Enhancement of Experimental Bacteremia and Endocarditis Caused by Dysgonic Fermenter (DF-2) Bacterium After Treatment with Methylprednisolone and After Splenectomy

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Received 18 July 1984/Accepted 24 September 1984

The dysgonic fermenter-2 bacterium is a newly recognized fastidious gram-negative bacillus that causes bacteremia and sometimes endocarditis in immunocompromised persons after they are bitten by dogs. To develop an experimental model of this infection, we placed polyethylene catheters across the aortic valves of New Zealand white rabbits, which were inoculated intravenously the next day with dysgonic fermenter-2 bacteria. After 1 week, the rabbits were killed and the endocardial vegetations were homogenized for quantitative culture. Large inocula (1.3 × 1010 to 2.1 × 1010 viable bacteria) were required to produce infected vegetations. All infected rabbits had negative blood cultures at the time of autopsy and most developed serum agglutinins against dysgonic fermenter-2 bacteria. Three daily injections of methylprednisolone (30 mg/kg), starting the day before inoculation, significantly increased the incidence of endocarditis and the number of bacteria per gram of infected vegetation (P < 0.05). Treatment with methylprednisolone prolonged the initial bacteremia and caused significant increases in the numbers of bacteria per gram of blood, spleen, and liver compared with those of untreated controls (P < 0.05). Rabbits that had previously undergone splenectomy showed prolongation of the initial bacteremia but no significant increase in the incidence of infected vegetations. These results showed that the dysgonic fermenter-2 bacterium is a pathogen that causes endocarditis in rabbits but that it requires a large inoculum and produces blood culture-negative infections. Treatment with methylprednisolone enhances infection by prolonging the initial bacteremia and probably by diminishing bactericidal activity in the vegetations.

The dysgonic fermenter-2 (DF-2) bacterium is a newly recognized pathogenic bacterium, which has been tentatively designated in this group by the Centers for Disease Control because of its slow growth and fermentation of carbohydrates (5). It is a gram-negative bacillus found naturally in dogs’ mouths (3); it requires CO2 for growth and differs from all other species of bacteria by its biochemical characteristics, which include being positive for catalase and oxidase and negative for indole, nitrate reduction, and urease. The medical literature records 26 patients from whose blood or spinal fluid DF-2 was isolated (5, 6, 10, 14, 18, 22, 24–26). Most of these patients developed fever and cellulitis after being bitten by dogs or after other contact with animals and several developed meningitis, endocarditis, or fatal septicemia. Most of the patients had impaired host defenses against infection; these were mostly caused by previous splenectomy (10 cases), alcoholism (6 cases), and corticosteroid therapy (1 case).

The objective of these experiments was to establish an animal model for this infection. The catheterized rabbit model for endocarditis (12) was chosen because it has proved useful and reliable in the study of other human pathogens, including another fastidious oral bacterium, Eikenella corrodens (2). Once the ability of the DF-2 bacterium to cause endocarditis was established, we sought to enhance the susceptibility of rabbits to infection by altering their immune defenses by corticosteroid treatment and splenectomy.

MATERIALS AND METHODS

Bacteria. In all experiments with the DF-2 bacterium, the same strain, C8936, obtained from Robert Weaver, Centers for Disease Control, Atlanta, Ga., was used. This strain had been obtained from the blood of a patient described in the original clinical report by Butler et al. (5). It was maintained in the laboratory on nutrient agar containing 5% sheep blood in a candle jar at 22°C and was serially passed through rabbits to maintain virulence. Inocula of the DF-2 bacterium were prepared by streaking single colonies from stock cultures onto fresh 5% sheep blood agar with a platinum wire loop, followed by incubation in a CO2 atmosphere in a candle jar at 35°C for 48 h. The resulting growth was harvested by pipetting 2 ml of a modified broth medium (devised by Manganiello et al. for cultivation of oral anaerobes [16]) onto a plate, scraping the colonies loose with a wire loop, and removing the suspended colonies with a Pasteur pipette. A fine suspension was made by homogenizing the bacteria in a glass mortar fitted with a Teflon pestle. The suspension was diluted in the medium to achieve a desired concentration of bacteria by a McFarland turbidity standard. The precise number of CFU was verified by performing serial 10-fold dilutions in medium, followed by plating 0.1-ml portions of the dilutions onto 5% sheep blood agar plates, spreading them with a hockey stick, and allowing them to incubate in a candle jar for 72 h before counting the colonies. The identity of characteristic colonies was verified by agglutinating suspensions of colonies in 0.9% NaCl with rabbit anti-

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DF-2 serum. This antiserum had been made by inoculating a rabbit five times, on days 1, 3, 4, 12, and 13, intravenously and subcutaneously with $10^8$ formalinized organisms of the C8936 strain of DF-2. On day 16, blood was taken to obtain immune serum.

Resistance of bacteria to serum. A suspension of bacteria was placed into Hanks balanced salt solution containing 10% rabbit serum, as previously described (8). Rabbit serum was heated inactivated to remove complement activity by heating to 56°C for 20 min. A serum-sensitive organism, Escherichia coli K-12 C600, was used as a control. Numbers of viable bacteria were counted after 1 and 2 h of incubation at 37°C in a water bath by performing serial dilutions and counting bacterial colonies on 5% sheep blood agar plates.

Susceptibility of bacteria to the bactericidal action of whole blood. Fresh whole blood in heparin (Lipo-hepin; Riker Laboratories, Inc., Northridge, Calif.), 20 U/ml, was obtained for use on the same day. At 22°C, DF-2 bacteria in medium were injected into blood in a ratio of one part bacterial suspension to nine parts blood in polystyrene culture tubes (Hecton Dickinson Labware, Oxnard, Calif.). After being mixed thoroughly with a Vortex mixer, duplicate specimens were removed for the zero time bacterial counts. Tubes containing the mixtures of bacteria and blood were placed onto a blood rotator (Scientific Industries, Inc., Bohemia, N.Y.) set at 50 rpm in an incubator at 37°C. Duplicate samples were taken again after 1 and 2 h for quantitative cultures.

Experimental infections. New Zealand white female rabbits weighing 2.2 to 2.5 kg were used to produce sterile endocardial vegetations, followed by intravenous injection of bacteria in the model described by Garrison and Freedman (12) with the modification of left-sided heart catheter placement described by Durack et al. (9). Each rabbit was anesthetized with an intramuscular injection of 35 mg of ketamine hydrochloride (Ketaset; Bristol Laboratories, Syracuse, N.Y.) and 5 mg of xylazine (Rompun; Chemagro, Kansas City, Mo.) per kg. The right carotid artery was exposed and ligated. A 19-gauge polyethylene catheter (Intracath; Deseret Pharmaceutical Co., Sandy, Utah) was inserted across the aortic valve into the left ventricle and tied into place. One day after surgery, the animals were injected intravenously with suspensions of the DF-2 bacterium. At daily intervals, arterial blood from the central ear arteries of the rabbits was obtained for quantitative culture; 0.1 ml of blood was plated and 0.1 ml was serially diluted. Rectal temperatures and body weights were recorded daily. Seven days after catheter placement, the rabbits were killed by intravenous injection of sterile sodium pentobarbital (Nembutal; Gane’s Chemical Works, Inc., New York, N.Y.). Endocardial vegetations were removed from each rabbit, pooled, weighed, and homogenized in tissue grinders which contained 1 ml of medium. Serial 10-fold dilutions were made in the medium, and 0.1-ml samples were plated onto sheep blood agar plates for colony counts after 72 h of incubation in a candle jar at 35°C. Autopsy blood was quantitatively cultured. The kidneys were examined for gross signs of ischemic or hemorrhagic infarction. Animals that died more than 24 h after inoculation but before the scheduled sacrifices were autopsied freshly, and the vegetations obtained from them were cultured in an identical manner.

In some experiments, rabbits were treated with methylprednisolone sodium succinate (Solu-Medrol; The Upjohn Co., Kalamazoo, Mich.) at a dosage of 30 mg/kg per day intravenously on three consecutive days starting on the day of catheter placement. In some experiments, rabbits were splenectomized or received a sham abdominal incision 1 week before the placement of catheters. At the time of autopsy, the abdominal cavities were carefully examined to verify the completeness of the splenectomies and the absence of accessory splenic tissue.

In some experiments, rabbits without catheter operations were inoculated with bacteria and killed with injections of Nembutal. Duplicate specimens of blood were quantitatively cultured. Duplicate spleen and liver specimens were removed, weighed, and homogenized in medium. The homogenates were serially diluted in medium and cultured.

Pathological studies. Endocardial vegetation and kidney specimens were fixed in 10% buffered Formalin and sectioned for staining with hematoxylin and eosin and Gram stains. Specimens of vegetation were also fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for electron microscopy.

Detection of DF-2 agglutinins in rabbit serum. Serum was separated from blood obtained at the time of the autopsies. The antigen consisted of colonies of DF-2 bacteria suspended into 0.2% NaCl from the blood agar plates. Equal volumes of undiluted serum and fourfold dilutions in 0.9% NaCl up to 1:64 were mixed on slides with antigen to examine titers of 1:2 through 1:128. After the slides were tilted by hand at room temperature for 3 min, microscopic agglutination was read.

RESULTS

Resistance of the DF-2 bacterium to bactericidal activity of fresh rabbit serum. To be effective pathogens for causing endocarditis, bacteria must be resistant to the bacteriolytic action of serum complement (8). In the presence of both fresh and heat-inactivated rabbit serum, DF-2 strain C8936 was not killed, whereas the serum-sensitive control organism E. coli K-12 C600 was killed by fresh serum but not by heat-inactivated serum (Fig. 1).

Inoculum of DF-2 bacterium required to produce experimental endocarditis. It was determined that inocula of $10^8$ to $10^9$ CFU injected intravenously into catheterized rabbits failed to result in positive cultures of vegetations when the
rabbits were killed 7 days after inoculation. A group of 18 catheterized rabbits in five experiments received doses ranging from $2.3 \times 10^8$ to $2.1 \times 10^{10}$ CFU of bacteria. Seven of the rabbits developed vegetation culture-positive endocarditis. These 7 rabbits were in a group of 10 rabbits that received $>10^{10}$ inoculated bacteria, whereas all 8 rabbits that received $<10^{10}$ bacteria had uninfected vegetations at the time of autopsy ($P < 0.05$ by Fisher’s exact test).

**Characteristics of endocarditis caused by the DF-2 bacterium.** Blood cultures obtained at the time of autopsy were negative for the DF-2 bacterium in all cases. Blood cultures had been obtained daily from all inoculated rabbits, and all 18 rabbits showed negative blood cultures 72 h after inoculation and later. Only two of the seven infected rabbits died sooner than 7 days after inoculation, and both had negative blood cultures at autopsy. Neither showed gross autopsy findings that suggested a specific cause of death.

The clinical course of inoculated rabbits showed that illness caused by the DF-2 bacterium, if any, was mild. The body weight of the rabbits did not drop, and only 4 of the 18 inoculated rabbits had temperatures above 40°C on any day during the week after inoculation.

Endocardial vegetations were present on the endocardial surfaces next to the catheters in all animals. Typically the vegetations were white, nodular excrescences, varying in diameter from 1 to 5 mm and in number from 2 to more than 50, located variously on the aorta above the aortic valve, on the aortic valve, or in the left ventricle. The weights of the combined infected vegetations from individual rabbits after removal for homogenization and quantitative culture ranged from 0.03 to 0.34 g. The results of quantitative cultures of the infected vegetations revealed a range of $7.1 \times 10^6$ to $2.7 \times 10^7$ CFU of DF-2 bacteria per g of vegetation, with a mean of $2.6 \times 10^6$ CFU per g. Examination of the uninfected vegetations by light and electron microscopy showed that they were composed of fibrin clumps admixed with macrophages and platelets. In the infected vegetations, there were variable numbers of bacteria, necrotic cell debris, and degenerated neutrophils (Fig. 2).

The kidneys of most infected rabbits showed ischemic infarcts. Histologically, the kidneys showed tissue coagulation and cell death of renal tubular cells and glomeruli. There was infiltration by neutrophils, indicating that the infarcts had occurred ca. 5 days earlier.

**Enhancement of DF-2 endocarditis and bacteremia by methylprednisolone treatment.** The incidence of infected vegetations at autopsy was significantly increased by methylprednisolone treatment from 7 of 18 controls to 14 of 18 methylprednisolone-treated rabbits ($P < 0.05$ by Fisher’s exact test). The intensity of infection, as indicated by the number of bacteria per gram of infected vegetation, was also significantly greater in the treated rabbits ($P < 0.05$ by Student’s $t$ test). The methylprednisolone-treated rabbits showed a higher incidence of bacteremia at 48 h or longer after inoculation than the controls, but the incidence of fever and the mortality rates were similar in both groups (Table 1).

Quantitative blood cultures were performed at different intervals after intravenous injection of bacteria into these same rabbits. At all times between 1 and 72 h after inoculation, the treated group showed a higher mean log_{10} CFU per

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**FIG. 2.** Electron micrograph of vegetation which grew bacteria in culture. Abundant bacteria (B) are enmeshed with fibrin and cell debris. Magnification, $\times 9,000$. 

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**INFECTION IMMUNITY**
TABLE 1. Effect of methylprednisolone treatment on the development of endocarditis caused by the DF-2 bacterium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rabbits with vegetations containing DF-2/total no. of rabbits</th>
<th>Mean $\log_{10}$ CFU of DF-2 ± SE per g of vegetation in culture-positive rabbits</th>
<th>No. of rabbits with bacteremia ≥ 48 h after inoculation/total no. of rabbits</th>
<th>No. of rabbits with fever ≥ 40°C at ≥ 48 h after inoculation/total no. of rabbits</th>
<th>No. of rabbits that died/total no. of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylprednisolone</td>
<td>14/18*</td>
<td>7.32 ± 0.84*</td>
<td>9/14*</td>
<td>4/18</td>
<td>3/18</td>
</tr>
<tr>
<td>None (controls)</td>
<td>7/18</td>
<td>6.41 ± 0.96</td>
<td>4/14</td>
<td>4/18</td>
<td>2/18</td>
</tr>
</tbody>
</table>

* Incidence of positive cultures greater than in controls by Fisher's exact test ($P < 0.05$).
* * Mean $\log_{10}$ CFU greater than in controls by Student's $t$ test ($P < 0.05$).
* * Incidence of bacteremia greater than in controls by the chi-square test ($P = 0.058$).

milliliter of blood (Fig. 3). Significant differences were evident 24, 48, and 72 h after inoculation ($P < 0.05$ by Student's $t$ test).

Blood bactericidal activity was tested in untreated rabbits and in rabbits injected with methylprednisolone (30 mg/kg per day for 3 days). The blood samples were inoculated with ca. $10^8$ DF-2 cells per ml. After 1 h of incubation, the logarithm of the counts of the DF-2 bacterium fell by 1 to 2 equally in both groups of rabbit blood samples (data not shown). This result was evidence against any diminution in blood bactericidal activity caused by methylprednisolone.

To examine the effect of steroid treatment on the removal of bacteria from blood by the spleen and liver, we inoculated three control rabbits and three methylprednisolone-treated rabbits with DF-2 intravenously and tested their tissues for the presence of bacteria 4 h later. Methylprednisolone treatment resulted in significantly higher bacterial counts in all tissues compared with those in control rabbits ($P < 0.05$ by Student's $t$ test) (Fig. 4).

Effect of splenectomy on experimental endocarditis and bacteremia caused by the DF-2 bacterium. In four experiments, 16 rabbits received splenectomies and 16 rabbits received sham abdominal operations 1 week before catheters were placed into their left ventricles. After excluding four rabbits that died less than 24 h after inoculation or developed superinfections, we found that the incidence of DF-2 endocarditis was higher in the splenectomized group (8/13) than in the control group (6/15), but this difference was not significant by Fisher's exact test ($P > 0.05$). Only one rabbit died before the time of sacrifice; it was a splenectomized animal that died 5 days after inoculation and was the only animal to show a positive postmortem blood culture. Nevertheless, the overall mortality rates, incidences of bacteremia 48 h or longer after inoculation, and incidences of fever were indistinguishable by statistical comparisons between the two experimental groups. Furthermore, the intensity of infection measured by the mean $\log_{10}$ CFU per gram of vegetation in infected rabbits was similar in both experimental groups.

At all times that blood was taken for culture, the splenectomized group showed a higher mean $\log_{10}$ CFU of DF-2 per milliliter than the sham-operated group (Fig. 5). Only at the 4-h point was the difference statistically significant ($P < 0.05$ by Student's $t$ test).

Immune responses after experimental DF-2 infection. Sera of 45 rabbits were tested 6 days after bacterial inoculation; 23 serum samples were from rabbits with vegetation cultures positive for the DF-2 bacterium and 22 were from rabbits with negative vegetation cultures. Positive agglutination reactions were found in 38 serum samples with titers ranging from 1:2 to 1:128. The seven serum samples with absent DF-2 agglutinins were obtained from rabbits that included

FIG. 3. Effect of methylprednisolone treatment to reduce clearance of the DF-2 bacterium from blood. Catheterized rabbits received methylprednisolone (30 mg/kg) intravenously on the day before, the same day, and the day after intravenous inoculation of DF-2 bacteria, which in four experiments ranged from $2.3 \times 10^8$ to $2.4 \times 10^8$ CFU. Mean values ± 1 standard error for 14 rabbits injected with methylprednisolone are compared with those for 13 control animals that did not receive the drug.

FIG. 4. Effect of methylprednisolone on the distribution of the DF-2 bacterium in rabbit tissues. Three rabbits treated with intravenous methylprednisolone (30 mg/kg daily for 3 days) (■) and three uninjected control rabbits (□) were inoculated intravenously with $4 \times 10^9$ CFU of DF-2 bacteria. Four hours after inoculation, the animals were sacrificed and duplicate blood, spleen, and liver samples were obtained for quantitative cultures. Methylprednisolone treatment resulted in increased numbers of bacteria in all tissues.
required large inocula to initiate infection. Serum-resistant strains of *E. coli* required inocula of ca. $10^8$ organisms (8), *Pseudomonas aeruginosa* required $10^7$ to $10^8$ organisms (1), *Neisseria gonorrhoeae* required $1 \times 10^8$ to $5 \times 10^8$ organisms (15), and *Eikenella corrodens* required $10^7$ to $10^8$ organisms (2). These large inocula are likely to be necessitated by factors governing the adherence of bacteria to vegetations as well as the ability to evade humoral immunity.

The negative blood cultures in rabbits with DF-2 endocarditis is a significant feature of this animal model. For the robust pathogens of endocarditis, a continuous bacteremia is usually demonstrable, indicating a rapid rate of bacterial multiplication in the vegetations and shedding into the blood or relatively slow clearance of the bacteria by reticuloendothelial tissues, or both. The infrequent finding of positive blood cultures in *Eikenella corrodens* endocarditis (2) and the finding of negative blood cultures in DF-2 endocarditis imply that these organisms may infect human endocardial vegetations and produce inflammatory damage there without being detectable in blood cultures.

The vegetations infected with the DF-2 bacterium showed in some areas a loose arrangement of bacteria with degenerated polymorphonuclear leukocytes. This structure is different from that of gram-negative coccal infections described by Durack (7), in which clusters of bacteria were usually separated from inflammatory cells by strands of fibrin. Our finding of polymorphonuclear leukocytes near bacteria in the vegetations is more consistent with the results of Meddens et al. (20) and Yersin et al. (28), who found that with streptococcal endocarditis, polymorphonuclear leukocytes were numerous in the vegetations.

Experiments to immunosuppress rabbits before infection with the DF-2 bacterium were undertaken because of the relatively weak pathogenicity of this organism and the association of human DF-2 infection with immunosuppression (5). Other workers have reported results with the catheterized rabbit model of endocarditis and treatment with nitrogen mustard (20, 28) and with dexamethasone (11). Meddens et al. (20) showed that, by depleting leukocytes, nitrogen mustard caused a higher density of streptococcal bacteria in rabbits with left-side cardiac vegetations than in control rabbits, but the infective dose of bacteria in treated rabbits was not changed. Francioli and Freedman (11) showed that dexamethasone prevented sterilization of intravascular vegetations infected with streptococci at sites other than the aortic valve. Our results with methylprednisolone and the DF-2 bacterium differ from those of Francioli and Freedman (11) by showing that vegetations in the left side of the heart contained increased numbers of bacteria per gram of tissue. This effect is likely to be the result of impaired local bactericidal mechanisms. Antibody production appeared not to be impaired in our rabbits, suggesting that cellular defenses in the vegetations were affected. Francioli and Freedman (11) also suggested that cellular mechanisms could have been impaired by dexamethasone to prevent sterilization of vegetations, and Meddens et al. (19) showed that granulocytes have a protective role in the development of experimental endocarditis caused by *E. coli*.

The mechanism by which methylprednisolone enhanced the susceptibility of rabbits to DF-2 infection can be partially attributed also to the heightened and prolonged bacteremia induced by the inoculum, giving more opportunity for contact between the sterile vegetations and circulating bacteria. The rate of killing of DF-2 bacteria by the blood appeared not to be diminished by methylprednisolone, and this is consistent with other work (13). The mechanism of the

**DISCUSSION**

This is the first reported animal model of disease caused by the newly recognized bacterial pathogen DF-2. In rabbits prepared by placing polyethylene catheters into the left side of their hearts to induce sterile endocardial vegetations, the intravenous injection of $>10^{10}$ CFU of DF-2 bacteria resulted in infected vegetations. The disease produced by the DF-2 bacterium in the rabbits, if any, was mild because most rabbits survived to the time of sacrifice 1 week later, rarely had fever, and showed negative blood cultures at all times after 48 h from the time of bacterial inoculation. The vegetations were composed of fibrin, platelets, neutrophils, macrophages, and microcolonies of bacteria. Most rabbits showed renal embolic infarctions.

In contrast to the endocarditis pathogen *Staphylococcus aureus*, which requires an inoculum of $10^5$ organisms and produces fatal and blood culture-positive infections in the rabbit model (23), the DF-2 bacterium was a less effective pathogen. This weaker pathogenicity could be explained by the slower growth of the DF-2 bacterium, which produces pinpoint colonies on sheep blood agar after 48 h of incubation at 35°C. Within 1 week after inoculation, most rabbits in our studies had developed agglutinating anti-DF-2 antibodies, which may have exerted their antibacterial action on circulating bacteria.

Some of the other gram-negative bacteria that have previously been used in the rabbit model of endocarditis have also

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**FIG. 5.** Effect of splenectomy to reduce clearance of the DF-2 bacterium from the blood of catheterized rabbits. Seven rabbits were splenectomized, and eight controls received a sham operation 1 week before catheters were placed in the heart. On the following day, rabbits were inoculated intravenously with $3 \times 10^8$ to $4 \times 10^8$ CFU of the DF-2 bacterium.

six with negative vegetation cultures ($P < 0.05$ by Fisher's exact test). Thus, there was a statistically significant correlation between vegetation culture results and the presence of serum-agglutinating antibodies.

The overall rate of animals showing negative agglutination reactions in those sera tested was 7/45, or 16%. The rates of negative agglutination reactions were 2/16 (13%) in control rabbits, 2/12 (17%) in methylprednisolone-treated rabbits, 2/8 (25%) in splenectomized rabbits, and 1/9 (11%) in sham-operated rabbits. Thus, neither methylprednisolone treatment nor splenectomy appeared to exert any detectable inhibitory effect on anti-DF-2 antibody production.
action of methylprednisolone may resemble that of the colloid thorotrust, which diminishes the removal of blood bacteria by producing a blockade of the reticuloendothelial system (17). Our results showed that methylprednisolone treatment caused a larger number of viable bacteria to inhabit the liver and spleen. This suggests that the reticuloendothelial tissues of steroid-treated animals could remove bacteria from the blood but failed to kill bacteria normally. The build-up of unkillled bacteria in the spleen and liver might create a saturation of the uptake mechanism for bacteria and then a slower rate of bacterial removal from blood.

The finding that 10 of the 26 human patients with DF-2 infection reported in the medical literature had had splenectomies (5, 6, 10, 14, 18, 24) makes the association of DF-2 sepsis with splenectomy more intimate than that of any other bacterial infection with this underlying condition. Furthermore, the fact that 8 of the 10 splenectomies in these patients had been performed after accidents or were incidental to abdominal surgery suggested that the spleen itself provides a protective function against illness caused by DF-2 infection. Thus, our studies of the effects of experimental splenectomy on the course of DF-2 bacteremia and endocarditis should be relevant to host mechanisms functional in human infection. Our experiments showed that splenectomy resulted in higher counts of bacteria in the blood 4 h after inoculation, but by 24 h after inoculation the levels of bacteremia were equal to those in the controls. Splenectomy caused a higher incidence of infection (62%) than occurred in the control group (40%), but with the numbers of animals used in these experiments, the difference was not statistically significant.

In other animal models that have been used to study the effects of splenectomy, Moxon et al. (21) showed that asplenic rats developed fatal infections with Haemophilus influenzae with smaller inoculum sizes and developed more bacteremia than did sham-operated controls. Asplenic mice inoculated intravenously with Streptococcus pneumoniae were shown by Whitaker (27) to have higher mortality rates and higher concentrations of blood bacteria than sham-operated controls. Furthermore, Brown et al. (4) showed that a virulent strain of S. pneumoniae produced a more marked increase in mortality in splenectomized guinea pigs compared with control animals than did a less virulent strain. Both H. influenzae and S. pneumoniae, which are the most common bacteria that cause overwhelming post-splenectomy sepsis in humans, differ from the DF-2 bacterium in this model of endocarditis by their more rapid multiplication in animal tissues after inoculation. The DF-2 bacterium is less virulent by virtue of its slow growth and the ability of the animal tissues, even after splenectomy, to contain the organism.

ACKNOWLEDGMENTS

We thank Shirley Badger for technical advice and Charles Carpenter and Oscar Ratnoff for reading the manuscript.

This work was supported in part by a grant from the American Heart Association, Northeast Ohio affiliate.

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