Experimental Herpes Simplex Virus Carditis in Mice

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Herpes simplex virus types 1 and 2 induced acute and chronic cardiac damage in suckling and weanling mice after intranasal inoculation. Signs of virus replication were detected by light, immunofluorescent, and electron microscopy techniques. Virtually all of the cardiac tissues appeared to be susceptible to herpes simplex virus. The myocardium, however, was most regularly affected. The viral lesions were discrete during the acute phase of infection. The cardiac damage, however, was more extensive in some of the chronically infected mice. Morphologically, these lesions either resembled the acute ones or were associated with inflammatory granulomatous and sclerotic changes.

The nature of virus-induced heart disease seems to depend upon the type of virus, and at least 18 different viruses, including herpesvirus zoster, have been isolated (1, 2, 8, 11). One of the most common viruses, however, herpes simplex virus (HSV), has not been observed to replicate in hearts of either experimentally or naturally infected animals or humans (10). On theoretical grounds this is an unexpected finding because, if one considers the ubiquitous nature of HSV, there is no obvious reason why it should not affect the heart tissues, particularly as it is known to replicate in endothelial cells (6). Furthermore, occasionally viremia has been detected in natural and experimental infections (6, 10, 13). For these reasons it seemed worthwhile to reexamine this question by carrying out a detailed study of the hearts of experimentally infected mice.

The findings described in this report have revealed evidence of HSV replication in the epicardium, myocardium, mural endocardium, heart valves, and cardiac ganglia by light, immunofluorescent, and electron microscopy techniques.

MATERIALS AND METHODS

Virus. The virus pools used in these studies were prepared in mouse brain, inoculated at the age of 1 day. Hanks balanced salt solution was used as diluent to which 250 U of penicillin per ml and 250 µg of streptomycin per ml were added before inoculation. The virus pools were titrated in African green monkey kidney (Vero) cells. The mean tissue culture infective dose (TCID₅₀) was calculated according to Karber's formula (7).

Two standard strains of HSV were used. The HF strain of HSV-1 was obtained from the School of Hygiene, University of Toronto, where it had been passed once each in chicken chorioallantoic membrane and human amnion cells, twice in HeLa cells, and an unspecified number of times in mouse brain. The virus was passaged eight more times in mouse brain before use. The titer of the stock suspension was 10⁸ TCID₅₀/ml. The MS strain of HSV was purchased from the American Type Culture Collection. The virus had a history of an unspecified number of passages in sheep choroid plexus and HeLa cultures, eight passages in primary rabbit kidney cultures, two passages in human foreskin cell cultures, and three passages in mice. Before use it was passed once more in human foreskin cell cultures and three times in mouse brain. The titer of the stock virus was 10⁸ TCID₅₀/ml.

Animal inoculation. Three- to fourteen-day-old, randomly bred, albino mice were used. These mice were of our own stock, which originated from Cottage Laboratories Limited colonies. Approximately 100 TCID₅₀ of virus, contained in 0.03 ml, was instilled into the nares of each mouse by using a blunt hypodermic needle. Before inoculation the mice were anesthetized with ether. Virus-free supernatants of mouse brain suspensions were instilled into the control animals. A total of 50 to 70 mice were inoculated for each experimental group.

Harvesting of tissue samples. During the acute stage of disease, the mice were harvested when moribund. The hearts were collected for light, fluorescent, and electron microscopy examination. The survivors were harvested at random at various intervals, 28 to 180 days after inoculation. Hearts from these animals were examined only with a light microscope. Before removing the hearts, the mice were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal).

Fluorescent antibody studies. Immediately upon removal the hearts were cut in longitudinal slices and quick frozen in a dry ice-isopentane alcohol slurry. The frozen blocks were sectioned in a Pearse-Slee cryostat. The sections were fixed in acetone, overlaid with rabbit anti-HSV serum (kindly provided by A. E. Kellen, Laboratory Center for Disease Control, Ottawa). After incubation at 37°C for

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30 min the slides were washed with several changes of phosphate buffer (pH 7.1) for 10 min, overlaid with fluorescein-conjugated anti-rabbit gamma globulin, and incubated for another 30 min. After washing with phosphate buffer followed by water, the slides were dried, mounted in buffered glycerol, and sealed with nail varnish. The usual controls were treated by the accepted method (4). The sections were examined in a Leitz Orthoplan microscope, equipped with a Ploem incident light illumination and BG12 excitation filter.

Light microscope studies. The hearts were fixed in Bouin fixative and embedded in paraffin, and multiple sections of each heart were stained with hematoxylin and eosin (3).

Electron microscope studies. The electron microscope studies were limited to 3-day-old mice infected with HSV-2 and the corresponding controls. The heart was quickly removed after first being flooded with 3% glutaraldehyde in Millonig buffer at pH 7.4 (9). The excised hearts were placed immediately into fresh, ice-cold fixative. First, each heart was dissected into nine anatomically distinct parts: right auricle, left auricle, right ventricle, left ventricle with epicardium, left ventricle with endocardium, orifice of the pulmonary artery, including the semilunar valve, orifice of the aorta with the semilunar valve, tricuspid valve, and mitral valve. Approximately 1-mm³ blocks were collected from each part and kept separately. These tissue blocks were fixed in the glutaraldehyde fixative for 3 h, followed by frequent rinses in the buffer for at least 24 h, and postfixed in 1% OsO₄ for 90 min. After fixation the blocks were rapidly dehydrated in graded ethanol solutions, passed through two changes of propylene oxide each 15 min long, and embedded in Araldite. Five tissue blocks were picked at random from each of the nine parts of the heart. Approximately 20 thick (0.5 µm) and 30 thin sections, at a minimum of five levels, were cut and collected from each block. The thin sections were stained with lead citrate (12) and 3% aqueous uranyl acetate stains and examined with a Philips EM200 electron microscope.

RESULTS

Signs of disease. Both viruses, HSV-1 (HF) and HSV-2 (MS), induced severe signs of disease in mice inoculated during week 1 of life. All of the infected animals in this age group became spastic and convulsive and developed paralysis shortly before death. The 3-day-old mice died within 3 days, and the 1-week-old mice died within 6 to 8 days after inoculation. The 2-week-old mice that were inoculated with HSV-1 showed no obvious changes at any time during the observations. By contrast, the 2-week-old animals that had received HSV-2 looked ruffled, slow, and smaller than the controls. About 30% of them died within 10 days. None of them had shown any signs of damage in the central nervous system. The survivors recovered completely and by the end of the experiment were indistinguishable from the controls.

Histopathological changes in the acutely infected hearts. In the acutely infected mice small, focal areas of degenerating and necrotic tissues were seen in the epicardium, myocardium, valves, and cardiac ganglia. Most of these lesions contained the characteristic Cowdry type A intranuclear inclusion bodies. Occasionally these inclusions were seen in polykaryocytes. This enabled one to detect even the very early virus-induced lesions, which frequently were confined to a few cells. The inflammatory infiltrate was scant and contained polymorphonuclear and mononuclear cells.

The heart appeared to be susceptible to the HSV in all three of the age groups examined (Table 1). The frequency and distribution of the lesions, however, varied somewhat, depending upon the virus type and the age of the mouse.

Table 1. Distribution and frequency of HSV-1 and HSV-2 cardiac lesions in experimentally infected mice

<table>
<thead>
<tr>
<th>Age when inoculated (days)</th>
<th>HSV type</th>
<th>Localization of HSV lesion</th>
<th>% of mice affected at day:</th>
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<tr>
<td></td>
<td></td>
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<td>3⁺</td>
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<td>3</td>
<td>2</td>
<td>Valves</td>
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<tr>
<td></td>
<td></td>
<td>Myocardium</td>
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<td>7</td>
<td>2</td>
<td>Valves</td>
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<td></td>
<td></td>
<td>Myocardium</td>
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<td>Epicardium</td>
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<tr>
<td>14</td>
<td>1</td>
<td>Myocardium</td>
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* Number of days after inoculation.
⁺ In groups of 30 mice.
NS, No survivors.
⁺⁺ In groups of 50 mice.
In general, HSV-2 seemed to be more cardiotropic than the HSV-1 strain used. The 3-day-old hearts were damaged only by HSV-2. Furthermore, valvulitis (Fig. 1) was induced only by HSV-2 and only in the suckling mice. By contrast, the epicardium seemed to be spared in the 3-day-old age group. In the 7- and 14-day-old mice the epicardium regularly showed foci of inflammatory cells. In these areas the nuclei of the epicardial mesothelial cells contained the characteristic inclusion. The myocardium was found to be affected by HSV at all ages. In well-localized, tiny areas the muscle fibers showed signs of degeneration. The nuclei were either swollen or pyknotic and frequently contained type A inclusions. Clumps of infected nuclei were common, particularly in the perivascular areas. The incidence of the HSV damage in the cardiac ganglia was omitted from Table 1 because in some cases they had been missed during sectioning. When present, they either looked completely normal or showed damage of varying severity (Fig. 2). In the latter case the characteristic HSV inclusions were present in both the neurons and satellite cells.

**Histopathological changes in the chronically infected hearts.** In the chronically infected mice, harvested 28 to 180 days after inoculation (Table 1), two types of changes were observed. In some mice the cardiac lesions were indistinguishable from those observed in the acute phase; in others the damage was associated with inflammatory granulomatous and sclerotic changes. Despite either the unmistakably chronic or subacute nature of these lesions, the presence of intranuclear inclusions was not unusual (Fig. 3). The distribution and frequency of the lesions are summarized in Table 1. The lesions were not observed in the controls.

**Immunofluorescence in the acutely infected hearts.** The infected heart tissues were obtained from two groups of mice; one group was inoculated at the age of 3 days, and the other group was inoculated at 14 days with either HSV-1 or HSV-2. The younger age group was affected only by HSV-2. In the older age group immunofluorescent cells were first observed on day 3 after inoculation. However, the numbers of the antigen-producing cells increased on the next day and were maximal on day 6 after inoculation. No specific fluorescence could be detected by day 8 of infection. Between days 3 and 6 of infection approximately 50 to 75% of the examined hearts appeared to contain HSV-producing cells. The incidence was higher and the cell involvement was more extensive in HSV-2- than in the HSV-1-infected hearts. The virus-specific fluorescence was observed most regularly in the myocardium. In the muscle fibers the fluorescence was confined to numerous nuclei throughout the heart (Fig. 4a). Furthermore, among the muscle fibers there were dispersed large cells with strongly fluorescing nucleus and cytoplasm (Fig. 4b). In sections stained with hematoxylin and eosin most of these cells looked like macrophages. In the epicardial and endocardial cells the fluorescence was granular and comparatively weak. By contrast, the subendocardial tissues in some areas contained strongly fluorescent cells. These appeared to be macrophages and possibly also Schwann cells, associated with unmyelinated nerves. In some hearts the cells in the aortic semilunar valve were strongly fluorescent. No comparable fluorescence was observed in any of the controls.

**Ultrastructural changes in the acutely infected hearts.** In the majority of cases the cardiac lesions in the acutely infected, 3-day-old mice were very small and well localized. HSV particles were identified, on the average, in one out of five tissue blocks. Most frequently the virus was present in the auricular tissues, near the origin of the aortic valve. A wide variety of cells appeared to be infected. Intranuclear herpesvirus particles were seen in cardiac muscle fibers (Fig. 5), fibroblast-like cells (Fig. 6), epicardial and endocardial lining cells (Fig. 7), Schwann cells (Fig. 8), and also macrophages (Fig. 5). Virions or incomplete viruses appeared singly or, less frequently, in small crystalline aggregates (Fig. 5). In some cases the infected nuclei had maintained a virtually normal appearance. Usually, however, the nuclear membrane was more or less crenated, lined with chromatin, and often showed duplications. In areas where the virus replicated the nucleoplasm was less electron dense. Intracytoplasmic viruses were of common occurrence. A possible exception was the sarcoplasm of the cardiac muscle fibers, which appeared to be free.

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**Fig. 1.** Valvulitis in a mouse heart infected with HSV-2 at the age of 7 days and harvested 6 days later (hematoxylin and eosin). (a) ×250. (b) Intranuclear Cowdry type A inclusions are present in several cells (arrows). Some nuclei show pyknotic or karyorrhectic changes. ×1,000.

**Fig. 2.** Cardiac ganglion in a mouse infected with HSV-2 at the age of 3 days and harvested 6 days later. Type A inclusions are present in the nuclei of both the ganglion and the satellite cells (hematoxylin and eosin, ×1,000).
from virus. The virus-induced cytoplasmic damage was of varying severity. In some of the infected cells the cytoplasm appeared normal, whereas in others the cytoplasmic organelles were less electron dense, enlarged, and distorted. Occasionally, however, one could see cells in which the normal structures were replaced by myelin figures and autophagic vacuoles, surrounded by an abnormally electron-dense cytoplasm. Severe changes, associated with marked interstitial swelling and the presence of macrophages, were observed in the subendocardial and subepicardial tissues. The macrophages appeared to support virus replication (Fig. 5). Because the cardiac lesions were comparatively sparse and varied from animal to animal, even within the same age group, an account of the temporal sequence and relative frequency of the cellular changes was not attempted.

**DISCUSSION**

HSV is not known to cause cardiac disease in natural infection. The experimental studies described in this report, however, have revealed ample evidence that both HSV-1 and HSV-2 may be cardiotropic to the murine heart. The fact that the ensuing lesions were very discrete may explain why they have been overlooked by other investigators. Although the virus was not very destructive during the acute phase of infection, large numbers of cells in the various cardiac tissues were producing HSV antigens. Studies are in progress to find out whether the HSV might persist in the muscle fibers that were obviously infected but not destroyed. It has been suggested by earlier investigators that other cells besides the sensory neurons might be associated with herpesvirus latency (5). As a result of the present findings two other
possible suspects of harboring latent infection emerged: the Schwann cell, which was seen in association with the unmyelinated axons in the heart, and the fibroblast-like cells in the myocardium. The infection in the neurons in the local autonomic ganglia was frequently associated with extensive structural changes. One suspects, therefore, that HSV infection in these cells may have resulted in destruction rather than latency.

The finding that the heart damage may be considerably aggravated in chronically infected mice was suggestive either of a continuous HSV pathogenesis or of recurrent infection(s). The disappearance of the specific fluorescence from the infected hearts after week 1 of infection excluded the possibility of a continuous infection. By contrast, the presence of the characteristic HSV inclusions in some cells within the subacute or chronic lesions was indicative of a reactivated infection. To prove the hypothesis that HSV may cause recurrent infections in a mouse heart, we are presently trying to detect and reactivate a latent HSV in an experimentally infected mouse heart.

Although virtually every one of the acute or chronic lesions might interfere with the normal function of a heart, the lesions in the semilunar valves and the autonomic ganglia, as well as the unmyelinated nerves, seem to merit particular attention for future studies. One also should not overlook the susceptible fibroblast-like cells in the myocardium. These cells had a marked resemblance to the Anitschkow cell, which in humans has been associated with rheumatic heart disease and the development
Fig. 5. Virus particles (arrows) are present in the nucleus of a heart muscle fiber (H) and in the nucleus and cytoplasm of a macrophage (M) 5 days after inoculation. ×11,400. Insert: Encircled virus particles in the nucleus of the heart muscle fiber. ×89,000.
FIG. 6. HSV (arrows) are seen in the nucleus (N) of a fibroblast-like cell in the myocardium of a mouse harvested 6 days after inoculation. ×13,650.

FIG. 7. Virus particle (arrow) in the nucleus of an endocardial cell 4 days after inoculation. L, Lumen of ventricle. ×22,000.
FIG. 8. Section through an unmyelinated nerve in a mouse heart 6 days after inoculation. Virus particles (arrows) are present in the nucleus of a Schwann cell (S) and in the nucleus and cytoplasm of a fibroblast (F). ×19,050.
of Aschoff body (14). Thus, the experimental herpetic carditis in mice might be used as a model for the elucidation of rheumatic heart disease and possibly other, yet unexplained, human cardiopathies.

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LITERATURE CITED