

Experimental Models of Splenic Dysfunction

Allan E. Dumont, M.D.

New York University School of Medicine, Department of Surgery, New York, USA

Summary

Animal simulations of splenic disorders are described including alterations in portal and splenic vein flow, induction of splenic sequestration, models of hyposplenism and murine hereditary spherocytosis.

Clinical studies limited to microscopic examination of spleens removed at autopsy or surgery, while providing morphologic data, offer limited insights into fundamental mechanisms underlying splenic dysfunction. Investigators are, therefore, turning increasingly to animal experimentation, which is likely to become the major source of new information about the spleen.

When some discrepancy exists between a traditional view of splenic dysfunction and the results of a specific experiment, it is tempting to attribute it to a wide gap between disease in man and the experimental maneuver in animals. Yet most of the experimental models of splenic dysfunction described here are in fact close counterparts of human disorders, although each model must be considered individually with respect to this question.

I. Splenic Response to Experimentally Induced Alterations in Portal and Splenic Venous Flow

A. Ligation of Splenic Vein

Interruption of splenic vein flow in animals has been used since 1910 (1) in attempts to simulate Banti's syndrome. While a simpler, more direct method of inducing congestion of the spleen can hardly be imagined, such attempts have failed to produce a sustained

increase in either spleen size or sequestering activity. Pressure in the ligated venous segment increases transiently accompanied by 2-3 fold increase in weight of the spleen and diminished number of circulating formed blood elements. But after several weeks venous pressure falls, the spleen begins to shrink, and the numbers of formed blood elements gradually return to normal. As the spleen continues to atrophy, splenic artery flow decreases and flow in collateral veins increases (2-5).

While obviously failing to duplicate Banti's syndrome, interruption of splenic vein flow does result in an immediate and sustained increase in the rate of splenic lymph formation (6), an alteration which appears to be characteristic of the congested spleen in patients with portal hypertension as well as in patients with severe congestive heart failure (7). The fact that splenomegaly and hypersplenism are conspicuously absent in most patients with even the most severe form of cardiac circulatory congestion indicates that in Banti's syndrome and in cirrhosis with portal hypertension, the origin of splenomegaly and hypersplenism is more complicated than the mechanism suggested by the convenient clinical term "congestive splenomegaly".

B. Experiments with Transplanted Spleen

In 1939 *Cameron* and *DeSaram* described a new experimental approach for investigating the effect of liver injury on the spleen (8). After transplanting the spleen out of the portal and into the systemic circulation, they injured the liver chemically and observed that the

transplanted spleen enlarged and increased its sequestering activity. Similar findings have since been noted by others who compared the growth of regenerating spleen fragments in the portal and systemic circulations after other forms of liver injury. In animals with portal hypertension secondary to experimentally induced chronic biliary obstruction for example, splenic transplants whether in the portal or systemic circulation grew faster than in controls (9). And in mice with portal hypertension secondary to experimentally induced schistosomal cirrhosis, fragments of spleen transplanted into the systemic circulation grew almost as fast as those placed in the congested portal circulation (10).

C. Related Observations

Attempts to induce enlargement of the spleen and/or to increase splenic sequestering activity by producing chronic hepatic vein outflow block have also failed. Such a hemodynamic derangement results in atrophy of the spleen (11).

Rousselot and Thompson succeeded in producing enlargement of the spleen by injecting particles of silica into the portal or splenic veins of dogs and rabbits (12). Although they believed that the resulting increase in portal pressure was the mechanism underlying splenomegaly, it seems more likely that a compensatory increase in splenic reticuloendothelial (RE) activity secondary to reduced transsinusoidal portal flow and Kupffer cell activity was responsible.

In acute experiments, even simple manipulation of the spleen may introduce significant alterations in transsplenic blood flow (13). The extreme lability of this flow was clearly described by *Barcroft and Florey* as follows: "If the spleen of the chloralosed cat be exposed through a small incision it would usually be large and of a dark blue color, the blood in the veins being dark blue. When the spleen is made to contract by handling, electrical stimulation or sometimes by the insertion of a needle into its pulp, the color immediately changes to a bright arterial red and the blood in the veins is now a bright

red color" (6). This observation probably signifies sudden "opening" and "closing" of the splenic microcirculation, a phenomenon known to follow infusion of highly dilute epinephrine into, or topical application onto, the spleen.

II. Experimental Induction of Increased Splenic Sequestering Activity

As an important component of the RE system, the spleen sequesters and removes macromolecules and aging and/or defective red cells from circulating blood. An experimentally induced increase in such activity is capable of producing splenic enlargement and panhypersplenism, i.e. the abnormal sequestration and removal of normal formed blood elements. Under these circumstances splenectomy regularly corrects or prevents this hematologic abnormality.

Splenic RE activity can be increased experimentally by the following methods: a) injection of colloidal substances known to be sequestered and phagocytosed in the spleen and other RE organs, b) injection of pharmacologically active materials which increase the rate of red cell destruction by phagocytosis in the spleen, c) injection of substances which enhance mechanical filtration in the spleen, and d) as mentioned earlier, decreasing hepatic RE activity to the point at which a compensatory increase in splenic activity occurs.

Methyl (14) and hemicellulose (15) are macromolecules often used to increase splenic phagocytic activity. It is clear that the resulting hyperplasia of RE elements in the spleen accounts for increased spleen weight and the associated anemia, leukopenia and thrombocytopenia.

A single injection of phenylhydrazine damages red cells and results in a severe hemolytic anemia. In treated animals, the spleen enlarges rapidly and by the 4th day may be three times normal size with a corresponding increase in sequestering activity (16, 17). Regenerating fragments of the transplanted spleen also respond to phenylhydrazine with a striking increase in growth and sequestering activity (18).

Trypan blue-induced increase in spleen size and sequestering function has been studied

in rats as a possible experimental counterpart of the enlargement and dysfunction associated with lymphoma in man (19). Histologic abnormalities resembling human lymphoma develop in rats receiving trypan blue for 6–17 months (20–22). The spleen enlarges (whether or not lymphoma-like tumors develop) and sequesters red cells at an abnormally high rate (23). When a suspension of tantalum powder, a physiologically inert radiopaque metal easily visible under the light microscope, is injected in these animals, it is possible to demonstrate that an increase in mechanical filtration alone (in contrast to phagocytosis) accounts for the trypan blue-induced enhancement of sequestering activity. Thus, increased demand for mechanical filtration may at times lead to enlargement and hyperfunction of the spleen (19).

III. Experimental Counterparts of Hyposplenism

Less commonly, splenic dysfunction in man takes the form of a reduction in functioning mass of splenic tissue. Studies in patients with sickle cell anemia (24) and in patients with celiac disease (25) for example disclose that uptake of macromolecules is depressed and that antibody response to intravenously injected sheep red cells is diminished. Experimentally, a decrease in the functioning mass of spleen tissue can be induced by interrupting arterial inflow to the spleen (26), by partial resection of the spleen (26) and by total splenectomy combined with auto-transplantation of splenic fragments (27). The results of a graded or partial splenectomy are particularly interesting and suggest that a reduction in functioning spleen mass to one-third of normal is associated with significant impairment of antibody production and of resistance to intravenous pneumococcal challenge (28).

IV. Genetically Based Animal Model of Spherocytosis

In patients with hereditary spherocytosis, the spleen sequesters an abnormal number of red cells and enlarges. The essential features of the disease are found in deer mice

homozygous for spherocytosis (29, 30). In these rodents the markedly enlarged spleen consists mostly of red pulp with an approximate three-fold increase in hemoglobin per unit weight. As in man, the hemolytic state in these mice is abolished by splenectomy although spherocytes with increased osmotic fragility persist in circulating blood. Transmitted by a single recessive gene, this disorder in deer mice appears ideally suitable for experimental studies aimed at determining whether the amount of spleen which must be removed to eliminate the syndrome is less than a total splenectomy and should this prove to be the case, whether the remaining spleen is sufficient to protect the animal immunologically.

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Allan E. Dumont, M.D., New York University School of Medicine, Department of Surgery, 550 First Avenue, New York, New York 10016, USA