Limitation of Splenic Growth as Studied by Heterotopic Splenic Implants

By Mehdi Tavassoli

Multiple spleens were implanted in the subcutaneous tissue or the peritoneal cavity of inbred rats which had undergone splenectomy. The total weight of regenerated implants was compared to the weight of implanted tissue. The weight of recovered tissue was not a function of implanted spleen mass; rather, it approximated the unit ratio of the animal’s own spleen. Those animals receiving more than ten spleens died by days 3–6 postimplantation, probably as a result of massive necrosis that occurred during the process of regeneration. When implants were made in a temporal sequence of two spleens every 3 days, the first set was uniformly successful, whereas regeneration was aborted in subsequent sets of implants. These observations are consistent with the conclusion that certain factors control the total mass of spleen in any one animal.

The total mass of certain tissues in any one animal appears to be controlled by some as yet unknown factors. For instance, after partial hepatectomy the remaining liver undergoes regeneration until the original hepatic mass is regained and regeneration then ceases.1 By contrast, the total mass of some other tissues appears to be free of such controls. Thus, Metcalf has been able to produce an artificial hyperthymic state in mice by isologous transplantation of as much as ten times the normal weight of thymus in a single animal.2 There are indications that certain factors control the total mass of spleen in any one animal3: splenic implants do not thrive in the presence of the animal’s own spleen,4 suggesting a negative feedback on the growth of implants. By contrast, implantation of small pieces of spleen into splenectomized rats results in the regeneration of splenic tissue that exceeds the weight of the original implants, suggesting an attempt on the part of the regenerating splenic tissue to attain the mass of the animal’s original spleen.5 A similar process has been observed after partial splenectomy where the splenic remnant undergoes compensatory hypertrophy.6 In the congenital anomaly associated with double spleen, the combined weight of the two spleens approximates the average weight of a single normal spleen (i.e., 60 g each).7

In the present study, ectopic implants of spleen have been used in splenectomized rats, and it was found that certain limitations were imposed on the growth of splenic tissue, keeping the total mass of regenerating spleens close to that of the original spleen.
MATERIALS AND METHODS

Male inbred Bartonella-free Fisher rats weighing 150-200 g were used in these studies. They were fed Purina lab chow and water ad libitum. All operations were carried out under aseptic conditions using intraperitoneal pentobarbital (25 mg/kg) for anesthesia. All recipients of splenic implants were splenectomized so that the implants were their only functional splenic tissue. Splenectomy was performed through a midline abdominal incision, and the spleens were weighed. Isologous implants were made using either intact spleens or pieces of spleens averaging 40-60 mg each. Implants were placed either in pockets made in subcutaneous tissue or, in some instances, dropped freely into the abdominal cavity. Six weeks after implantation complete blood counts were obtained and the animals explored. The implanted splenic tissue was removed and placed in 10% buffered formalin and processed for routine histology. All tissues were weighed prior to implantation and again after removal.

RESULTS

Figure 1 shows the total weight of regenerated implants as a function of the total weight of implanted tissue. The weight of recovered tissue is expressed as the ratio of the weight of the recipient's own spleen. This internal control seemed necessary, as splenic weight may vary considerably from animal to animal. It is clear from Fig. 1 that the total weight of regenerated tissue is not a function of the implanted tissue mass; rather, it approximates closely the unit ratio of the weight of the animal's own spleen.

Figure 1 incorporates data from the experiments in which intact spleens were implanted and those in which splenic fragments were implanted. A separate plot of the two sets of data did not suggest a difference in the results. Likewise, the supporting bed for implants (intraperitoneal versus subcutaneous) did not affect the results. There was no significant change in blood counts of these animals as compared to their preoperative counts. Histologic examination of implants revealed normal splenic tissue indistinguishable from the original spleen. Although morphometric studies were not done in these experiments, there did not appear to be a disproportionate change in the ratio of red to white pulp.

In those animals given multiple implants of spleen fragments subcutaneously, a consistent finding at the time of exploration was that some implants were reduced to a few strands of fibrous tissue, whereas others had regenerated to approximately 50%-100% of the original weight of implanted tissue. Furthermore, most animals who received more than ten spleens from inbred littermates died within 3-6 days after operation. Histologic examination of these splenic implants revealed massive central necrosis and a peripheral shell of granulation tissue. Since massive necrosis could have contributed to the death of these animals, an experiment was carried out in a different group of six ani-
mals who received 2–3 spleens every 3 days for a total of up to 15 spleens. None of these animals died; they tolerated multiple operations well. However, only the first two to three transplanted spleens survived, the rest being reduced to strands of fibrous tissue.

DISCUSSION

The data presented here indicate that the total spleen mass in any one animal cannot be increased above a certain limit by isologous transplantation of splenic tissue. This finding is consistent with the conclusion that certain factors control the total splenic mass in any one animal and that this mass approximates the weight of the animal’s own spleen.

From a practical point of view, this conclusion should be taken into consideration when ectopic splenic implantation is used as an experimental model. In this respect, spleen differs from thymus but is similar to liver, the total mass of which also appears to be under certain controls.

The regenerative pattern of splenic implants has previously been described. Such implants undergo almost total necrosis and then regenerate into splenic tissue with a microscopic structure indistinguishable from that of the original organ. The regenerative process is apparently triggered by penetration of capillaries from the surrounding tissue by day 3 and originates from a shell of surviving splenic tissue at the surface of the implant. This zone first consists of almost monotonous connective tissue cells interspersed with red cells, and it develops into the red pulp. As capillaries develop, the structure of small arteries, periarterial lymphatic sheaths appear, and soon the formation of splenic white pulp becomes evident.

The nature of the factors controlling total spleen mass are as yet obscure. In analyzing the regulation of the proliferative activity of a given tissue, Metcalf has indicated that three components should be considered: the proliferative stimulus, possible inhibitory influences suppressing proliferation, and the reactivity of target cells to such stimulatory and inhibitory factors. In the present experiments, there is no reason to believe that the reactivity of splenic cells was altered, since all the implants were normal tissues. The total mass of the splenic implants was maintained irrespective of the site of implantation, suggesting that the proliferative stimulus or inhibitory factor was of a general nature and not a local phenomenon. There is evidence in endocrine glands that inhibitory factors are effective in controlling the total mass of such tissues through a feedback mechanism. Similar factors have been incriminated in maintaining the growth of liver after partial hepatectomy, and it is possible that a circulating factor of similar nature influences the proliferative activity of splenic tissue. Because the presence of the animal’s own spleen suppresses the growth of isologous splenic implants, one could assume that the source of such a circulating factor is the functioning splenic tissue.

If this assumption is correct, the observations presented here suggest that the regenerating spleen is capable of elaborating such an inhibitory substance by day 3–6 after implantation because, when the implants are made in a temporal sequence of every 3 days, the first set is always successful, whereas regeneration is aborted in subsequent sets of implants. The connective tissue cells at the sur-
face of implants may therefore be responsible for elaboration of this inhibitory factor. There is evidence to indicate that these cells arise from stromal cells of splenic tissue. Because the penetration of implants by capillaries from the surrounding tissues does not occur for the first 3 days, it is only at this time that the inhibitory factor can be released into the circulation and exert its influence.

Furthermore, when multiple pieces of spleen are placed in a single animal, some undergo the complete regenerative process, whereas regeneration is aborted in other pieces, reducing them to strands of fibrous tissue. Because the regenerative process is not synchronous in all these pieces, it is possible that, when an adequate mass of tissue is in the definitive stages of regeneration, the controlling factors may act on those pieces that have not entered the definitive stages of regeneration to abort the process. This possibility is strengthened by the observation that, if the implants are made in a temporal sequence of every 3 days, the first set is always successful, whereas regeneration in subsequent sets of implants is aborted.

The transplantation of more than six spleens at one time causes the animal to die, probably as the result of massive necrosis of the implants occurring after 3–6 days. This time coincides with the necrotic phase of splenic regeneration. When multiple implants are made at 3-day intervals, death can be prevented.

The limitations imposed on total splenic mass should be considered in relation to clinical states associated with splenomegaly in humans. The spleen is basically a filter, made up by a reticular meshwork that traps circulating cells and provides a place for their interaction, division, and differentiation. Splenomegaly does not usually result from an increase in the mass of splenic tissue per se, but from an accumulation of other cellular elements—trapped red cells in hemolytic states, hemopoietic cells in myeloproliferative disorders, and lymphocytes in lymphoproliferative disorders. Ultimately, however, hypertrophy or even hyperplasia of fixed cellular elements of the spleen may contribute to splenic enlargement induced initially by accumulation of extraneous elements. By analysis of DNA synthesis and mitotic activity, Jandl et al. have demonstrated that reticuloendothelial elements proliferate in the spleen after induction of hemolysis.

REFERENCES

Limitation of splenic growth as studied by heterotopic splenic implants

M Tavassoli