

## The Protective Effect of Intraperitoneal Splenic Autotransplants in Mice Exposed to an Aerosolized Suspension of Type III *Streptococcus Pneumoniae*

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Splensitis has been shown to occur after traumatic injury to the spleen. It is postulated that this is the mechanism for the low incidence of bacterial infection in this group of patients when compared to those who undergo splenectomy for other reasons. Therefore, we studied the effect of exposure to an aerosolized suspension of type III *Streptococcus pneumoniae* on splenectomized mice who had either all or half of their splenic tissue cut up and reim-

planted into the abdominal cavity 8 wk prior to bacterial exposure. It was determined that the mortality experience of these two groups of mice was similar to each other and no different from the sham control group, although all three groups had a statistically significant lower mortality experience than the splenectomized control group. This study demonstrates that splensitis in mice can protect against aerosolized bacterial infection.

REVIEWS of clinical studies in humans,<sup>1-3</sup> as well as laboratory experiments in animals,<sup>4-7</sup> have demonstrated an increased susceptibility to pneumococcal infection in the asplenic host. Perla and Marmorston-Gottesman have shown that autochthonous splenic tissue implanted in the abdominal wall of albino rats protected them against endogenous infection with *Bartonella muris*.<sup>8</sup> Crosby and Benjamin were unable to duplicate their work using different experimental conditions.<sup>9</sup> Schwartz et al. reported that peritoneal splenic autotransplantation did not protect splenectomized rats challenged 18 wk later with intravenous pneumococci.<sup>10</sup> Cooney et al. were able to demonstrate an increase in survival for only 24 hr when comparing rats with retroperitoneal splenic autografts exposed to intravenous pneumococci 4-5 wk postsplenectomy with sham and splenectomy controls.<sup>11</sup> Tesluk and Thomas were not able to show a protective effect in rats exposed to intravenous pneumococci 5 wk after placement of autotransplanted splenic tissue into intramuscular abdominal wall pockets.<sup>12</sup> Recently, however, Likhite was able to show protection against intravenous infection with pneumococci in splenectomized mice who were given subcutaneous splenic autotransplants 3 mo previously.<sup>13</sup>

Pearson and associates have demonstrated that children who had splenectomy because of traumatic injury showed evidence of splenic regeneration.<sup>14</sup> Their group suggested that this in vivo expression of splensitis was responsible for the low frequency of overwhelming sepsis encountered in these patients.

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Employing an experimental model previously described,<sup>7,15</sup> we have now shown a significant protective effect against aerosolized type III *Streptococcus pneumoniae* in splenectomized mice who had autochthonous splenic tissue implanted in their peritoneum 8 wk prior to exposure. This work provides experimental confirmation of Pearson's study<sup>14</sup> and, moreover, does so with a model that closely approximates the route of human infection and the mechanism of production of human splenosis following splenic injury.

## MATERIALS AND METHODS

### *Animals*

One-hundred and nineteen Swiss White male mice, CD-1 strain (purchased from Canadian Breeding Laboratory, a subsidiary of Charles River Farms), weighing 19–20 g at operation and 25–30 g during exposure, were used in these experiments. Prior to exposure, the mice were group-housed in filter-top cages. Postexposure, the animals were randomized, housed three to a cage and fed food and water ad libitum.

### *Preparation of Bacterial Suspension.*

The organism employed for this experiment was type III *Streptococcus pneumoniae* from the same stock culture described previously.<sup>7,15</sup> One frozen vial (2 cc) of this stock culture was thawed and added to 250 cc Todd-Hewitt media, which was then stored at  $-70^{\circ}\text{C}$  in 2-cc vials. For the experiment, 2 vials of this culture (4 cc) were thawed and added to 250 cc Todd-Hewitt media and incubated at  $37.5^{\circ}\text{C}$  for 15–18 hr prior to use. The broth was concentrated by centrifugation at 8000 rpm for 45 min at  $4^{\circ}\text{C}$  and the precipitate resuspended in 10 cc of Todd-Hewitt media. The total number of bacteria per cubic centimeter of the resuspended 10 cc was determined by serial dilution and was  $2.5 \times 10^9$  colony-forming units/cc.

### *Operative Procedure*

The mice were anesthetized with 1.7 mg of pentobarbital given intraperitoneally. The sham and splenectomized groups were operated on in a manner described previously.<sup>7,15</sup> The whole-spleen autotransplant group had their spleens removed in a manner similar to the splenectomized group. The spleen was then cut up into 4–6 pieces and placed back into the peritoneal cavity. The half-spleen autotransplant group was treated in a similar manner except that only half of the spleen was cut into 2–3 pieces and replaced. There was no operative mortality.

### *Animal Exposure*

All animals were aerosolized at the same time with type III *Streptococcus pneumoniae* 8 wk after surgery. The apparatus used for the aerosolized exposure (Model A-42 Tri-R Airborne Infection Apparatus purchased from Tri-R Instruments, Inc., Rockville Centre, N.Y.), and the methods used to effect that exposure have been described elsewhere.<sup>7,15</sup> The only difference in the present work was that a 6-cc, instead of a 7-cc, aliquot of the final suspension was placed in the nebulizer, and it was completely used up.

### *Statistical Analysis*

The mortality experiences of the four groups (sham-operated, splenectomized, whole-spleen autotransplant, and half-spleen autotransplant) were compared through the use of the proportional hazards model. This model has been used by us in previous studies.<sup>7,15</sup> For a given group of animals, the hazard function at time  $t$  is the conditional probability of death in the next unit of time for those animals still alive at time  $t$ . Under the proportional hazards model, the hazard functions for various groups are assumed to be proportional to one another. Thus, the ratio of the hazard functions for any two groups gives a measure of the relative risks experienced by the animals in the two groups. A ratio significantly different from unity means that at any particular time point, the animals in one group tend to be at greater risk than the animals in the other. These ratios can be estimated from the data. A computer

**Table 1. Comparison of Hazard Functions Between Groups**

Groups Compared	Ratio of Hazard Functions
Splenectomized versus sham-operated	2.27 ( $p < 0.005$ )
Splenectomized versus whole-spleen autotransplant	2.49 ( $p < 0.001$ )
Splenectomized versus half-spleen autotransplant	2.25 ( $p < 0.005$ )
Whole-spleen autotransplant versus sham-operated	0.91 (NS)
Half-spleen autotransplant versus sham-operated	1.01 (NS)
Whole-spleen autotransplant versus half-spleen autotransplant	0.92 (NS)

NS, not significant.

program (kindly furnished by N. Breslow, University of Washington, Seattle, Wash) based on the theoretical work of Cox<sup>16</sup> and Breslow<sup>17</sup> was used to estimate the ratios (and associated standard errors) of the hazard functions between various pairs of groups.

## RESULTS

Table 1 shows the results. The splenectomized group had a significantly greater hazard function than any of the other three groups, nearly two and one-half times that for the whole-spleen autotransplant group and over twice that for the sham-operated and half-spleen autotransplant groups. Comparing the splenectomized group with the sham-control group confirms our previous findings.<sup>7,15</sup> The whole-spleen autotransplant, half-spleen autotransplant, and sham-operated groups showed no significant differences in hazard function among themselves, with all ratios close to one. In Fig. 1, these findings are reflected in the higher mortality curve for the splenectomized group as compared to those for the other three groups, which are close together. All animals who died during the experiment had gross and microscopic evidence of pneumonia and lung cultures from these animals grew pneumococci. At the end of 20 days, the remaining animals were sacrificed and showed no gross or microscopic evidence of pneumonia. Splenic tissue from the autotransplant groups was found adherent to the peritoneum throughout the abdominal cavity. The splenic tissue from both autotransplant groups was examined histologically and differed from the spleens of the controls in only two respects: (1) the splenic tissue was distributed in multiple nodules surrounded by a thickened, fibrotic pseudocapsule, and (2) the blood supply to each of the nodules consisted of several small vessels entering at one side of the implant.

## DISCUSSION

Experimental studies,<sup>13,18-20</sup> have shown that autotransplanted splenic tissue is histologically indistinguishable from that of normal spleen. Functionally, the

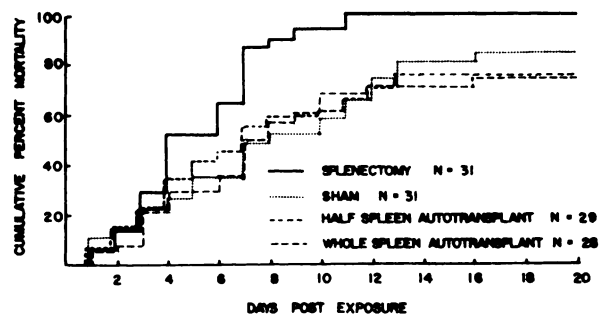


Fig. 1. Dose of pneumococci ( $2.5 \times 10^8$  colony-forming units/cc).

autotransplanted spleen can produce antibodies to sheep red blood cells,<sup>21-23</sup> maintain normal opsonin and leukophilic  $\gamma$ -globulin activity,<sup>24</sup> and remove Howell-Jolly bodies from erythrocytes.<sup>25</sup> In addition, cells from autotransplanted spleens and normal spleens demonstrate the same degree of migration inhibition when mixed with sheep red blood cells, thus showing no difference in cell-mediated immunity as tested in this system.<sup>21</sup>

Our results clearly show that autotransplanted splenic tissue can function immunologically and restore protection to asplenic mice exposed to pneumococci. Furthermore, the route of infection and the method used to effect splenosis closely parallels the human experience. We have provided experimental support for the clinical observations of Pearson and associates<sup>14</sup> that the splenosis that occurs following splenic trauma may indeed be responsible for the low, but still very real, incidence of overwhelming bacterial infection in these patients. The failure of most investigators to demonstrate that splenic implants in animals can protect against pneumococcal infection might be related to the intravenous route of bacterial challenge used in these studies.<sup>10-12</sup> The lung is an important organ in terms of host defense against infection.<sup>26</sup> Circumventing it by the use of intravenous bacteria probably overwhelms the animal to such an extent that differences between those animals who have autotransplanted splenic tissue and their controls may not be appreciated because of the high mortality rate. Pneumococcal infections in humans are acquired via the respiratory tract,<sup>27</sup> and anyone attempting to study this problem must take this fact into consideration.

Nonoperative management<sup>28</sup> and conservative surgery<sup>29</sup> have recently been suggested as alternatives to splenectomy for splenic trauma. If the clinical situation demands total splenectomy in cases of splenic trauma, autotransplantation of healthy splenic tissue at the time of surgery, as suggested by others,<sup>11,13,30</sup> may help to protect the host against postsplenectomy sepsis; pneumococcal vaccine and prophylactic antibiotics should still be used because of the uncertainty that splenosis is completely protective.<sup>31</sup>

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