Lack of Protective Effect of Autotransplanted Splenic Tissue to Pneumococcal Challenge

By Allen D. Schwartz, Jane F. Goldthorn, Jerry A. Winkelstein, and Andrea J. Swift

Studies in animals and clinical experience in patients have demonstrated that splenectomy may lead to an increased susceptibility to infection. The infections are usually caused by encapsulated bacteria such as pneumococcus. It has been shown in a variety of experimental animals that autotransplanted splenic tissue is capable of regenerating into implants that are microscopically indistinguishable from normal spleen and of restoring a number of normal splenic functions. The response to intravenous challenge with Streptococcus pneumoniae, type 25, was therefore studied in control, asplenic, and autotransplanted Sprague-Dawley rats. Despite previous observations that a number of immune functions can be restored in this animal model by autotransplanted splenic tissue, the present study indicates that splenic tissue autotransplants do not restore the ability to resist intravenous pneumococcal challenge.

Studies in animals and clinical experience in man have demonstrated that splenectomy leads to an increased susceptibility to infection. The infections characteristically are blood-borne and fulminant, cause a high mortality rate, and are usually caused by encapsulated bacteria such as the pneumococcus. It has been shown in a variety of experimental animals that autotransplanted splenic tissue will regenerate into implants microscopically indistinguishable from normal spleen. These implants are able to remove Howell-Jolly bodies from erythrocytes and can take up intravenously injected radiocolloid. Furthermore, there is evidence that autotransplanted splenic tissue is capable of restoring a number of immunologic activities.

These observations suggested that autotransplanted splenic tissue might be able to restore protection against the pneumococcus and would therefore be of clinical value to patients requiring splenectomy following abdominal trauma, a staging laparotomy for lymphoma, or as a part of a shunting procedure. Accordingly, in the present experiments the ability of autotransplanted splenic tissue to restore protection against the pneumococcus was studied.
MATERIALS AND METHODS

Experiments were performed using 1-mo-old 150-g female Sprague-Dawley rats housed under normal laboratory conditions. Forty-eight animals underwent splenectomy, 48 underwent sham-operations, and 48 had multiple fragments of their own splenic tissue macerated and re-implanted into the peritoneal cavity immediately following splenectomy.

Eighteen weeks following the surgical procedure, the animals in each group were challenged intravenously via tail vein injections with Staphylococcus pneumoniae, type 25. The animals were observed at 12-hr intervals for 10 days to determine the number and rate of deaths. The mean lethal dose (LD₅₀) of the organism for each group was determined using previously described methods.¹⁰

RESULTS

All deaths occurred within 48 hr. There was no evidence that controlled splenosis conferred any protective effect against bacterial challenge (Table 1). In addition, the rate of death in the autotransplanted group of animals was similar to that in the asplenic animals (Fig. 1).

Postmortem examinations on the rats with splenosis revealed 46 of 48 to have large amounts of autotransplanted splenic tissue that grossly appeared to be from half to the same size as the spleens in the control animals. In 2 of the 48 rats, no splenic tissue could be found. Accurate weights of the splenic implants could not be obtained because the tissue was enmeshed within the omentum and the mesenteries of the upper abdominal viscera. In many instances, multiple splenic fragments were present. The technique of crushing fragments of splenic tissue before placing them into the peritoneal cavity probably resulted in the multiple growths found in some of the animals. The blood supply to the implants arose from the omentum and the other peritoneal surfaces.

DISCUSSION

A number of abnormalities in immunologic function have been described in asplenic hosts. Clearance of blood-borne pneumococci is impaired,¹¹ antibody formation to intravenous heterologous erythrocytes is deficient,¹² IgM levels are reduced,¹³ and serum opsonic and leukophilic gamma globulin activities are low.¹⁴

Subcutaneously autotransplanted splenic fragments in mice challenged intraperitoneally with sheep erythrocytes have been shown to be capable of both humoral and cell-mediated immune responses.⁹ In addition, subcutaneous splenic autotransplants in rats restore deficient opsonic and leukophilic gamma

<table>
<thead>
<tr>
<th>No. of Bacteria Injected</th>
<th>Control Rats (No. Deaths/No. Animals)</th>
<th>Asplenic Rats (No. Deaths/No. Animals)</th>
<th>Autotransplanted Rats (No. Deaths/No. Animals)</th>
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<tbody>
<tr>
<td>4 x 10⁸</td>
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<td>4 x 10⁴</td>
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<td>4 x 10³</td>
<td>0/8</td>
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<tr>
<td>LD₅₀</td>
<td>8 x 10⁴</td>
<td>&lt; 4 x 10³</td>
<td>&lt; 4 x 10³</td>
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Fig. 1. Number of deaths in control, asplenic, and autotransplanted animals following intravenous challenge with *S. pneumoniae*, type 25 at concentrations of (A) $4 \times 10^6$, (B) $4 \times 10^5$, (C) $4 \times 10^4$, and (D) $4 \times 10^3$ organisms.

globulin activity. Finally, splenic autotransplants in this same animal model using surgical techniques identical to those in the present study have been shown to produce antibody in response to intravenous antigenic challenge with sheep erythrocytes.

Our data indicate that despite reconstitution of a number of other splenic functions by autotransplanted splenic tissue the ability to resist intravenous pneumococcal challenge was not restored. It is possible that if lower concentrations of bacteria had been used in our studies a small difference between asplenic animals and those with the autotransplants might have been demonstrated. However, the greater than 2000-fold difference in LD$_{50}$ between control and autotransplanted animals clearly demonstrates that transplanted splenic tissue did not restore to the animal the same protection against pneumococcal septicemia as is conferred by a normal spleen.

Studies in young children with sickle cell anemia who have functional asplenia have demonstrated a lack of correlation between the presence of a number of splenic functions. The large spleens of these children are unable to remove Howell-Jolly bodies from erythrocytes, cannot take up intravenously injected radiocolloid, and have a poor antibody response to intravenous sheep erythrocyte challenge. However, they retain their splenic reservoir function to pool platelets. In addition, hypertransfusion in these children restores the spleen’s ability to clear radiocolloid but not the ability to produce antibody following intravenous sheep erythrocyte injection. Our studies further support
the concept that there is a separation of a number of functions of the spleen by
demonstrating that the restoration of some splenic functions can be achieved by
splenic autotransplantation without the restoration of others.

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