MORPHOLOGICAL ANALYSIS OF AUTOGRAFTING OF SPLENIC FRAGMENTS

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The number of patients undergoing enforced splenectomy has increased in recent years. This situation arises during surgical operations on the pancreas and proximal part of the stomach, and also after traumatic injury to the spleen [1, 5]. Meanwhile recent investigations have shown that splenectomy increases the probability of development of postoperative infectious complications, including the GPSI syndrome (general postsplenectomy infection) [7]. This fact was first noted in a publication [6] whose authors stated that sepsis arises in children after splenectomy. Furthermore, removal of the spleen leads to the development of a syndrome of hyposplenism [1], persistent thrombocytosis and monocytosis [8] and, consequently, a tendency toward thrombus formation, in the early postoperative period.

The facts described above fully justify a search for ways of preserving the spleen or of adquately compensating its functions. A report of autografting of homogenized splenic tissue has been published in the Soviet literature [2]. An important role in the restoration and normal functioning of the cells of immunogenesis is played by preservation of their microenvironment, namely cells of the reticular stroma of the spleen, or mechanocytes [3]. Interaction between lymphocytes and phagocytes of the spleen is evidently also more effective if the structure of the organ is preserved. Hence it follows that transplantation of splenic fragments can be considered to be more rational than implantation of homogenized pulp.

There have been many investigations to study the morphological characteristics of regeneration in splenic tissue, but the time course of regeneration and survival of autographs of splenic tissue has been insufficiently studied. Only a few communications have dealt with this matter [4, 9].

The aim of the present investigation was to study the time course of restoration of the tissue structure of the spleen and of its fragments transplanted intraperitoneally in experiments on guinea pigs, and the time course of survival of splenic grafts, and also to study whether distal resection of the pancreas has any effect on the course of regeneration of splenic tissue after transplantation.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male guinea pigs aged 2-3 months and weighing 300-350 g. Depending on the character of the operation, the animals were divided into two groups (75 guinea pigs in each group): group 1) splenectomy and autografting of splenic fragments; group 2) splenectomy, resection of the pancreas, and autografting of splenic fragments. The operation was performed with observation of the rules of asepsis and antisepsis. The animals were anesthetized by intraperitoneal injection of 1% hexobarbital solution (4 mg/kg) and of a solution of relanium (0.1 ml/100 g). In the animals of group 1, after removal of the spleen its tissue was used for reimplantation, the method of which was as follows. The spleen was decapsulated, rinsed with warm physiological saline with the addition of kanamycin (1 g in 50 ml), after which three or four pieces of splenic tissue up to 1 mm thick and weighing 80 mg for young guinea pigs and 100 mg for adult animals, or 25% of the weight of the spleen at the corresponding age, were cut out of its middle segments in the radial direction. Fragments of spleen prepared for autograting were placed in the peritoneal cavity, with their tissue wrapped in greater omentum. For this purpose, pieces of splenic tissue were arranged regularly on the anterior surface of the lower part of the greater omentum, which was folded

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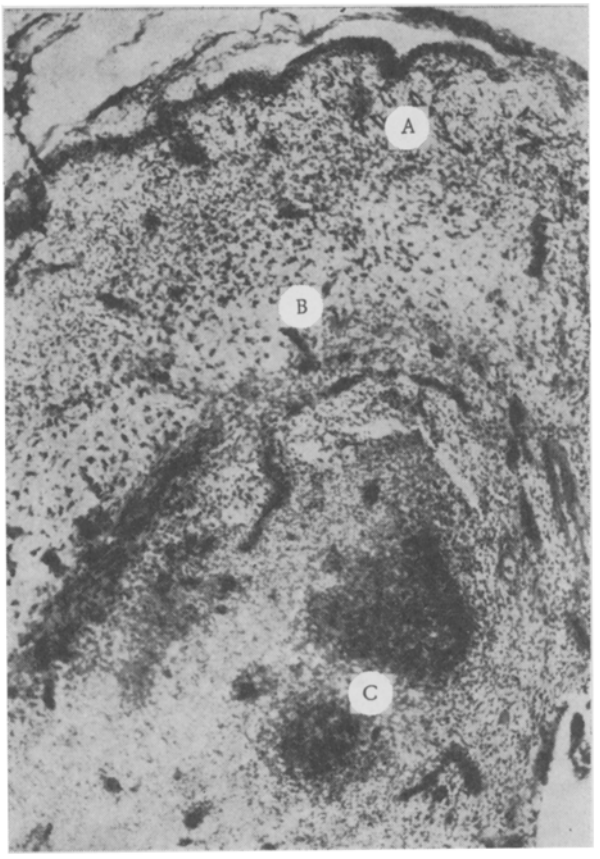

Fig. 1. General appearance of splenic graft 14 days after implantation of fragment in greater omentum. A) Zone of regeneration; b) zone of necrosis; c) zone of surviving tissue. Van Gieson, 63×.

in two and fixed in that position with silk sutures. In other cases (half of all the experimental animals) fragments of spleen were implanted into the omentum of the small intestine, for which purpose one leaf of the omentum was divided and a tunnel of appropriate size made in it.

Splenectomy was performed on the animals of group 2, with distal resection of the pancreases and reimplantation of splenic fragments into the omentum folded in two as described above. The animals were kept under observation for 4-5 months. Material from the splenic autografts for morphological study was taken 14, 20, and 30 days and 4-5 months after autografting. The material was fixed in 10% neutral formalin, embedded in paraffin wax, and sections were stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

From 14 to 20 days after autografting the fragments of implanted splenic tissue had a zone consisting of widely spaced reticular cells and connective-tissue trabeculae, the spaces between which were filled with an albuminous fluid, erythrocytes at different stages of lysis, and siderophages (Fig. 1). Changes of this kind were observed most frequently at the periphery of the autograft. Less frequently, the zone with widely spaced cellular structures extended in the form of tongues into the depth of the graft. It was in this part of the autograft that the necrotic cells underwent lysis. It will be recalled that fragments of splenic tissue implanted in the peritoneal cavity, but without any blood supply, undergo necrobiotic changes, and some of their cells become nectoric. By 14-20 days after the beginning of vascularization of the fragments (ingrowth of capillaries into them from the surrounding tissues — the bed of the autograft) lysis of the dying cells takes place, and the surviving cells appear to be widely spaced. In the central part of the autograft at this time the cells are more compactly arranged, but changes due to deficient blood supply can be seen in them: the decrease in the number of lymphocytes is noteworthy. Although the fibrous and reticular stroma is comparatively well preserved, lymphocytes are completely absent in the red pulp and the follicles appear greatly reduced in volume and have no pale centers. Pycnosis of the nuclei and cytoplasm of both lymphocytes and reticular cells is noteworthy. Histological investigations of the transplanted fragments of splenic tissue after 14-20 days revealed, besides the two zones described above, a narrow band which can be regarded as a zone of regen-

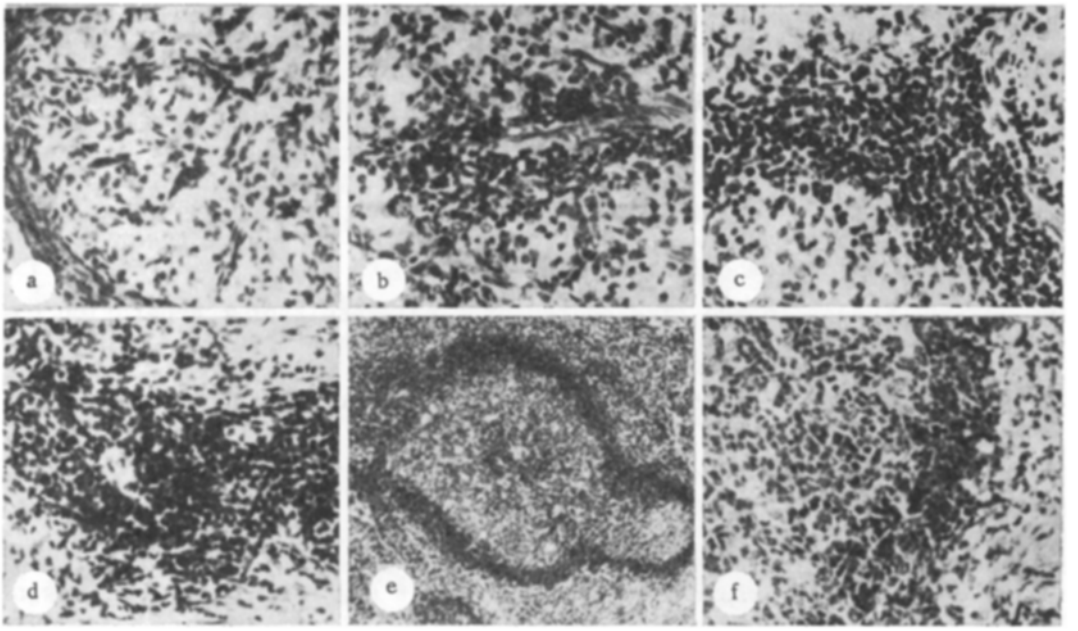

Fig. 2. Time course of restoration of splenic autografts: a) zone of regeneration containing capillaries and reticular cells. Van Gieson; b) beginning of follicle formation around a. Centralis. Hematoxylin and eosin; c) primitive follicle in restored splenic tissue, surrounded by reticular cells. Hematoxylin and eosin; d) further increase in size of follicles. Hematoxylin and eosin: e) complete restoration of splenic tissue; lymphatic follicle with pale germinal center. Van Gieson; f) detail of previous figure — fragment of lymphatic follicle. Magnification: a-d, f) 250 ×; e) 63 ×.

eration. It consists of a network of relatively widely spaced reticular cells with nuclei that are increased in volume and often hyperchromic. Mitotic figures are frequently found in these cells. Many capillaries with swollen endotheliocytes also are present here (Fig. 2a). The zone of regeneration lies at the periphery of the fragments, and is bounded by areas of surviving splenic tissue with widely spaced cells. Less frequently the zone of regeneration is found in the depth of the fragments, in which case it is bounded by zones of surviving splenic tissue with widely spaced and compact cellular structures. The zone of regeneration, as already mentioned, contains many blood vessels of capillary type: blood vessels and young connective tissue invade the graft tissue in bands from the surrounding tissue of the omentum (or mesentery of the small intestine). In some parts of the strip of regeneration, it is beginning to be filled with lymphocytes, which are arranged either diffusely or in small groups, resembling primitive primary follicles. The nuclei of the lymphocytes here are larger than those of lymphocytes in the surviving zone of the graft, where pycnosis of the cell nuclei are observed. Consequently, on the basis of the results of histological investigations it can be concluded that regeneration begins exclusively in the revascularized areas of the graft. The character of the histological changes at this time was the same in animals of both groups (with and without resection of the pancreas).

One month after autografting the process of clearance of necrotic cells in animals of both groups has spread to the central part of the fragments, where only a few (viable) reticular cells are still preserved, mainly around blood vessels and trabeculae, and where groups of lymphocytes with pycnomorphic nuclei are less frequently preserved. At the same time, widening of the zone of regeneration (from periphery to center) can be seen. The number of lymphocytes, distributed both diffusely and in primitive lymphatic follicles, is increased in the zone of regeneration (Fig. 2b, d). Reticular cells in the zone of regeneration are acquiring a spatial orientation typical of the spleen. The degree of swelling of the nuclei in these cells is reduced. Some small splenic fragments at this stage appear to consist entirely of a zone of regeneration. However, only the reticular stroma of the organ is restored, and the number of lymphocytes in it, as before, remains sharply reduced compared with normal.

After 3 months the reticular stroma is now completely restored in what are already relatively large splenic fragments. The peripheral zones of the graft contain many concentrations of lymphocytes, in the form of primitive or developing follicles, which have as yet no pale germinal centers (Fig. 2c).

After 4-4.5 months the graft in animals of both groups has the histological structure of an unchanged, intact spleen. Large lymphatic follicles with germinal centers in them (Fig. 2e, f) and a red pulp containing many diffusely distributed lymphocytes can be identified in them. These restored autografts are rather smaller in volume than the splenic fragments when implanted in the peritoneal cavity initially.

There were no differences in the time course or final result of regeneration when the splenic fragments were transplanted into the folded greater omentum or into the mesentery of the small intestine, i.e., the effect in both cases was satisfactory. Distal resection of the pancreas did not significantly affect the course of regeneration of the splenic tissue or the chances of survival of the graft.

Autografting of fragments of decapsulated spleen into a fold in the greater omentum and into the mesentery of the small intestine in male guinea pigs thus terminates, as histological investigation shows, with survival of the autografts but some reduction in their size. The autografts acquire the typical histological structure of intact spleen after 4-4.5 months. The autograft survives through a radical reconstruction of the splenic tissue: restoration of the histological structure of the autograft is preceded by necrobiotic changes with lysis of the lymphocytes and of a large volume of the reticular cells. The lysis of dying cells and regeneration "move" from the periphery toward the center of the autograft.

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