Rat Model of Experimental Endocarditis

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A simple model of infective endocarditis was produced in rats. With the aid of a guide wire, polyethylene catheters were passed into the left ventricle through the right carotid artery of Sprague-Dawley rats weighing 300 to 350 g. A volume of 1 ml of an overnight culture of Streptococcus mitis, Staphylococcus aureus, or Streptococcus faecalis was intravenously injected 1 to 2 days after catheterization. Bacterial titers of Streptococcus mitis in vegetations were about 10^4-fold greater than in other tissues. Blood cultures were always positive after 6 h. Mortality was 19% at 1 week and 82% at 2 weeks. Catheters were pulled 24 h after infection, and vegetation titers of >7.0 log_{10} colony-forming units per g were sustained at 5 days. In intravenously infected rats without catheters, blood and tissues were sterile after 3 to 5 days. With Staphylococcus aureus, vegetations had >9.0 log_{10} colony-forming units and with Streptococcus faecalis 8.8 ± 0.3 log_{10} colony-forming units per g at 2 days. The rat model of infective endocarditis should prove to be suitable for further pathological and therapeutic studies.

The study of experimental infective endocarditis has been greatly enhanced in recent years by the rabbit model (1-6). Rats, however, offer advantages over rabbits as laboratory animals because rats are more easily housed and are less expensive. The following study was undertaken to evaluate experimentally produced infective endocarditis in rats.

MATERIALS AND METHODS

Microorganisms. Microorganisms (a strain each of Streptococcus mitis, Staphylococcus aureus, and Streptococcus faecalis) isolated from the blood of patients with endocarditis were used in all experiments. Stock cultures were maintained by storing aliquots of an 18-h culture in heart infusion broth (Difco Laboratories, Detroit, Mich.) at -20°C. For each experiment, the stock culture was thawed and incubated in 10 ml of heart infusion broth overnight at 37°C. Sheep blood (2%) was added to broth for growth of Streptococcus mitis.

Production of endocarditis. Male Sprague-Dawley rats (Blue Spruce Farms, Inc., Altamont, N.Y.) were anesthetized with 18 mg of sodium pentobarbital given intraperitoneally. The right carotid artery was exposed through an anterior incision just slightly right of midline above the clavicles. Polyethylene tubing (Intramedic PE 10; Clay Adams, Parsippany, N.J.) was placed over a 0.008-inch (ca. 0.2032 mm) guide wire (American Wire Co., Philadelphia, Pa.) and passed within the right carotid artery into the left ventricle until resistance was met. The guide wire was then removed, and the catheter was secured as previously described for the rabbit model (4). Cardiac pulsations of the catheter indicated proper placement of the catheter tip at the apex of the heart. After 24 to 48 h, 1 ml of the overnight cultures was injected through a tail vein. Rats were sacrificed by intraperitoneal injection of 60 mg of sodium pentobarbital from 6 h to 1 week later. Vegetations and tissue samples from lung, liver, kidney, and spleen were excised, weighed, and homogenized in heart infusion broth with a Teflon tissue grinder (Tri-R Instruments, Inc., Rockville Center, N.Y.). Blood was obtained for culture by puncture of the right ventricle at the time of sacrifice. Rats without catheters were infected, sacrificed, and cultured in the identical fashion as described above for rats with intracardiac catheters.

Microbial enumeration. Serial 10-fold dilutions of the inoculum or homogenized tissue suspension were made in heart infusion broth. The number of colony-forming units (CFU) of bacteria per gram of tissue was determined by plating 0.1 and 1.0 ml of inoculum or homogenized tissue and 0.1 ml of each dilution onto plates containing Trypticase soy agar with 5% sheep blood (BAP). Plates were read after 48 h of incubation at 37°C. This method permitted the detection of as few as 10^1 CFU/g of tissue and 10^2 CFU/g of vegetation. A quantity of 1 ml of blood was placed in broth and 0.1 ml was placed on BAP.

Survival studies in streptococcal endocarditis. Rats were catheterized and infected as above. Animals were observed daily and autopsied within 24 h of death to establish the presence of endocarditis. Vegetations were homogenized in 1.0 ml of heart infusion broth and cultured semiquantitatively on BAP with a 0.001-ml platinum loop (at least 10^5 CFU/g). After 2 weeks, all surviving animals were sacrificed and autopsied in similar fashion.

Removal of catheters. Rats were catheterized and inoculated with streptococci as above. Catheters were removed after 24 h of infection, and animals were sacrificed 5 days later to determine whether endocar-
diagnosis persisted without the continuous presence of an intracardiac catheter.

RESULTS

Streptococcus mitis endocarditis. Only when catheters were placed across the aortic valve into the left ventricle were sterile macroscopic vegetations reliably produced (Table 1). Upon intravenous injection of Streptococcus mitis, endocarditis was produced in all rats in which the catheter crossed the aortic valve leaflets. As early as 6 h after infection, the mean log$_{10}$ CFU/g (± standard error of the mean) of vegetation was 6.9 ± 0.5 (three rats), whereas valves in three rats without catheters contained <2.0, 2.0, and 3.7 log$_{10}$ CFU/g of valve leaflet, respectively, although vegetations were not visible. The vegetations in catheterized rats at 6 h were quite small; the mean weight ± standard error of the mean was 7.6 ± 1.8 mg. However, this value may be falsely elevated because of the difficulty in excising the vegetation without also including small amounts of valvular tissue. These falsely elevated weights may also have falsely lowered titers of organisms in the vegetations. By 24 h, vegetations were much larger; mean weight ± standard error of the mean was 24.2 ± 2.6 mg. The mean log$_{10}$ CFU ± standard error per gram of vegetation in five catheterized rats at 24 h (7.3 ± 0.2) remained similar to that found at 6 h, whereas valves 1 or more days after infection in 10 control animals were sterile (<2.0 log$_{10}$ CFU/g). In contrast, catheterized rats had vegetation that weighed 50.6 ± 11.8 mg, 35.0 ± 4.0 mg, and 43.3 ± 8.5 mg and had mean Streptococcus mitis titers of 7.5 ± 0.5, 8.4 ± 0.1, and 8.0 ± 0.4 log$_{10}$ CFU/g at 3 days (seven rats), 5 days (seven rats), and 7 days (three rats), respectively. Vegetations on the aortic valve from an animal infected for 7 days can be seen in Fig. 1.

For the first 3 days of infection, titers of bacteria in lung, kidney, liver, and spleen of catheterized rats tended to parallel those found in control animals (Fig. 2). On day 5, when tissues from control animals were sterile, however, tissues from catheterized rats had between 10$^3$ to 10$^9$ CFU/g. These titers were maintained on day 7. Only one of three rats with catheters had positive blood cultures at 6 h, but thereafter all blood cultures were positive, whereas two of three control rats had positive blood cultures at 6 h, and blood thereafter was sterile. There were usually less than 10 organisms per ml in the rats with positive blood cultures.

Survival studies. Sixteen rats were catheterized and infected with Streptococcus mitis 24 h later. All had infected vegetations at death. Of these rats 81% were alive after 1 week of infection, but survival diminished to 56% at 10 days and 18% at 2 weeks.

Removal of catheters. Six animals were catheterized and infected with Streptococcus mitis 24 h later. After 24 h of infection, animals were again anesthetized, and the catheters were removed. After 5 days, the six rats were sacrificed. In two of the six rats, no vegetations were present on the valves, and there was no evidence that the catheter ever crossed the aortic valve. Four of the six rats had obvious endocarditis, and in three the titers exceeded 7.0 log$_{10}$ CFU/g. In one animal, although the vegetation weighed 21 mg, it contained <2.0 log$_{10}$ CFU/g.

Staphylococcal and enterococcal endocarditis. Staphylococcus aureus was injected intravenously in five rats with catheters in place. Two animals died after 48 h of infection, and the remaining three were sacrificed at 48 h. All five animals had vegetations weighing, on the average, 32.2 mg and had titers of >9.0 log$_{10}$ CFU/g. Three animals were similarly infected with Streptococcus faecalis and at 48 h had vegetations weighing, on the average, 29.0 mg and had a mean titer of 8.8 ± 0.3 log$_{10}$ CFU/g.

DISCUSSION

The rabbit model of bacterial endocarditis has been useful in elucidating some of the pathophysiological and therapeutic aspects of the disease. Previous studies of left-sided endocarditis on the aortic valve of rabbits using viridans streptococci have produced similar bacterial populations in vegetations (6). Durack and Bee- son produced right-sided Streptococcus viridans endocarditis in rabbits and monitored titers of bacteria in vegetations, liver, spleen, and blood for 72 h (3). This study showed rapid, apparently unimpeded growth within the tricuspid valve vegetation; by 24 h, about 10$^9$ CFU/g of vegetation existed. This rate of growth of bacteria in vegetations, similar to that observed under favorable conditions in broth, contrasts with falling bacterial populations in liver and spleen (to <50 CFU/g at 72 h) and small numbers or no bacteria in blood. Similarly, in rats, numbers of

<table>
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<th>Time</th>
<th>No. of animals</th>
<th>Catheterized$^a$</th>
<th>Control: valve weight (mg)</th>
<th>Vegetation weight (mg)</th>
<th>Vegetation (log$_{10}$ CFU/g)</th>
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<tr>
<td>6 h</td>
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<td>6.9 ± 0.5</td>
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<td>1 day</td>
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<td>24.2 ± 2.6</td>
<td>7.3 ± 0.2</td>
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<tr>
<td>3 days</td>
<td>7</td>
<td>50.6 ± 11.8</td>
<td>7.5 ± 0.5</td>
<td>&lt;2.0</td>
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<tr>
<td>5 days</td>
<td>7</td>
<td>35.0 ± 4.0</td>
<td>8.4 ± 0.1</td>
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<tr>
<td>7 days</td>
<td>3</td>
<td>43.3 ± 8.5</td>
<td>8.0 ± 0.4</td>
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$^a$ Mean ± standard error.
**FIG. 1.** Aortic valve vegetation (indicated by arrows) 7 days after intravenous inoculation of *Streptococcus mitis* in a rat with an indwelling catheter across the aortic valve.

**FIG. 2.** Vegetation and organ populations of *Streptococcus mitis* in rats with indwelling catheters across the aortic valves and in control rats without indwelling catheters.

Organisms in aortic valve vegetations within 6 h after infection were about $10^7/g$, whereas tissue populations of viridans streptococci in these rats were $10^2$- to $10^3$-fold less in the liver and spleen and $10^4$- to $10^6$-fold less in the kidney and lung. Bacterial titers in tissues of rats with endocarditis tended to fall for the first 72 h and closely parallel tissue populations in control animals. However, beyond 72 h, when tissues in control rats were sterile, titers of streptococci once again rose in rats with endocarditis. Because of persistent bacteremia in the rats with endocarditis, continued seeding of bacteria may have resulted in infection at these sites. The mortality of viridans streptococcal endocarditis was similar to that described in rabbits (2). Titers of enterococci and *Staphylococcus aureus* in aortic vegetations were also comparable to the respective rabbit models (1, 5). Left-sided endocarditis in rats was reliably and easily produced. However, right-sided infection is extremely difficult to produce because of technical problems in catheter-
izing the right side of the heart (unpublished data). The rat model is suitable for further pathophysiological and therapeutic study.

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LITERATURE CITED