Heterotopic Splenic Autotransplantation in the Prevention of Haemophilus influenzae Meningitis and Fatal Sepsis in Sprague-Dawley Rats

By E. Richard Moxon and Allen D. Schwartz

It has been suggested that autotransplantation of splenic tissue following trauma may result in splenic implants that protect the human host from severe infection with encapsulated bacteria. To test this hypothesis, Sprague-Dawley rats underwent sham operation, splenectomy, or splenectomy followed by autotransplantation of splenic fragments into the peritoneal cavity. Three months later, they were inoculated intranasally with H. influenzae b. The incidence and severity of bacteremia and meningitis were determined subsequently in 15 randomly selected rats from each group. Splenosis did not appear to confer significant protection against bloodstream dissemination. However, significantly more (p = 0.005) asplenic rats (13/15) developed meningitis than did splenosed rats (6/15). None of the rats with normal splenic tissue developed CNS infection. Thus, the occurrence of meningitis was reduced in autotransplanted rats as compared to asplenic rats. Ten remaining rats from each group were followed for 3 wk after inoculation and the number of deaths was recorded. All sham-operated and autotransplanted rats survived, whereas 7 of 10 asplenic rats died (p = 0.003). These studies indicate that surgically created splenosis offers the potential for reducing the risk of life-threatening sepsis.

STUDIES IN ANIMALS and clinical observations in man have established that the spleen is important in defense against systemic infection with encapsulated bacteria such as Streptococcus pneumoniae and Haemophilus influenzae.1-4 The asplenic individual is thus at increased risk for developing severe and often life-threatening bacterial infection. Spontaneous regeneration of autotransplanted splenic tissue (splenosis) occurs, however, very commonly in persons splenectomized for trauma.5 It has been suggested that this “born-again spleen” affords protection against the occurrence and consequences of bloodstream invasion with encapsulated bacteria.6 We have studied the effect of splenectomy and splenosis on susceptibility to H. influenzae b bacteremia and meningitis in a rat model. In this model, nasopharyngeal colonization, hematogenous dissemination, and meningeal invasion by H. influenzae resembles very closely the pathogenesis of naturally occurring infection in man.4,7,8 Because the asplenic human and the asplenic rat have a marked increase in the frequency of meningitis caused by encapsulated bacteria, rats challenged intranasally with H. influenzae appear to afford a convenient and biologically relevant model in which to study the potential protective efficacy of regenerated autotransplanted splenic tissue.

MATERIALS AND METHODS

One-month-old female albino COBS/CD Sprague-Dawley rats were purchased from Charles River Breeding Labs Inc. Rats, randomly assigned to 3 groups of 25 each, underwent sham operation, splenectomy and splenosis, or splenectomy using previously described surgical techniques.9 Briefly, splenosis was performed by cutting the spleen into multiple fragments, macerating it with the scalpel handle, and returning this tissue into the peritoneal cavity at the time of surgery. The source, characteristics, culture presence, and techniques of splenic implants. The source, characteristics, culture presence, and techniques of splenic implants in the peritoneal cavity are described in detail in previous publications.4,7,8

RESULTS

Fourteen of 15 splenosed rats had gross evidence of splenic implants in the peritoneal cavity at the time of sacrifice. Accurate weights of the splenic implants could not be obtained because the tissue was enmeshed within the mesenteries of the abdominal viscera. In most instances, multiple splenic implants were present. No splenic implants were found in the splenectomized group. Bacteremia was detected in 73% of sham-operated, 100% of splenosed, and 100% of asplenic
Asplenic splenosis. Sham operation

Fig. 1. Incidence of meningitis on days 1, 2, and 3 following intranasal inoculation.

Fig. 2. Mortality rate (% deaths) during the 21 days following intranasal inoculation.

**Table 1. Incidence and Severity of Bacteremia**

<table>
<thead>
<tr>
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<th>Sham Operation</th>
<th>Splenosis</th>
<th>Asplenic</th>
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<tbody>
<tr>
<td>No. bacteremic (%)</td>
<td>11/15 (73)</td>
<td>15/15 (100)</td>
<td>15/15 (100)</td>
</tr>
<tr>
<td>Mean log_{10} bacteremia ± SD</td>
<td>3.32 ± 1.40</td>
<td>5.22 ± 0.79</td>
<td>5.48 ± 0.85</td>
</tr>
<tr>
<td>Geometric mean bacteria/ml of blood</td>
<td>2.1 x 10^3</td>
<td>1.7 x 10^4</td>
<td>3.07 x 10^4</td>
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rats (Table 1). Splenosed and asplenic rats had significantly more intense bacteremia (about 100-fold) than did sham-operated rats with intact spleens (p < 0.001; Wilcoxon rank sum test). Based on these data, splenosis did not appear to confer significant protection against bloodstream dissemination. However, 13 of 14 asplenic rats had positive CSF cultures: 3 on day 1, 5 on day 2, and 5 on day 3 (Fig. 1). One asplenic rat died spontaneously on day 1 before the CSF could be evaluated. In contrast, only 6 of 15 splenosed rats had positive CSF cultures: 1 on day 1, 1 on day 2, and 4 on day 3 (Fig. 1). This difference was statistically significant by Fisher's exact test (p = 0.005). Thus, the occurrence of meningitis was reduced, or at least delayed, in splenosed as compared to asplenic rats. In contrast to the occurrence of meningitis among splenosed and asplenic rats, none of the 15 rats possessing normal splenic tissue developed CNS infection.

Additional evidence for the protective effect of splenosis was evident when mortality rates were compared (Fig. 2). All sham-operated and splenosed rats survived, whereas 7 of 10 asplenic rats died. The excess of deaths among asplenic rats was statistically significant by Fisher's exact test (p = 0.003).

**DISCUSSION**

The asplenic individual is unusually susceptible to overwhelming bacterial infection. The present studies have shown that splenosis in rats affords protection against *H. influenzae* b meningitis and fatal sepsis. Caution must be exercised in extrapolating observations from laboratory animals to man. The rat model of encapsulated *H. influenzae* b meningitis, however, simulates the human infection with respect to mode of bacterial entry, bloodstream dissemination, concentrations of bacteria in blood and CSF, and the histopathologic features of meningeal inflammation.7,8 Furthermore, the spleen apparently plays a critical role in the clearance of bacteria from the bloodstream of both man and rat. In the rat, splenectomy resulted in an approximately 10–100-fold increase in maximal concentrations of bloodstream bacteria (Table 1). However, peak bacteremia in the rat occurs significantly later following intranasal as opposed to intravenous inoculation.4 This temporal difference in the evolution of the infection could permit a variety of immunologic responses to exert a critical effect on the ultimate outcome of the interaction between host and pathogen. For these reasons, previous animal studies that used systemic inoculation of bacteria may have underestimated the potentially protective role of splenic autotransplants. Although Likhite reported that mice with splenosis had a lower mortality following intravenous challenge with *S. pneumoniae* than did asplenic mice,10 other studies were unable to demonstrate any significant protective effect of splenosis on ultimate survival following intravenous or intraperitoneal bacterial challenge.9,11,12 Other studies yielded conflicting data regarding the effect of splenosis on the clearance of *S. pneumoniae* from the bloodstream after intravenous challenge.11,13 Recently, Dickerman et al. showed no difference in the mortality of autotransplanted and control mice, but a signifi-
cantly higher mortality in asplenic mice following exposure to an aerosolized suspension of *S. pneumoniae*. Dickerman's data and the present studies suggest strongly that splenosis affords significant protection against systemic infection with encapsulated bacteria when a natural route of infection is employed.

Prophylactic measures presently used to protect asplenic patients from infection include antibiotics and immunization. Prophylactic antibiotics, mainly penicillin, have been used following splenectomy, but the patient remains prone to infection with penicillin-resistant organisms such as *Haemophilus influenzae*. Also, strains of pneumococci resistant to penicillin and other antibiotics have been reported within the last decade. Polyvalent pneumococcal vaccine does not protect against all pneumococcal serotypes or other encapsulated bacteria to which the patient is susceptible. Also, it does not always result in an adequate antibody response in children under age 2 and, therefore, may not afford protection in a population that is at high risk. Vaccine failures in older children have been reported, and the efficacy of pneumococcal vaccine is justifiably under critical scrutiny at present. The limitations of these prophylactic measures indicate that certain protective mechanisms are most efficiently performed by the spleen. Thus, as a practical consequence, many surgeons prefer surgical repair of the spleen or careful observation following trauma. Nonetheless, there are inevitably instances where these measures are not possible and the spleen must be removed. Splenic tissue has an excellent ability to regenerate when implanted into the subcutaneous tissue or the abdominal cavity. These implants are able to remove Howell-Jolly bodies from erythrocytes and can take up intravenously injected radiocolloid. Furthermore, there is evidence that these splenic implants can perform a number of immunologic functions. Although splenosis does not seem to offer as good a protection against intravenous or intraabdominal challenge with large numbers of organisms as does the normal spleen, splenic implants may prevent or modify most episodes of septicemia in man to be of major clinical benefit. This decrease in the protective effect of splenosis as compared to normal spleen may be due to a smaller mass of splenic tissue than normal, but rare cases of overwhelming sepsis in patients with masses of autotransplanted tissue of near normal size suggest that other factors, such as blood supply, may be important. Hemisplenectomized animals, however, do not appear to have as good a protection against intravenous bacterial challenge as do normal animals, despite the fact that some protection is achieved. This indicates that, in addition to a normal blood supply, splenic tissue mass also is an important factor. Despite these limitations of abnormal blood supply and smaller mass, splenosis still affords protection against bacterial infection. Thus, when faced with a decision to perform total splenectomy after trauma, surgically created splenosis would seem to offer the potential for reducing the risk of life-threatening sepsis.

ACKNOWLEDGMENT

We thank David Sevdalian for his excellent technical assistance in this study.

REFERENCES

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