Antibody Response to Intravenous Immunization Following Splenic Tissue Autotransplantation in Sprague-Dawley Rats

By Allen D. Schwartz, Mahboubeh Dadash-Zadeh, Richard Goldstein, Susan Luck, and James J. Conway

The response to intravenous challenge with sheep erythrocytes was determined in Sprague-Dawley rats following autotransplantation of splenic tissue into the subcutaneous tissue, peritoneal cavity, or a surgically created omental pouch. There was a marked rise in heterophil antibody titer following intravenous challenge in ten control animals, and no rise in titer in nine of ten asplenic animals. Heterophil antibody titers increased in all of the animals with transplants, but the response in some was less than that in control animals. Thus autotransplanted splenic tissue demonstrated immunologic responses similar to those of normal, intact spleen.

The spleen is essential for the formation of antibody in response to small doses of intravenously administered particulate antigen. It has been shown in a variety of experimental animals that autotransplanted splenic tissue will regenerate into implants microscopically indistinguishable from normal spleen. The present study investigates the ability of splenic autotransplants to respond to intravenous antigenic challenge.

MATERIALS AND METHODS

Experiments were carried out with adult male Sprague-Dawley rats weighing 350-400 g. The animals were housed under normal laboratory conditions and were allowed unlimited access to Purina rat chow. Splenectomy was performed on 23 animals through a left lateral subcostal incision under aseptic conditions. The animals were anesthetized with intraperitoneal sodium pentobarbital at a dose of 5 mg/100 g body weight. In 13 of the splenectomized animals, approximately one-third of the spleen was cut into multiple fragments, macerated with the scalpel handle, and reimplanted into the animal. In three animals the splenic fragments were returned to the peritoneal cavity in the splenic fossa, in eight the fragments were placed into a surgically created omental pouch, and in two they were placed into a pocket created by blunt-tipped scissors in the subcutaneous tissue of the abdomen. Ten animals had no surgery performed and were used as normal controls.

Between 5 and 6 mo following the surgical procedures, the asplenic, autotransplanted, and control animals were challenged with 1 ml of a 0.25%, suspension of fresh sheep erythrocytes in saline administered into the tail vein. Serum heterophil titers were determined just prior to the challenge and at weekly intervals for 3 wk. Heterophil testing was performed in a conventional manner utilizing a 2%, suspension of washed sheep red cells added to serial dilutions of the animals' sera. After incubation at room temperature for 2 hr, the degree of agglutination was determined macroscopically.

Radionuclide liver-spleen imaging was performed 15 min after intravenous injection into the
tail vein of 250–300 μCi 99mTc sulfur colloid which was prepared from a commercial kit (Squibb Tesuloid). Imaging was done using a Searle Pho Gamma H.P. III scintillation camera with a pinhole collimator at a 3-inch distance. Imaging was performed on a number of control and asplenic animals, and was performed periodically on all of the animals who received autotransplants but, because of technical difficulties due to placement of splenic tissue near the liver, this was not systematically carried out. One animal with splenic tissue autoimplanted in a splenic pouch was sacrificed following radionuclide imaging and the splenic tissue was examined to determine its anatomical location. One animal with splenic tissue transplanted into an omental pouch and one with splenic tissue transplanted subcutaneously were sacrificed 15 min following radionuclide injection. The autotransplanted splenic tissue and a small piece of liver were removed from the animals and imaging was performed on these organs.

RESULTS

The ten control animals had elevations in heterophil antibody titers following intravenously administered sheep erythrocytes. Of the ten asplenic animals, nine had no rise in antibody titers and one had a rise, but lower than that seen in the controls. Two of the three animals with tissue placed intraperitoneally into the splenic fossa had antibody titer rises similar to those seen in

Fig. 1. Heterophil antibody response to intravenously administered sheep red cells in (A) asplenic animals, (B) animals with omental pouch splenic implants, (C) animals with intraperitoneal implants, and (D) animals with subcutaneous splenic implants. Shaded area, values obtained in the control animals.
the controls, and the third had a delayed rise that eventually reached the normal range. One of the eight animals who had splenic tissue placed into an omental pouch died two days following surgery. Of the seven surviving rats, three had a normal rise in antibody titer, two had a delayed rise that eventually reached normal levels, and two had a delayed rise that did not reach the normal range. The two animals with subcutaneous implants experienced antibody titer rises, but lower than those seen in the control animals. The rises in antibody titers of the various groups of animals are shown in Fig. 1.

Reticuloendothelial localization of $^{99m}$Tc sulfur colloid in the spleen was visualized in the control animals, while the spleen was not visualized in the asplenic animals. The splenic fragments were anatomically placed in areas so close to the liver in the autotransplanted animals that visualization of splenic reticuloendothelial activity could not be distinctly separated from hepatic activity. Because of this technical problem, radionuclide imaging was not systematically repeated. One animal was sacrificed shortly after radionuclide imaging performed 6 mo after splenic autotransplantation into an omental pouch, and tissue microscopically identical to normal spleen was found in the area of radionuclide localization. The splenic tissue removed from one animal with an omental transplant and one with a subcutaneous transplant also had evidence of reticuloendothelial uptake of the radionuclide.

All of the animals were sacrificed following the study. Autotransplanted splenic tissue was found in all of the transplanted animals. No splenic tissue was found in the splenectomized animals, including the one who had a rise in heterophil antibody titer following antigenic challenge.

**DISCUSSION**

The splenectomized rat has an antibody response to particulate antigen when challenged intraperitoneally, intraportally, intramuscularly, or intradermally but is incapable of responding with a rise in antibody titer to the same dose and type of antigen administered intravenously. The splenectomized human has been demonstrated to have an immunologic response to antigen administered subcutaneously, but the splenectomized adult or the child with hereditary splenic hypoplasia is unable to respond with a significant rise in antibody titer to intravenous challenge with small doses of intravenously administered particulate antigen. Thus, in both man and experimental animal the spleen is essential for the formation of antibody in response to intravenous challenge by particulate antigen.

Studies in animal models have demonstrated that autotransplanted splenic fragments initially undergo almost complete necrosis and then regenerate into tissue that is microscopically indistinguishable from the normal spleen. Despite the fact that the growth and microscopic appearance of autoimplanted splenic tissue have been well studied, few studies have been performed regarding its function. 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, and subcutaneous splenic autotransplants in rats protect against the deficient opsonin and leukophilic gamma globulin activity observed in the splenectomized
animal. Thus there is evidence that transplanted splenic tissue is capable of immunologic activity. However, the ability of splenic transplants to respond to intravenous antigenic challenge, an immune response indicative of normal splenic function, has not been previously examined in animals with autotransplanted splenic tissue.

Failure of the anatomically present spleen to clear intravenously administered radiocolloid has been termed “functional asplenia” by Pearson et al. The large spleens of young children with sickle cell disease are usually unable to take up $^{99m}$Tc sulfur colloid or produce heterophil antibodies in response to intravenous challenge with sheep erythrocytes. These children are also unusually susceptible to overwhelming septicemia, a predisposition also seen in the splenectomized or congenitally asplenic child. Functional asplenia also has been observed in children with cyanotic congenital heart disease, supradiaphragmatic splenic transposition, reticulum cell sarcoma treated with cyclophosphamide and splenic radiation, and following thorotrast injection. Therefore, the presence of splenic tissue does not guarantee the presence of normal splenic function. It would not have been surprising to find a state of functional asplenia in animals with splenic autotransplants in view of the difference of the vascular supply between the transplanted and normal spleen. Such a finding would strongly suggest that such transplants would offer no protection against bacterial infection. The fact that transplanted splenic tissue has been found to produce antibody in response to intraperitoneal injection of sheep erythrocytes does not ensure a response to intravenous challenge, since previous studies have shown the route of administration of antigen to be a critical factor.

The observation that one asplenic rat had an antibody titer rise following antigenic challenge can best be explained by infiltration of the sheep erythrocytes into the subcutaneous tissues. No splenic tissue was found in this animal at postmortem examination, and previous studies have shown that such a challenge was followed by a rise in heterophil antibody titer. A poor response by the animals with subcutaneous implants as compared to those with peritoneal implants was noted, suggesting that subcutaneous splenic tissue may not be as immunologically responsive as intraperitoneal transplants to intravenous challenge. Splenic tissue found in the transplanted animals was indistinguishable from normal splenic tissue. The description of splenic transplants has been reported in detail by other investigators and is not the purpose of this report.

The majority of individuals who undergo splenectomy do so because the spleen is damaged due to abdominal trauma or because the splenic vessels are needed to perform a shunting procedure to relieve portal hypertension. Such patients have a small, but statistically significant increase in their susceptibility to severe, overwhelming bacterial infection. Our observations that autotransplanted splenic tissue is capable of taking up radiocolloid and responding to intravenous antigenic challenge suggest that such implants may protect against the overwhelming bacterial infection to which such asplenic individuals are prone. Further studies in animals to evaluate the protective effect of splenic implants to overwhelming infection are clearly indicated.
ACKNOWLEDGMENT

The authors thank Dr. Jane Goldthorn and Susan Schindler for their technical assistance.

REFERENCES

Antibody response to intravenous immunization following splenic tissue autotransplantation in Sprague-Dawley rats

AD Schwartz, M Dadash-Zadeh, R Goldstein, S Luck and JJ Conway

Updated information and services can be found at:
http://www.bloodjournal.org/content/49/5/779.full.html

Information on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml