
<tr>
<td>Microscopic findings</td>
<td>4/1</td>
</tr>
<tr>
<td>% Gross findings</td>
<td>0</td>
</tr>
<tr>
<td>% Microscopic findings</td>
<td>80</td>
</tr>
<tr>
<td>C. Controls</td>
<td></td>
</tr>
<tr>
<td>Gross findings</td>
<td>0/6</td>
</tr>
<tr>
<td>Microscopic findings</td>
<td>0/4</td>
</tr>
<tr>
<td>% Gross findings</td>
<td>0</td>
</tr>
<tr>
<td>% Microscopic findings</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: ND = not done.

A.\footnotesize{FIGURE 1. A) The heart of a 19-day-old mouse on day 17 after an intraperitoneal inoculation of 10^4 TCD_{50} of coxsackievirus B1 (magnification × 5). The dilatation of the freshly autopsied specimen (arrow) and B) dimpling (arrow) of the left ventricular wall due to the aneurysm after fixation is visible (magnification × 10).}
coxsackievirus myocarditis had been studied. Since then, most observations have been limited to coxsackievirus B3, which is most cardiotropic in weanling mice and, in our experiments, has not produced ventricular aneurysms. In the latter case, cytolytic thymic derived lymphocytes directed against a virus induced, but non-virion neo-cardiac antigen, have been implicated. Cytolytic lymphocytes have been suspected in massive myocardial necrosis in a 33-year-old American woman. An association with an earlier “virus” infection was suspected.

In this study, coxsackieviruses B1 and B4 induced...
severe necrotizing transmural myocarditis in suckling mice, resulting in aneurysmal dilatation of a fibrosing scar. Localized abnormalities in cardiac contraction have been documented by ventriculographic studies in 10 of 34 patients with primary myocardial disease, and in six of 10 patients with Bantu cardiomyopathy. Similar kinetic abnormalities may have initiated the aneurysmal dilatation at the areas of transmural fibrosis which we have demonstrated. Certainly these convalescing mice did not rest, and, after transmural myocardial infarctions in humans, early ambulation and hypertension facilitate formation of ventricular aneurysms.

Coxsackievirus group B human infections are common. There is serologic evidence implicating concomitant coxsackievirus infection in some patients with myocardial infarction. Coronary vessels may be patent in human myocardial infarctions. Some ventricular aneurysms in humans may be complications of unrecognized coxsackievirus B necrotizing myocarditis.

Acknowledgment

We received coxsackievirus B1 as a gift from Dr. Dorothy Horstmann, Yale University School of Medicine.

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