HISTOLOGICAL EVALUATION OF THE REGENERATION OF SPLENIC AUTOTRANSPLANTS. EXPERIMENTAL STUDY IN RATS

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SUMMARY: The aim of this study is to analyze the histological regeneration of splenic tissue autotransplanted in a pouch created by gastrocolic omentum in rats. Forty-eight Wistar rats were used, adult, male, weighing 160 to 210g. The surgical procedure consisted of two stages: at first a total splenectomy was made, transverse section of the spleen in four slices and finally was made the autotransplantation of two slices harvested from the waist of the spleen with 3-5 mm thick in the omental pouch. The animals were then, after this common procedure divided into 4 groups of 12 animals each and evaluated after 7, 14, 21 and 28 days. The autotransplanted splenic tissue were withdrawn after this period of time and submitted to histological examination. The splenics fragments recovered on the 7th day revealed a tissue necrosis in the central two thirds with initial regeneration in the one third outer with macrophages. On the 14th day, the histological examination revealed necrosis in the central one third of the autotransplanted splenic tissue with initial development of the red pulp, sinuses, capsule and trabeculae. The autotransplanted splenic tissue recovered on the 21st day were well developed at microscope examination with evident vascular supply, defined red pulp with the presence of lymphoid follicles. The histological examination on the 28th day, the autotransplanted splenic tissue revealed complete regeneration compared to normal spleen. The present study showed that splenic fragments autotransplanted in a omental pouch in rats regenerate completely in a period of 28 days.


INTRODUCTION

The role of the spleen in the immunological system has been the objective of many clinical studies, since it is one of the most important organs involved in defense against microorganisms. Through its network and lymphoid follicles circulate various cells from immunological system. The spleen was considered, for some time, to be a mysterious organ without any function. Its surgical removal, as a result of trauma or other diseases, was not considered to harm the patient.

In 1919, the studies of MORRIS and BULLOCK showed that the absence of the spleen was associated with an increased susceptibility to infection. Even so, splenectomy was largely used as an elective treatment for trauma and hematological problems. In 1952, KING and SHUMACKER demonstrated an association in children, between overwhelming...
infective meningitis and splenectomy suggesting a reevaluation of the basis for splenectomy, mainly in traumatic ruptures\textsuperscript{37,44}. Since then, the risk of fulminant post splenectomy infection has been recognized, not only in children, but also in adults, mainly during the first two years after surgery\textsuperscript{1,13,41}.

The incidence of overwhelming infection in splenectomized patients by trauma (0.5 - 1\%) is 58 times greater than in the normal population with present mortality level of 50 a 80\%\textsuperscript{18,36}. In these patients the involvement of the phagocytic function, depression of the level of sera immunoglobulin (IgM class), properdin and T lymphocytes, and also alterations on the complement activity and deficiency of tuftsin were observed\textsuperscript{39}. The most common microorganism isolated in these infectious processes is Streptococcus pneumoniae, but other encapsulated microorganisms such as Haemophilus influenzae, Neisseria meningitidis and Escherichia coli were also found\textsuperscript{38}. Based in these observations, attempts of preserving the spleen and its function were become important and were carried out when possible\textsuperscript{21,25}.

A great number of alternatives, mainly in polytraumatized patients were proposed, in order to prevent complications related to the spleen’s absence, such as conservative treatment\textsuperscript{19}, suture of the lacerated spleen\textsuperscript{2}, application of hemostatic agents\textsuperscript{34} and partial splenectomy\textsuperscript{4}. However, in approximately 10\% of the patients, total splenectomy was inevitable. In these cases, besides the use of vaccines and antibiotic with the object of prophylaxis\textsuperscript{18}, the autotransplant of splenic tissue is viable option, in terms of preservation of immunological function, contributing to the resistance of the host against infection\textsuperscript{1,2,26,30}. Some experimental studies show that autotransplanted splenic fragments have a capacity of regeneration, without the necessity of vascular anastomosis, after an initial period of necrosis\textsuperscript{33}. There has been described in the literature various methods for the preparation of spleen fragments, to be transplanted, and also a variety of topographical regions for the auto-transplantation, but the autotransplantation of approximately 30\% of the weigh of the original spleen in an omental pouch results in a satisfactory regeneration of tissue, viability and a protective functional action against infections, not only in experimental levels, but also in clinical ones\textsuperscript{22,29,40}.

Autotransplanted splenic tissue goes through different phases of evaluation until having normal spleen characteristics. The aim of this study is to verify development of the autotransplanted splenic tissue in a pouch created by gastrocolic omentum in rats, define the regeneration phases from this tissue after 7, 14, 21 and 28 days and compare the histological characteristics of these phases with those of normal splenic tissue.

**METHOD**

Forty-eight Wistar rats, male, adult, weighing from 160 to 210 g were used. After 12 hours fasting before the actual experiment, the animals were submitted to the surgical procedure, carried under aseptic conditions. Superficial inhalatory anesthesia was with sulphuric ether and spontaneous ventilation. Subsequently, trichotomy on the ventral region, medium incision and examination of abdominal cavity were carried out in order to check the presence of accessory spleens of the left hypochondria with the pushing back of the stomach and spleen, and partial ligation of the abdominal cavity. Total splenectomy was realized after isolation, nipping and ligation of the splenic pedicle using 4/0 cotton thread (Figure 1).

![Fig. 1 - Spleen exposure and nipping of splenic pedicle.](image1)

The spleen was transversally sectioned in respect to its craniocaudal axis, into 4 slices, and two 3 - 5 mm thick slices from its medial portion were also utilized for the autotransplantation (Figure 2).

![Fig. 2 - Transversal section of spleen giving rise to 2 slices in its medial portion.](image2)

A pouch was made from the fold of the gastrocolic omentum. Autotransplantation from 2 spleen slices was effected close to the sides of the pouch with interrupted stitches of 4/0 polyglyconate thread (MAXON\textsuperscript{R}). Fragments composed of cranial and caudal poles were placed in 10\% formalin buffer and submitted to an
histological examination for characterization of the control. At the end of the procedure, the abdominal wall was sutured into layers.

Animals were divided into 4 groups of 12 each, named A, B, C and D, and evaluated respectively after 7, 14, 21 and 28 days following autotransplantation. After these periods, they were sacrificed and submitted to the abdominal cavity examination, to determine recovery of autotransplantation splenic tissue by their resection (Figure 3). They were fixed in 10% formalin buffer and submitted to histological examination.

![Fig. 3 - Recovered autotransplantated splenic tissue involved by the omentum.](image)

Histological examination consisted in dehydration with ethanol, xylol diaphanization, impregnation with paraffin at 58°C and inclusion with formation of blocks. After paraffin treatment, the block was cut into 4 to 6 mm thick slices, and stained with hematoxylin-eosin and by the Masson trichromic techniques. Histological examination was realized using an optical microscope for examination of the splenic capsule, white pulp (lymphoid follicles and its components), red pulp, structure of the splenic tissue and vascularization, and also the presence of necrosis and sclerosis were evaluated and histologically compared with normal splenic tissue.

![Fig. 4 - Photomicrograph of normal splenic tissue showing: 1. capsule; 2. trabeculae; 3. lymphoid follicle; 4. germination center; 5. marginal zone; 6. centrofolicular arteriole; 7. red pulp (HE, 40x).](image)

**Autotransplantation Histology**

**1. Group A (7 Days)**

The splenic tissue recovered after this time showed recent ischemic central necrosis area enveloped by neutrophiles, filling two thirds of the internal part of the transplanted splenic tissue with indications of regeneration activity, also with reticular cells and large amount of hemossiderine pigments. The splenic tissue structure, in this phase, was irregular, being granular, with capillaries and histiocyte phagocytic cells. In the other third, there occurred initial development of reticular fibers which could eventually become a concentric standard. Among the lymphoid elements in the differentiation phase, a type of primitive red pulp was observed, with plasmatic cells, macrophages and lymphocytes in process of formation. Few well-defined sinuses were present in this phase, and were markedly congest, with erythrocytes in their interior. The capsule, poorly developed, contained granular tissue, dense fibers of collagen, fusiform cells and few trabeculae, which extended to the

**RESULTS**

Obits among animals submitted to spleen autotransplantation did not occur. In the abdominal cavity examination few intraperitoneal adhesions and the absence of hematoma or abscess were observed. Autotransplanted splenic tissues were readily identified and recovered in all animals.

**Histology of normal splenic tissue**

It was found that splenic tissue of the reticular type, with reticular cells, lymphocytes and macrophages as well as fibroelastic capsule with trabeculae pointing towards the central region. Between the capsule and the trabeculae a few consistent tissue with a large quantity of blood vessels were observed which were red and white pulps. Red pulp was formed mainly by the sinuses which formed an extensive plexus of irregular blood spaces with dilatations. In the white pulp, lymphoid follicles occurred and in this region there were centrofolicular arterioles, branches of trabeculae arteries existing not only as eccentric in relation to the follicle, which they were, but also as being enveloped by a lymphatic covering cape composed of a network of reticular cells and by a framework of reticular fibres with a large number of lymphocytes. Present, were distinct different lymphocyte phases, macrophages and plasmocytes, originating the germination center. The marginal zone was constituted mainly of small lymphocytes and reticular cells (Figure 4).
central area. No development of lymphoid follicles was observed (Figure 5).

2. Group B (14 Days)

In this phase, the persistence of a small necrosis in the central one third of the autotransplant was observed, with areas of hyaline material, the presence of phagocytosis and hemossiderine pigments. The splenic tissue structure was not well-defined, but lymphoid follicles were already present, with an absence of a germination center and marginal zone. Connective tissue cells in process of differentiation in the splenic reticular cells were observed and with capillaries formation. The few sinuses found in the red pulp were dilated. In the regeneration area there were small cells with dark nuclei, being erythrocytes precursors. The capsule, now with a greater development, was presented in almost all the periphery of autotransplanted splenic tissue (Figure 6).

3. Group C (21 Days)

A great vascular proliferation was present with arteries and veins forming the final appearance of the red pulp. The regeneration tissue of the periphery of splenic autotransplantation was dilated towards its center with a gradual substitution of the necrotic central zone. The sinuses were well-developed and congest, with its white pulp not yet well-defined. The lymphocytes occurring around the arteries formed lymphoid follicles with germination centers having a moderate development and a largely observable marginal zone. The regeneration of the capsule was in an advanced stage, covering almost all the surface of the autotransplanted splenic tissue, with trabeculae, plasmatic cells and phagocytosis with macrophages (Figure 7).

4. Group D (28 Days)

In this phase, an important similarity was observed between the autotransplanted splenic tissue and the normal spleen in all its details. The tissue surface was covered by mesothelial cells with a thin capsule of fibroelastic tissue. The red pulp had a splenic tissue structure, with trabeculae in most of the tissue, some of cicatrization area, and many macrophages. The white pulp consisted of small lymphoid follicles, with mature lymphocytes, active germination centers, periarterials lymphoid sheaths and well-differentiated marginal zones. The centrofollicular artery was not well-differentiated (Figure 8).

Fig. 5 - Photomicrograph of autotransplanted splenic tissue after 7 days: 1. necrotic central zone; 2. irregular structure of splenic tissue; 3. granular tissue; 4. red pulp (HE, 40x).

Fig. 7 - Photomicrograph of autotransplanted splenic tissue after 21 days: 1. vascularization; 2. red pulp; 3. sinuses; 4. lymphoid follicles; 5. capsule (HE, 100x).

Fig. 6 - Photomicrograph of autotransplanted splenic tissue after 14 days: 1. capsule; 2. trabeculae; 3. splenic tissue structure (MASSON’S trichromic, 100x).

Fig. 8 - Photomicrograph of autotransplanted splenic tissue after 28 days: 1. normal splenic tissue structure; 2. capsule; 3. red pulp; 4. lymphoid follicle; 5. centrofollicular arteriole; 6. marginal zone (HE, 100x).
DISCUSSION

The spleen has a central position in circulatory blood distribution, receiving a volume per minute of approximately 5% of the total. Moreover, the spleen represents an important point of interaction between antigenic information transported by the blood and the immunological system. Two important functions of the spleen can be recognized, namely its capacity of being an efficient phagocyte filter and as an antibodyproducing organ. Knowing that patients without spleen, mainly children, have a greater risk of develop overwhelming pneumococcal infections, alternatives for preserving spleen should be consider in order to maintain its immunological function, when possible8,10,15,19,26.

The confection of a pouch from the gastrocolic omentum as a region of a splenic tissue autotransplantation is appropriate since the omentum has a rich vascular supply and allows easy reintegration of tissue. It impedes the migration of this autotransplanted tissue and maintains the spleen into the portal circulation, where it is normally located. Some studies show a higer capacity of splenic function, with high antibody levels when compared with other implantation locales4,38.

The utilization of thin slices of splenic tissue, 3 - 5 mm thick, allows the maintenance of the spleen's structure, an important factor on its function as a filter of bacteria in the blood circulatory system. This occurs since the autotransplanted splenic tissues submit an early partial necrosis and is regenerated from the peripheral layers of remaining cells. In autotransplantations where the splenic tissues are thicker, necrosis progresses and the central area of the tissue becomes totally enveloped before that regenerative process can begin, demonstrating that the thickness of the fragment from the autotransplanted splenic tissue is a fundamental factor in preserving its viability7,23,31.

Technically, spleen autotransplant is not difficult. The formation of a pouch, using the gastrocolic omentum does not significantly increase the duration of surgery and is not associated with post-operative complications. Other procedures, such as partial splenectomies, occupy a longer time and increase the morbimortality. Autotransplantation using a omental pouch results in a good tissue viability, with low complication rates. There were no technical difficulties during the execution of the present autotransplantation study5,6,20,42.

The immunological and hematopoietical tissues have some structural and functional characteristics in common. From the functional point of view, they are vascular filters with a reticular network that store blood cells and allow a complete regeneration capacity after autotransplantation in ectopic area. Reticular cells associated with the reticular network are responsible for this regenerative potential. The growth of autotransplanted splenic tissue consists of a degenerative phase, followed by a regeneration one. These tissues undergo an almost total necrosis, which can be observed in the center of the tissue, similar to that of coagulation necrosis, being observed in cases of an abrupt interruption in circulation. However, a strip of splenic tissue becomes feasible, assuming its disposition in the external zone of autotransplanted tissue. The viability of this region is maintained by nutrients perfusing through the vessels of the pouch33. Splenosis is the term used to define the capacity of regeneration which the splenic tissue has in any region, in or outside of the peritoneal cavity, when dislodged from its natural position, in an accidental or intentional manner. This development, in a small period of time, becomes microscopically similar to that of the normal splenic tissue and is clinically recognized after spleen is withdrawn by traumatic rupture11, 32.

The observation of splenic function in the laboratory is demonstrated by the return to normality of seric levels of substances such as opsonin, tufsin, platelets and IgM immunoglobulins; clearance of the blood circulation of substances like target-cells and HOWELL-JOLLY bodies17, 43.

During the execution of a splenic autotransplant, reticular cell proliferation is the dominant response, followed by their differentiation in lymphocytes, reticuloendothelial monocytes, platelets and endothelial of the sinous venous. The differentiation in the cells of primitive connective tissue occurs through the penetration of capillaries from the large omentum, which invade the autotransplanted tissue, arising from the splenic capsule1.

In relation to the factors responsible for regeneration of splenic tissue, TAVASSOLI, in 197538, observed that when the splenic autotransplants were only remaining functional tissues of the spleen, the development occurred better and more rapidly, suggesting that the absence of any other splenic tissue could be the factor responsible for the regeneration of splenic autotransplantation.

The hypothesis that the spleen tissue is capable of regeneration, even in the presence of residual spleen tissue as occurs in partial splenectomy or in presence of accessory spleens constitute a variable that has been observed by other investigators and which requires studies in the future34.

CONCLUSIONS

We conclude that, in rats, the histological regeneration of autotransplanted splenic tissues in a pouch created by a gastrocolic omentum is a satisfactory procedure. The phases of the regeneration process vary according to time elapsed after autotransplantation, since almost complete necrosis of the autotransplanted splenic tissue occurred up to 7 days. This passed to a phase of centripetal vascular neoformation, after 14 days, followed after 21 days

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RESUMO

O presente estudo objetiva analisar a regeneração histológica do tecido esplênico autotransplantado em uma bolsa criada a partir do omento gastrocêntrico em ratos. Foram utilizados 48 ratos da linhagem Wistar, adultos, machos, pesando entre 160 e 210g. O procedimento constou de duas etapas: inicialmente realizou-se a esplenectomia total, seccionou-se transversalmente o baço em 4 pedaços; em segundo lugar realizou-se o autotransplante de duas fatias retiradas da porção média do baço com 3-5mm de espessura em uma bolsa de omento. Os animais foram, a partir deste procedimento comum, divididos em 4 grupos, contendo 12 animais cada grupo, e avaliados após 7, 14, 21 e 28 dias. Os tecidos esplênicos autotransplantados foram recuperados após este período e encaixados à análise histológica. Os fragmentos esplênicos retirados no 7º dia demonstraram uma necrose tecidual envolvendo os 2/3 centrais com início de regeneração no 1/3 periôfico e com presença de macrófagos. Ao 14º dia, a avaliação histológica mostrava necrose no 1/3 central do tecido esplênico autotransplantado, com desenvolvimento inicial da polpa vermelha, sinusóides, cápsula e trabéculas. O tecido esplênico autotransplantado recuperado no 21º dia apresentou-se bem desenvolvido à análise microscópica, com suprimento vascular evidente, polpa vermelha definida e folículos linfoides presentes. À análise histológica no 28º dia, o tecido esplênico autotransplantado apresentou-se com uma regeneração completa, podendo ser comparado ao baço normal. O presente estudo demonstra que fragmentos de baço autotransplantados em uma bolsa de omento, em ratos, regeneram-se completamente em um período de 28 dias.